Exploring Neurocognitive Factors Underlying Autism and Hyperphagia in Prader-Willi Syndrome

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by

Sarah-Marie Feighan BA, MSc

Supervised by: Professor Louise Gallagher and Dr Ciara Molly

Department of Psychiatry
School of Medicine
Trinity College Dublin
University of Dublin
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Declaration

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Summary

Background

Prader-Willi Syndrome (PWS) is a rare complex multisystem genetic disorder recognised as the most common genetic cause of life-threatening obesity in humans (Butler, 1996). The PWS genotype gives rise to a complex behavioural phenotype. Autism traits and hyperphagia, a feeling of extreme hunger, are prevalent and impairing features of the PWS behavioural phenotype. Understanding the neurocognitive nature of autism behaviours in people with PWS will clarify if autism behaviours are related to social cognitive impairments, which will be imperative for understanding if autism supports may be suitable for some people with PWS. Identifying neurocognitive factors underlying hyperphagia will determine if eye tracking can be used as an objective measure of hyperphagia. A lack of objective measures of hyperphagia is currently a significant barrier to evaluating potentially life-altering drug therapies for hyperphagia.

Aims and structures of thesis:

The primary objective of this thesis was to enhance comprehension of PWS by profiling the mental health and behaviour needs of individuals with PWS and contributing critical knowledge to understanding neurocognitive factors underlying autism behaviours and hyperphagia using eye tracking technology.

Chapter 1 introduces the PWS behavioural phenotype and discusses findings in the literature supporting the exploration of neurocognitive factors in neurodevelopmental conditions using eye tracking methodology. The thesis aims, and hypotheses are introduced.

Chapter 2 describes the different methodologies used to investigate each chapter's hypotheses.

Chapter 3 describes the results from a national survey carried out with PWS parents/caregivers and summarises findings in relation to psychiatric comorbidities, behaviours of concern, service needs and the impact of caring in families of an individual with PWS.

Chapter 4 presents a study investigating social cognition in PWS by examining preferences for social stimuli and their relationship with autism and social functioning using a well-established passive viewing eye tracking paradigm, the face pop task.

Chapter 5 describes the Food Attentional Bias (FAB) task, an innovative eye tracking paradigm, to explore variations in visual attention towards food stimuli as a potential measure of hyperphagia. This chapter develops and tests the task in a health-weight cohort.

Chapter 6 describes the participatory approach used to conduct focus groups with PWS stakeholders to determine what adaptations would be required to implement the FAB task protocol in PWS.

Chapter 7 discusses the results of implementing the adapted FAB task protocol from Chapter 6 in individuals with PWS and an age and gender-matched neurotypical comparison group.

Finally, Chapter 8 presents a discussion of the findings.

Results:

The survey results from Chapter 3 revealed a high prevalence of psychiatric disorders and behaviour challenges in PWS, highlighting the complex mental health and behavioural needs of individuals with PWS. Caregivers expressed that their employment, family relationships, and emotional well-being were significantly affected, highlighting the notable impact experienced by them.

The investigation of social cognition in Chapter 4 revealed that PWS participants looked at social stimuli relatively more than the comparison group as measured by the proportion of fixations to face stimuli. However, higher levels of autism traits in the PWS group were associated with reduced sustained attention to faces, which has also been reported in autism cohorts. These findings suggest that endogenous deployment of attention to social stimuli may be reduced in individuals with co-occurring PWS and autism.

In Chapter 5, an objective measure of satiety was investigated using an eye tracking task modified from the 'face pop' task described in Chapter 4. The FAB task measured interest in food stimuli across two meal conditions, pre-and postmeal. Healthy-weight participants showed a clear reduction in attention to food stimuli in the postmeal condition, as evidenced by shorter durations and fewer fixations on food stimuli. This finding suggested that changes in attention to food stimuli from premeal to postmeal may be a marker of satiety in typically developing populations.

In Chapter 6, the main theme to emerge from the focus groups with PWS stakeholders was the importance of communication and cooperation between researchers and participants' parents/caregivers when scheduling and organising the PWS research visit. Key adaptations were made to the protocol concerning this theme, such as the design of the premeal and postmeal study condition, the standardised meal approach taken, and the behavioural questionnaires used.

In Chapter 7, the adapted FAB task protocol was implemented in PWS and showed that participants with PWS did not display a significant decrease in the number and duration of fixations on food stimuli in the post-meal condition. This differed from the comparison group, which showed reduced visual attention to food stimuli, similar to the healthy-weight group in Chapter 5. This outcome validated the primary objective of the FAB task protocol, showing that participants with PWS maintained their interest in food stimuli even after eating. The absence of reduced visual attention to food stimuli in PWS may reflect an atypical satiety response, a critical aspect of hyperphagia. Therefore the FAB task protocol has potential as a neurocognitive marker of hyperphagia and warrants further investigation.

Conclusion

The results from this work provide novel insights into the neurocognitive nature of autism behaviours and hyperphagia in PWS. An improved understanding of social cognitive factors in PWS can inform treatment approaches for autism symptoms and social functioning in PWS. Further investigation of visual attention to food stimuli has the potential as an objective marker of hyperphagia in PWS that could be used to monitor the treatment effectiveness of novel drug therapies.

Contributions to this Work

All studies were designed in collaboration with my supervisors, Professor Louise Gallagher and Dr Ciara Molloy. I was responsible for data collection, data analysis and interpretation of the work under the supervision of Professor Louise Gallagher and Dr Ciara Molloy. Dr Eleisa Heron, Dr Clare Kelly and Dr Cathal Ormond provided consultation on statistical methods for the studies.

For the study described in Chapter 3, Dr Marguerite Hughes helped recruit for the survey. For the study described in Chapter 4 and Chapter 7, Dr Ciara Molloy and Ms Aine McNicholas helped with clinical recruitment and Ms Meg Young helped with data entry. For Chapter 5, Ms Katie McArdle helped with task design and clinical recruitment, and Ms Linda Lisanti helped with clinical recruitment. For chapter 6, Ms Aine McNicholas helped with study design, recruitment, and analysis. For Chapter 7, Dr Ciara Molloy helped with clinical recruitment.

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The Prader-Willi Syndrome Association of Ireland inspired my journey into PWS research. I consider myself incredibly fortunate to have been at the right place and time where I had the opportunity to work with PWSAI on their Assessment of Needs project. PWSAI generously funded my first-ever trip to Florida for the PWS-USA Conference in 2017, an experience that ignited the ideas for this PhD. This thesis would never have happened without this organisation. Their unwavering support throughout has been indispensable, and I am immensely grateful to Dr Marguerite Hughes, Gary Brennan, Don Tallon, Emma Walshe-Tierney, Anthony Carr and all the members for their continuous encouragement and backing.

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List of Abbreviations

AB	Attentional Bias
ABA	Applied Behaviour Analysis
ADI-R	Autism Diagnostic Interview, Revised
ADOS-II	Autism Diagnostic Observation Schedule, Second Edition
AIC	Akaike's Information Criterion
AIMS-2-TRIALS	Autism Innovative Medicine Studies-2-Trials.
ANOVA	Analysis of Variance
AOI	Area of Interest
ASC	Autism Spectrum Conditions
BMI	Body Mass Index
CM	Centimetres
COM	Comparison
CSS	Calibrated Severity Score
DEL	Deletion
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EEG	Electroencephalogram
EU-AIMS	European Autism Interventions
FAB	Food Attentional Bias Task
FDA	The United States Food and Drug Administration
fMRI	Functional magnetic resonance imaging
FPWR	Foundation for Prader Willi Research
FPWR-CTC	Foundation for Prader Willi Research Clinical Trials Consortium
FRPQ	Food-Related Problem Questionnaire
FSIQ	Full-Scale Intelligence Quotient
HQ	Hyperphagia Questionnaire
ICD-10	International Classification of Diseases, Tenth Edition
ID	Intellectual Disability
KG	Kilogram
LLM	Linear Mixed Model
LSD	Least Significant Difference
MRI	Magnetic Resonance Imaging
MSEL	The Mullen Scales of Early Learning

mUPD	Maternal Uniparental Disomy
OCD	Obsessive Compulsive Disorder
PPI	Public and Patient Involvement
PRI	Perceptual Reasoning Index
PWS	Prader-Willi Syndrome
PWSAI	Prader-Willi Syndrome Association of Ireland
REML	Restricted Maximum Likelihood
SCQ	Social Communication Questionnaire
SD	Standard Deviation
SE	Standard Error
SLIM	Satiety labelled Intensity Magnitude
SSRIs	Selective Serotonin Reuptake Inhibitors
VABS-II	Vineland Adaptive Behaviour Scales, Second Edition
VCI	Verbal Comprehension Index
VMPFC	Ventromedial Prefrontal Cortex
VR	Virtual Reality
WASI-II	Wechsler Abbreviated Scale of Intelligence, Second Edition

Contents

Chapter 1: Introduction

1 Introduction

1.1. Overview

This chapter provides the necessary background information relevant to the interconnected research aims of each experimental chapter in this thesis. It begins with an exploration of the clinical presentation of Prader-Willi syndrome (PWS) and its genetic underpinnings. The literature on treatment approaches in the clinical management of PWS is reviewed. Emphasis is placed on the complex behavioural phenotype of PWS, underscoring the importance of unravelling the neurocognitive mechanisms behind these behaviours to inform future treatments and interventions. Introducing eye tracking as the chosen methodological approach for investigating cognition, the chapter lays the foundation for subsequent chapters by presenting specific study hypotheses and aims for each part of the thesis.

1.2 Background

1.2.1 Clinical Presentation of PWS

PWS is a rare complex multisystem genetic disorder recognised as the most common genetic cause of life-threatening obesity in humans (Butler, 1996). The birth incidence rate of PWS is approximately 1:25,000 (Smith et al., 2003; Vogels et al., 2004; J. E. Whittington et al., 2007). The main early features of the syndrome are extreme hypotonia at birth (i.e. babies are born with low muscle tone and appear “floppy”) and failure to thrive, followed, in early childhood, by the development of hyperphagia. Hyperphagia in PWS presents as an extreme, unsatisfied drive to consume food, accompanied by a lack of satiety, which results in severe obesity if food management is not in place (Schwartz et al., 2021).

PWS affects multiple systems, with many varying clinical features including growth hormone deficiency, leading to growth and developmental issues. Hypogonadotropic hypogonadism in PWS (low levels of gonadotropins leading to decreased production of sex hormones and impaired reproductive function) can result in delayed or absent puberty, infertility, and other related issues. Sleep disturbances are also common (Cassidy, 1997; Höybye, 2013). Another characteristic is reduced pain sensitivity, resulting in a diminished response to pain stimuli. Gastrointestinal function is compromised with decreased gastrointestinal motility, affecting digestion. Scoliosis, an abnormal lateral curvature of the spine, may also be observed. Developmental delays in achieving milestones and intellectual disabilities are part of the clinical spectrum (Cassidy, 1997; Höybye, 2013). Socially and behaviourally, individuals with PWS often encounter challenges such as anxiety, repetitive behaviours, a strong need for routine, difficulties in social interactions, temper tantrums, and skin picking (Cassidy, 1997; Höybye, 2013; Veltman et al., 2004; J. Whittington & Holland, 2018). Individuals with PWS face an elevated risk of mental illness, with higher rates of depression, bipolar disorder, and psychosis than the general population (Dykens & Shah, 2003; Whittington & Holland, 2018). In a systematic review and meta-analysis exploring the mental health of children with neurogenetic disorders linked to intellectual disability, it was found that 74% of those with PWS exhibited mental health symptoms, according to data from four studies using the Child

Behaviour Check List (Glasson et al., 2020). In adults with PWS, a meta-analysis of 13 studies reported mood disorders affected 10 to 20% of adults with PWS and one-third of PWS adults experienced psychotic symptoms, with a higher occurrence seen in individuals with maternal uniparental disomy (Yang et al., 2013).

1.2.2 Genetics of PWS

PWS is an imprinting disorder resulting from the absence of paternal expression of maternally imprinted genes at chromosomal locus 15q11-13 (Butler, 1996). Genetic imprinting is an epigenetic phenomenon that results in the differential expression of genes based on their parental origin. In mammals, including humans, each individual inherits one set of chromosomes from the mother and one from the father. While most genes from parental chromosomes are actively expressed, specific genes undergo epigenetic modifications during gamete formation, resulting in silencing or suppressing expression from one parent's allele (Butler, 1996). Chromosome 15 has two imprinted regions: the Prader-Willi region, maternally imprinted and paternally expressed, and the Angelman Syndrome (AS) region, paternally imprinted and maternally expressed. AS, PWS' sister disease results from the lack of maternal expression of paternally imprinted genes at chromosomal locus 15q11-13 (Figure 1.1). The absence of maternal expression in the PWS region can arise through four genetic defects

1. De Novo Deletion on the Parental Chromosome (DEL): Approximately 70% of PWS cases are attributed to de novo deletions on the paternal chromosome 15. A De Novo genetic deletion refers to the loss of a segment of DNA from a chromosome that is newly occurring in an individual and is not inherited from their parents (Butler et al., 2018). These deletions are categorised into Type 1 and Type 2, distinguished by their size and specific breakpoints on chromosome 15. Type 1 deletions span approximately six megabases (Mb) between breakpoints one to three, while Type 2 deletions cover around 5.6 Mb between breakpoints two and three (Butler et al., 2018).

2. Maternal uniparental disomy (mUPD): Approximately 25% of cases are attributed to the presence of two maternally inherited chromosome 15s without any contribution from the father due to non-disjunction during meiosis (Butler et al., 2018). During meiosis, the chromosomes in a cell are supposed to separate properly, resulting in gametes (sperm or eggs) with a complete set of chromosomes. In cases of non-disjunction, an error occurs during this process, causing chromosomes to fail to separate correctly. In the case of mUPD, both copies of a chromosome 15 come from the mother instead of one from each parent. The loss of paternal expression of maternally imprinted genes also causes an excess of paternally imprinted and maternally expressed genes (Butler et al., 2018). Notably, the proportion of PWS cases resulting from mUPD appears to have increased recently, possibly due to advanced maternal age at conception (Cho et al., 2013).

3. Imprinting Centre Defect (ICD) is a rare genetic anomaly in PWS caused by microdeletions or changes in DNA sequences, leading to errors in the imprinting process. ICD accounts for only 2-4% of PWS cases (Bittel & Butler, 2018). The pattern of phenotypic expression in ICD is often similar to mUPD.

4. Translocations are a highly uncommon genetic subtype, representing less than 1% of PWS cases (Butler et al., 2018). A translocation refers to the relocation of a segment of

genetic material from one chromosome to another (Butler et al., 2018). Individuals with PWS due to translocations may experience additional symptoms due to the loss of expression from the other translocated region (Bittel & Butler, 2018)

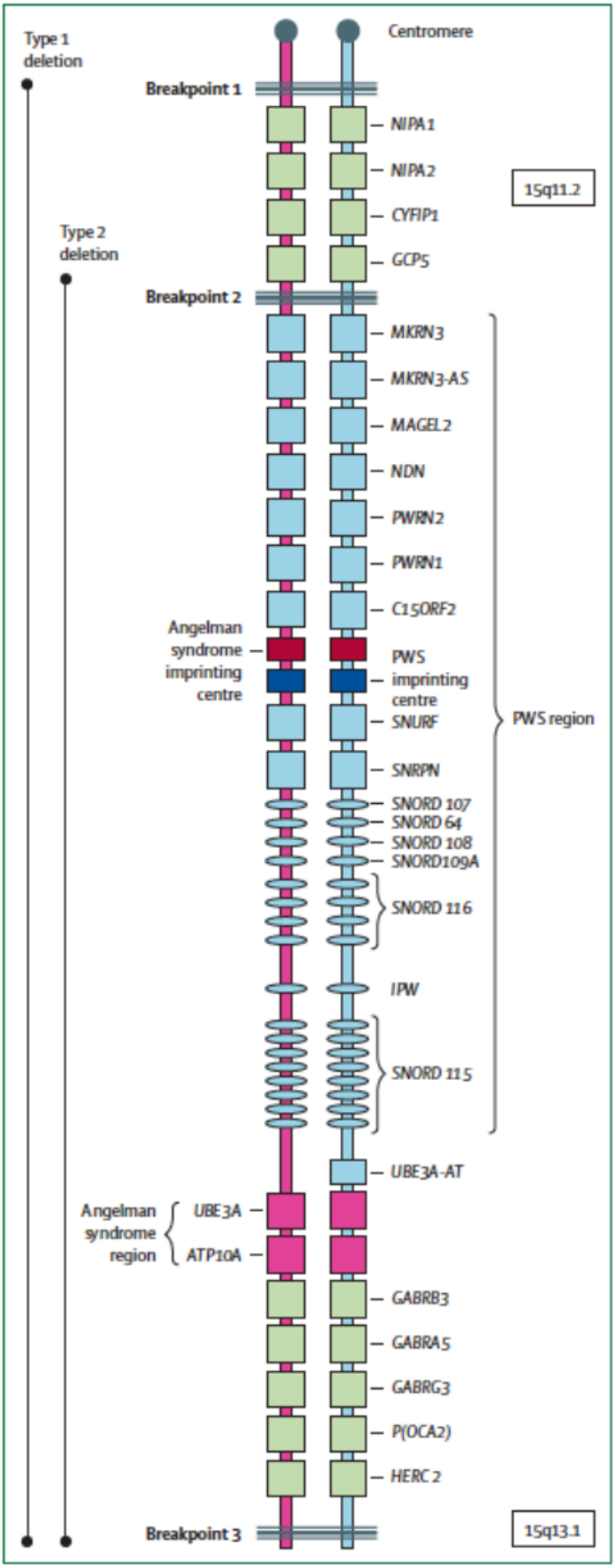

Figure 1.1: Genetics of Prader-Willi Syndrome (Figure from Aman et al., 2018).

1.2.3 Treatments Approaches in PWS

1.2.3.1. Physical Health Interventions

There is currently no cure for PWS. Due to the intensity and the range of clinical manifestations of PWS, input from a large variety of professionals and services is needed to ensure adequate care, prevent secondary disability, and optimise the quality of life for this patient population (McCandless et al., 2011). In infancy, special nipple or nasogastric tube feeding (the insertion of thin tube through the nose and down the throat into the stomach) is used to ensure adequate nutrition, while physical therapy is used to improve muscle strength and mobility. Optimising body composition, growth and development are key therapeutic targets. Growth hormone replacement therapy is used not only for its effect on stature but also for its metabolic effects and impact on body composition (improving muscle strength, increasing lean body mass to normalise height while decreasing fat mass). However, this treatment requires daily injections and frequent endocrine evaluations. Additional hormonal treatments at puberty can be considered to develop or induce secondary sexual characteristics and optimise bone health (McCandless et al., 2011).

1.2.3.2. Developmental, behavioural and mental health interventions

Hyperphagia begins to emerge in childhood and requires around-the-clock supervision to ensure the person with PWS is not seeking/eating food they should not consume (Schwartz et al., 2021). Effective treatments for hyperphagia in PWS remain elusive, and the current standard treatments, including dietary modifications, exercise, and growth hormone replacement, have shown limited efficacy in appetite and weight control (Tan et al., 2020). Bariatric surgery, while being considered in some cases, still lacks long-term safety and effectiveness for PWS patients. Several drug interventions have been investigated to target hyperphagia and associated symptoms in PWS, but unfortunately, they have not shown consistent and robust benefits (Tan et al., 2020). Although promising results from a phase-III clinical trial of carbetocin, an oxytocin analogue, indicated clinically meaningful improvements in hyperphagia and anxiousness and distress behaviours in participants with PWS as measured by caregiver reports (Roof et al., 2023).

Environmental interventions are currently the most effective approach to managing hyperphagia. In the absence of effective interventions for hyperphagia, restricting access to food and controlling food intake "food security" are paramount for the safety of persons with PWS and a 24/7 concern for families (Schwartz et al., 2021). Food security may include restricting access to food with locks on fridges or presses. Food security must be implemented across all settings, i.e., home, school, work, holidays and social events, placing a considerable burden on caregivers (Kayaajardan et al., 2018). Food security also includes schedules and plans for all meals and food consumption. Changes to those plans can lead to significant behavioural challenges for the person with PWS (Schwartz et al., 2021).

In terms of development, speech therapy is required, particularly in infancy, to support the management of feeding difficulties (McCandless et al., 2011). Educational planning and behaviour management are often necessary to manage behavioural problems and support an appropriately structured environment. Additionally, individuals with PWS require support and interventions related to cognitive delays and behavioural problems, such as managing and coping with emotional outbursts (McCandless et al., 2011). Beyond environmental and behavioural treatments, selective serotonin reuptake inhibitors and atypical antipsychotics

have been used with children with PWS to treat comorbid symptoms of depression and psychosis (Bonnot et al., 2016). While known to be effective in these conditions in the general population, research into their effectiveness in PWS is limited (Whittington & Holland, 2010).

1.2.4 The Behavioural Phenotype of PWS

The PWS genotype gives rise to a distinctive "behavioural phenotype," encompassing specific cognitive, social, and behavioural traits and increased susceptibility to behavioural disorders and psychiatric problems (Schwartz et al., 2021). The complexity of the interrelated behavioural features in PWS poses challenges in conducting studies, mainly due to the lack of consensus on defining the intricate behaviours associated with the syndrome. The PWS-CTC "Behaviour Outcomes Working Group" was formed in response to this issue, consisting of PWS scientists, clinicians, and representatives from patient advocacy organisations. The primary objective of this group was to establish consensus definitions and descriptions of the behavioural features associated with PWS. In the following section, I will introduce the key behavioural features identified by this working group (Schwartz et al., 2021) to give an overview of the complex PWS behavioural phenotype.

1.2.4.1 Hyperphagia

Hyperphagia in PWS manifests as an extreme desire to eat and a lack of normal satiety (Schwartz et al., 2021). Hyperphagia is proposed to overlap with binge eating disorder, addiction, and obsessive-compulsive features (preoccupation with food) (Holland et al., 2019; Salles et al., 2020). PWS-related hyperphagia is incessant for families and caregivers, with individuals constantly seeking food. As mentioned above, food security is vital for their safety, as is educating everyone interacting with the person with PWS (Schwartz et al., 2021). Hyperphagia is an overwhelming, life-threatening force requiring lifelong environmental control and a restricted lifestyle for the person with PWS, their caregivers and their siblings (Mazaheri et al., 2012). entire family. Constant vigilance is required regarding food exposure in those with hyperphagia, as uncontrolled or unsupervised access to food may result in obesity or even gastric rupture and death (Tan et al., 2020). Treating hyperphagia is the caregivers' highest priority (FPWR, 2014; Tsai et al., 2018).

Individuals with PWS typically progress through 7 different nutritional phases during their lifetime, as revealed through a longitudinal multicentre natural history study (Miller et al., 2011). Phase 0 occurs in utero with decreased foetal movements and growth restriction compared to siblings. In Phase 1, the infant is hypotonic and without obesity. Subphase 1a is characterised by poor appetite, hypotonia and difficulty feeding. This phase is followed by sub-phase 1b, when the infant's appetite and feeding significantly improve, and weight gain occurs. Phase 2 is associated with an abnormal weight gain; in sub-phase 2a, the weight increases without a significant change in appetite or caloric intake, while in sub-phase 2b, the weight gain is associated with a simultaneously increased interest in food, but the child can feel satiated. Nutritional Phase 3 is characterised by an insatiable appetite typically accompanied by aggressive food-seeking behaviour and a lack of satiety (see Figure 1.2).

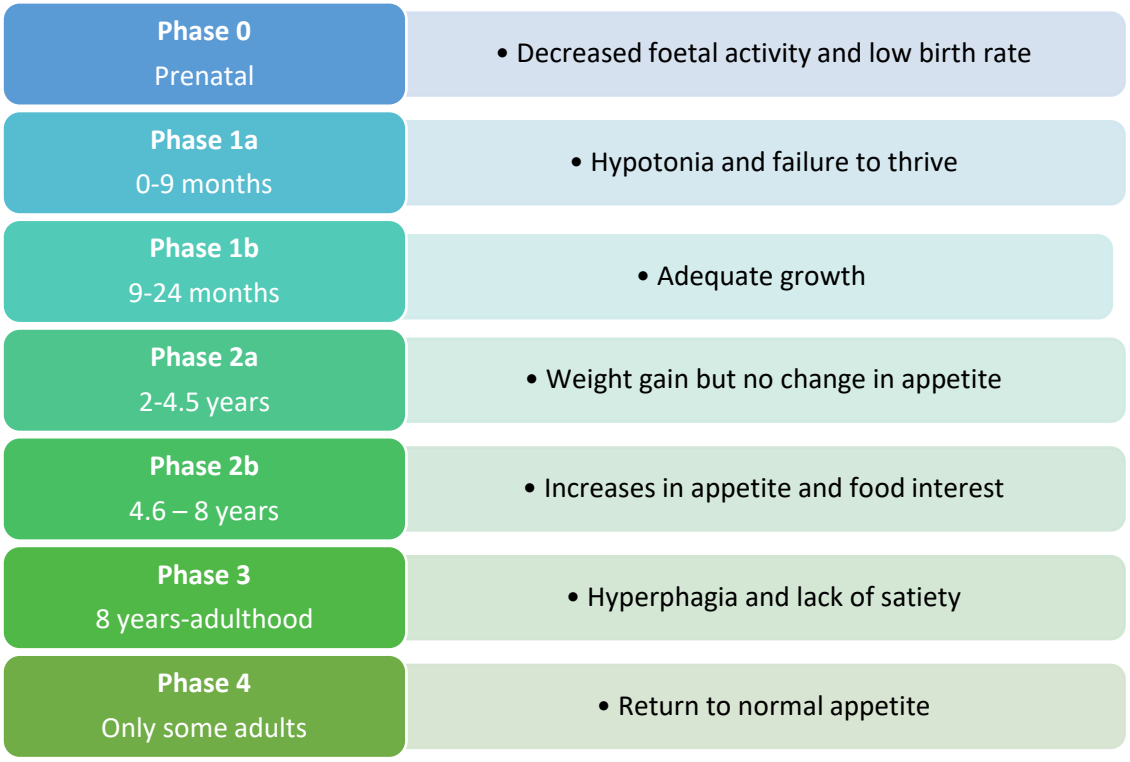

Figure 1.2: Nutritional phases of PWS (based on Miller et al., 2011)

While the precise mechanism of hyperphagia is not fully understood, evidence from MRI points to dysfunction within the feeding centre of the hypothalamus and its associated hormones, which have been strongly linked to uncontrollable food intake in PWS (Qaddra et al., 2023). Changes in several brain areas (hypothalamus, amygdala, hippocampus, orbitofrontal and medial prefrontal cortex) play an important role in regulating abnormal food intake in PWS. Functional magnetic resonance imaging showed higher activity in reward/limbic regions (nucleus accumbens, amygdala) in subjects with PWS (Holsen et al., 2006). Mainly, subjects with PWS exhibited greater activation in response to food in limbic and paralimbic regions (hypothalamus, amygdala, hippocampus) and lower activation in cortical inhibitory regions (orbitofrontal cortex, medial prefrontal cortex) (Holsen et al., 2006; Xu et al., 2017). Reduced functional connectivity between the ventral striatum and limbic structures (hypothalamus and amygdala) was reported in subjects with PWS and associated with obsessive eating behaviour (Xu et al., 2017). These studies suggest that dysfunction in food reward-related circuit areas and impairments in inhibitory control areas contribute to hyperphagia and extreme obesity in PWS.

1.2.4.2 Autism

Autism is highly heritable and characterised by distinctive behavioural challenges across three primary domains: social interactions, communication, and restricted, repetitive behaviours/interests (Tick et al., 2016; APA, 2013). The spectrum of autism presents a significant degree of phenotypic heterogeneity, encompassing a wide array of observable traits and characteristics. These traits manifest diversely in each individual, resulting in a varied spectrum of social interactions, communication styles, and behaviours (Loth et al. 2018).

In the pursuit of understanding the heterogeneity within the autism phenotype, research has increasingly turned to individuals with genetic syndromes, where a notable diversity in autistic characteristics is often observed (Bozhilova et al., 2023). Individuals with genetic syndromes linked to intellectual disability are more likely to display autistic traits compared to the general population (Richards et al., 2015). The prevalence and nature of autism behaviours in PWS have been a subject of debate among clinicians and researchers (Schwartz et al., 2021). Estimates of autism prevalence in PWS using the SCQ range from 29% to 49%. However, when direct assessment of autism with the ADOS-2 was used by PWS, autism diagnosis reduced to 12.3% (18 out of 146 children).

Individuals with PWS encounter challenges in areas of social communication comparable to those seen in autism. Differences between PWS and typically developing groups have been reported in social perception, face processing, understanding personal space and theory of mind i.e. interpreting others' mental states (Debladis et al., 2019; Key et al., 2013, Lo et al., 2013). Studies have consistently reported that individuals with the mUPD subtype demonstrate more pronounced social cognitive differences compared to those with deletion subtypes (Debladis et al., 2019; Key et al., 2013; Key & Dykens, 2017). While the existing literature on autism and social cognition within PWS will be reviewed in more detail in Chapter 4, it is important to note that the nature of autism behaviours within the context of PWS remains poorly understood. Therefore, conducting a comprehensive evaluation of autism behaviours and social communication in PWS is important in developing our understanding autism in the context of PWS.

1.2.4.3. Temper outbursts

Temper outbursts (also referred to as emotional outbursts or meltdowns) are common maladaptive behaviours observed in individuals with PWS, impacting their quality of life as much or even more than hyperphagia (Rice et al., 2018). In a study involving 248 individuals diagnosed with one of four rare genetic syndromes—Down syndrome, Fragile X syndrome, PWS and Williams syndrome—it was revealed that PWS (n=87) had the highest incidence of temper tantrums, with 80% of individuals exhibiting this behaviour as measured by the Developmental Behaviour Checklist (DBC) (Rice et al., 2015). These outbursts may reduce social opportunities, limit living options, and hinder employment prospects (Rice et al., 2018, Achenbach, 1999). The triggers for these outbursts can include a blocked goal, violation of social expectations, perceived injustice, or difficulty coping with changes in routine. The sequence of behaviours and emotions within these outbursts is similar to those seen in typically developing young children, but in PWS, they start slightly later in life and continue throughout adulthood (Rice et al., 2018). While it was initially hypothesised that temper outbursts might be related to hyperphagia, research now shows possible mechanisms driving these outbursts include deficits in task-switching ability and dysregulation of the autonomic nervous system (Manning et al., 2019; Tunnicliffe et al., 2013; Woodcock et al., 2010). A small pilot study demonstrated that vagal nerve stimulation reduced temper outbursts in some individuals with PWS, pointing to the role of the autonomic nervous system in this behavioural feature (Manning et al., 2019).

1.2.4.4 Anxiety

Anxiety is prevalent in individuals with PWS, characterised by excessive worry and tension, particularly concerning schedules, routines, food planning, food security, and individuals or items of special interest, such as teachers, caregivers, or pets (Schwartz et al., 2021). In a cohort study of PWS participants (n = 46), Einfeld et al. (1999) found that 43% of individuals with PWS assessed with the DBC had significant levels of anxiety. A 2018 review of data from the PWS Global Patient Registry reported that caregiver report of anxiety symptoms to be similar between males and females but higher in individuals with the UPD genetic subtype of PWS compared to the deletion subtype (73% vs. 32%) (FPRW, 2020). Anxiety in PWS is characterised by excessive worry and tension, particularly concerning schedules, routines, food planning, food security, and individuals or items of special interest, such as teachers, caregivers, or pets (Schwartz et al., 2021). A 2018 review of data from the PWS Global Patient Registry reported that anxiety symptoms in PWS appears to be similar between males and females but higher in individuals with the UPD genetic subtype of PWS compared to the deletion subtype (73% vs. 32%) (FPRW, 2020).Anxiety in PWS can share similarities with Generalised Anxiety Disorder (GAD) but also exhibits distinctive elements, such as significant worry related to food planning/security and specific items or individuals of interest. These unique features may not be fully captured by existing definitions based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5). Investigations have shown moderate to high anxiety levels in cohorts of individuals with PWS (Schwartz et al., 2021). Caregivers have consistently rated anxiety as a top concern for PWS treatment, reflecting its negative impact on individuals with PWS (Tsai et al., 2020). Unlike in the general population, where anxiety is more prevalent in females, anxiety prevalence in PWS individuals appears to be similar between males and females but higher in those with the UPD genetic subtype compared to the deletion subtype (Dykens & Roof, 2008). While age-of-onset studies for anxiety in PWS are lacking, clinical observations suggest that anxiety often emerges during preschool to school age, with peak symptoms during adolescence and early adulthood (Schwartz et al., 2021).

1.2.4.5 Obsessive-compulsive behaviours

In a cohort study involving 91 individuals with PWS 46 participants (51%) exhibited symptoms of obsessive-compulsive (OC) behaviours as assessed by the Yale-Brown Obsessive Compulsive Scale (Goodman et al., 1989; Dykens et al., 1999). However, when applying the diagnostic criteria for obsessive-compulsive disorder (OCD) from the DSM-5 in a longitudinal study, only 8% of the PWS sample met the full criteria for an OCD diagnosis (Schwartz et al., 2021). This variance in results suggests that while obsessive-compulsive behaviours are prevalent, they represent only one facet of the broader OCD phenotype and do not reach the threshold for a clinical OCD diagnosis (Whitting & Holland, 2019). Obsessions in PWS often differ from typical OCD preoccupations, and repetitive questioning is a prominent OC behaviour in PWS, possibly linked to resistance to change (Woodcock et al., 2009). These behaviours may provide enjoyment and comfort for individuals with PWS, unlike the distressing nature of classic OCD (Novell-Alsina et al., 2019). The age of onset and possible genetic subtype differences in OC symptoms remain underexplored. However, clinical observations suggest that OC symptoms in PWS may begin around age 3, with higher rates of compulsive behaviour in young children than in typically developing peers (Dimitropoulos et al., 2001). The research on differences in OC symptoms among PWS genetic subtypes is inconclusive. One study found higher overall OC

behaviours in the PWS deletion subgroup, while individuals with PWS UPD exhibited higher rates of OC behaviours resembling those commonly seen in autism (State et al., 1999).

1.2.4.6 Rigidity

People with PWS often exhibit rigid thinking styles and behaviours that disrupt daily functioning. They commonly resist change and have challenges with task switching, which involves efficiently transitioning between different tasks (Chevalère et al., 2015; Haig & Woodcock, 2017; K. A. Woodcock et al., 2010). Clinical observations suggest that these behaviours emerge young, even before starting school. Compulsivity and insistence on sameness in routines or events have been observed in many individuals with PWS across a range of intellectual functioning and overlap with behavioural characteristics of autism (Dykens et al., 2017). The impact of these rigid behaviours on adaptive functioning is significant and poses challenges for parents and caregivers to manage effectively. However, there is limited knowledge about the variations of these behaviours over time, differences based on gender, or specific genetic subtypes of PWS (Schwartz et al., 2021)

1.2.4.7 Psychotic Illness

A significant observation in PWS is the notably high occurrence of psychotic illnesses, particularly prevalent among those with the mUPD genetic subtype (Boer et al., 2002). According to case studies and cohort investigations, the estimated lifetime prevalence of psychosis in individuals with PWS ranges from 60% to 100% (Aman et al., 2018; Yang et al., 2013). However, due to the rarity of PWS, conducting large-scale systematic studies is challenging, resulting in limited evidence. A meta-analysis of data from five studies concluded that individuals with mUPD are particularly at risk for psychosis (Yang et al., 2013). Hypothetically, the higher prevalence of psychosis in the mUPD genetic subtype may be attributed to genetic factors. Absent expression of specific maternally imprinted genes at 15q11-13 combined with excess maternally imprinted or paternally expressed genes on chromosome 15 may affect the γ-aminobutyric acid-glutamatergic pathways. The associated neural networks are known to underpin mood regulation and sensory processing and are implicated in psychotic illness (Aman et al., 2018).

1.2.5 Impact of the PWS Behavioural Phenotype

Hyperphagia has been reported as the primary treatment priority within the PWS community, distinguishing it from caregiver priorities related to obesity (Tsai et al., 2018). Additionally, recent findings have shed light on other significant aspects of the PWS behavioural phenotype, specifically behavioural challenges and anxiety, which profoundly impact the well-being of caregivers (Kayadjanian et al., 2021). These challenges encompass oppositional behaviours like arguing, inflexibility/rigidity, temper tantrums, meltdowns, poor emotional control, and aggression (Kayadjanian et al., 2021). Notably, these behavioural challenges also significantly affect individuals with PWS themselves, making it challenging for them to develop and sustain friendships and interact harmoniously with peers and others (Dykens et al., 2021). Current interventions for behaviour in individuals with PWS often rely on psychiatric medications, which may lack a specific evidence base in the context of PWS. These may provide some therapeutic effects but do not meet the comprehensive needs (Bonnot et al., 2016). Urgently, there is a pressing need to develop more effective and targeted interventions specifically tailored to

address the complex and challenging behaviours characteristic of PWS (Schwartz et al., 2016). Enhancing the management of behavioural challenges and implementing interventions that target the core symptoms of PWS are paramount to significantly improving the well-being and quality of life of individuals with PWS.

1.2.6 The search for neurocognitive biomarkers for neurodevelopmental disorders

A lack of effective treatments for behaviour and psychiatric symptoms is a challenge for PWS and other neurodevelopmental disorders (NDDs). NDDs are heterogeneous childhood-onset conditions that result from disrupted brain development and functioning (Sahin et al., 2018), including autism spectrum condition (ASC), intellectual disability and attention-deficit/hyperactivity disorder (Diaz-Caneja et al., 2021). NDDs show varying clinical manifestations and severity and are associated with differing degrees of cognitive and adaptive functioning and disability (Thapar et al., 2017). Drug development and therapeutic interventions for neurodevelopmental conditions are particularly challenging due to several factors. First, neurodevelopmental conditions encompass various disorders with diverse underlying causes and symptomatology, leading to significant heterogeneity. This makes it difficult to identify specific drug targets that would be effective across the entire population (Wetmore & Garner, 2010). Second, the underlying pathophysiology of many neurodevelopmental conditions remains poorly understood, making the development of targeted drugs more challenging without clear mechanistic insights (Ghosh et al., 2013). Lastly, many neurodevelopmental conditions lack reliable biomarkers, which are objective measures that indicate the presence or severity of a disease. The absence of biomarkers complicates the assessment of potential drug efficacy during clinical trials. The search for biomarkers for autism has recently intensified with increased efforts to identify novel pharmacotherapies (Molloy & Gallagher, 2021).

Efforts are underway to unravel the heterogeneity within the autism population and identify stratification biomarkers that define subgroups based on shared biology (Loth et al., 2017). Considerable advances are currently being made to identify eye tracking biomarkers with tractability for clinical trials in autism (Loth et al., 2016). These typically use up-to-date paradigms with greater ecological validity and are accessible across various ages and abilities. Previous social cognition studies in PWS are limited by methodologies and tools designed for higher-functioning individuals. Using accessible and validated methods to assess social cognition in PWS will create new knowledge of social cognition in PWS and how it impacts social functioning and behaviour and may highlight new avenues for treatment. The application of eye tracking as a tool to investigate processes associated with hyperphagia in PWS is also relevant. The absence of biomarkers for hyperphagia in PWS poses a significant barrier to evaluating potentially life-altering drug therapies for this condition. The Food and Drug Administration (FDA) in the USA has questioned what represents a meaningful improvement in hyperphagia to inform novel clinical trials in PWS. As a result, the PWS clinical trials consortium has prioritised the development of new biomarkers specifically for hyperphagia. Eye tracking methodologies were identified as a potential avenue to explore as novel endpoints in this pursuit (FPWR-CTC, 2019).

1.2.7 Eye tracking - A valuable tool in neurocognitive research

Eye tracking has emerged as a valuable tool in neurodevelopmental research. By monitoring and recording eye movements, valuable insights into cognitive processes such as attention, perception, memory, and decision-making can be gained (Eckstein et al., 2017). This non-invasive technique allows for the objective measurement of visual attention and provides valuable data on how individuals process information and interact with their environment. This methodology has a rich history, dating back two centuries, where cognitive psychologists have effectively utilised eye tracking to study the mechanisms underlying behaviour (Wade, 2015). Despite facing competition from brain imaging research over the past two decades, recent advancements in eye tracking technology have revitalised its significance. Improved hardware, software, accessibility and analytic approaches have contributed to the resurgence of eye tracking in cognitive research (Holmqvist et al., 2011).

The most commonly utilised ocular measure is eye gaze, where researchers observe how participants fixate on specific stimuli, providing valuable information about attention. Notably, eye tracking achieves high temporal resolution, comparable to Electroencephalogram (EEG), allowing moment-by-moment assessment of participants' responses to task demands (Holmqvist et al., 2011). Researchers have observed how eye movements reflect attention shifts, and fMRI studies demonstrate a close link between attention and gaze, implicating frontal eye fields in both eye movements and covert visual attention (Awh et al., 2006).

The use of eye tracking has been particularly revealing in studies of social cognition in autism. Two recent meta-analysis of studies measuring social interest in autism reported a reduced preference for social stimuli in the context of non-social stimuli in autistic people, with such differences relating to social communication impairments (Frazier et al., 2017) and restricted and repetitive behaviours (Chita-Tegmark, 2016). Gaze and eye movement differences can be detected as early as infancy in individuals with autism (Elsabbagh et al., 2013; Navab et al., 2012). Eye tracking has transformed our ability to gain insights into cognitive functioning in various clinical populations, primarily due to its accessibility across a broad range of developmental and intellectual abilities. This accessibility makes it a valuable tool in researching neurogenetic syndromes like PWS.

1.2.8 Thesis Aims and Hypotheses

The primary objective of this thesis was to enhance comprehension of PWS by profiling the mental health and behaviour needs of individuals with PWS and contributing critical knowledge to understanding neurocognitive factors underlying autism behaviours and hyperphagia using eye tracking technology.

The thesis's first aim was to conduct a comprehensive investigation to profile the mental health and behavioural needs of individuals diagnosed with PWS within the specific context of Ireland (Chapter 3). It hypothesised that individuals with PWS would exhibit a complex and age-dependent behavioural phenotype, characterised by a high prevalence of psychiatric comorbidities.

I then focused on a prevalent and impairing feature of PWS, autism behaviours. I wanted to investigate if social cognitive processes known to be altered in autism are also altered in PWS and associated with autism traits. Therefore, the second aim of this thesis was to investigate

social cognition, specifically preference for social stimuli in PWS (Chapter 4). I aimed to compare differences between PWS and a neurotypical comparison group, between the genetic subtypes, and to investigate the relationship between social cognition and autism behaviour. I hypothesised that individuals with PWS would display a reduced preference for social stimuli compared to the matched comparison group. I anticipated a more pronounced reduction within the mUPD group considering the higher prevalence of autism diagnosis in this subtype and that reduced interest in faces would be associated with greater autism symptom severity and poorer social functioning.

Next, I aimed to address the lack of urgently required objective measures of hyperphagia that impede progressing research into interventions for hyperphagia. This third aim was to develop and test a potential novel eye tracking paradigm, the Food Attentional Bias (FAB) task, to measure visual attention towards food stimuli under conditions of hunger and satiety in typically developing participants (Chapter 5). I hypothesised that visual attention to food stimuli in a healthy-weight cohort would decrease from the premeal "hungry" condition to the postmeal "satiated" condition. If correct, it would support further investigation of whether a lack of change in attention to food stimuli from premeal to postmeal could serve as a marker of typical satiety in PWS.

After testing the FAB task protocol in typically developing participants, it was necessary to adapt the protocol to ensure its feasibility and accessibility to participants with PWS. Therefore, the fourth aim of this thesis was to conduct focus groups with PWS stakeholders and to use codesign approach to adapt the FAB task protocol. The goal was to create an adapted version of the FAB for children and adults with PWS, informed by parents/caregivers and professional experts while maintaining scientific rigour. I hypothesised that there would be specific challenges that caregivers and experts would identify in implementing the protocol in PWS since it required fasting and alterations in the typical eating routine.

The final aim of this thesis was to implement the adapted FAB ask protocol in PWS and compare differences in performance between PWS participants and a neurotypical comparison group. I hypothesised that the PWS group would not show a reduction in attention to food in the postmeal condition compared to the premeal condition. I also investigated if attention bias to food stimuli would correlate with hyperphagia symptoms in the PWS group.

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Chapter 2: Materials and Methods

2.1. Introduction

To comprehensively address this thesis's objectives, a range of methodological approaches were used across the different experimental chapters. This chapter introduces the study designs implemented in each experimental chapter, accompanied by an overview of the ethical approval process and participant recruitment procedures. The clinical recruitment process for this thesis encountered significant delays due to the emergence of the COVID-19 pandemic. Consequently, adaptations were made to the study protocols, which will be elaborated upon below. The various clinical, behavioural, and neurocognitive assessments used throughout the thesis are described, and a comprehensive overview of the eye tracking methodology is provided, including detailed explanations of the data acquisition protocols and data processing techniques employed. The data analysis approach used in each experimental chapter is introduced.

2.2. Ethical Approval

Ethical approval was obtained from the following research ethics committees: School of Medicine Ethics Committee, Trinity College Dublin (REC: 20160105) (Chapter 3); the St. James's and Tallaght University Hospital's Research Ethics Committee (REC: 20160105) (Chapters 4 and 7); The School of Psychology Research Ethics Committee, Trinity College Dublin (REC: 2021117) (Chapter 5); The Faculty of Health Sciences Research Ethics Committee, Trinity College Dublin (REC: 210906) (Chapter 6). Patient information leaflets and consent forms are included in Appendix 1.

2.3. Study Designs

The study designs employed in this thesis are tailored to address specific research aims. In Chapter 3, the study design involved a cross-sectional approach using a caregiver survey, which aimed to profile the mental health and behavioural needs of individuals with PWS within the context of Ireland. In Chapter 4, a cross-sectional, between-group design was implemented to compare individuals with PWS to age and gender-matched typically developing comparison group. This design enabled examining eye movements on a social cognition task and behavioural measures, facilitating a direct comparison between the two groups. In Chapter 5, a within-subjects design was used to investigate the effects of hunger and satiety on an eye tracking paradigm involving food stimuli by analysing eye movements before and after a meal in typically developing participants. In Chapter 6, a qualitative research approach was taken that used focus groups with key stakeholders to gather in-depth insights into adapting an eye tracking paradigm tested in Chapter 5 for use in the PWS population. Finally, in Chapter 7, a mixed design was used with between-group factors (PWS vs age and gender-matched comparison group) and within-subject factors (premeal vs postmeal) to compare performance on an eye tracking paradigm with food stimuli across conditions of hunger and satiety. Please note that Chapter 4 and Chapter 7 data were collected at the same research visit and comprised of individuals with PWS and age- and gender-matched comparison participants. A separate

cohort of typically developing adults was recruited for Chapter 5 to test the newly developed eye tracking paradigm.

2.4. Participant Recruitment

PWS participants and PWS caregivers were recruited through leaflets emailed via the Prader-Willi Association of Ireland (PWSAI; https://pwsai.ie/) and distributed to carers attending the national paediatric centre for PWS Clinical Care, Children's Hospital Ireland (https://www.orpha.net/PWS/ire). The recruitment approach may have unintentionally excluded individuals with PWS who are not connected with the patient organisation or clinic, potentially leading to a sampling bias as this might have resulted in an overrepresentation of individuals with better access to information and resources about PWS. Participants for the comparison groups were recruited through two separate campaigns. Facebook advertisements were used to recruit children, teenagers and adults for studies described in Chapter 4 and Chapter 7. Posters advertisements that targeted students and staff at Trinity College Dublin Campus, Dublin 2 and the St James's Hospital Campus, Dublin 8, were used to recruit comparison participants for Chapter 5.

All potential participants were invited to contact the research team if interested in hearing more information about the study. A phone call was scheduled with each potential participant/participant's caregiver to talk through the study and to provide a chance to ask any questions. Each participant underwent a screener on the phone to check suitability (see Appendix 1). Participant information sheets, consent forms and, where relevant, assent forms (children under 18 years of age) were sent via post or email to all suitable participants in advance of their research visit to read. Written informed consent was obtained from participants via post or on the day of their research visit.

2.4.1 Inclusion and Exclusion criteria

Inclusion criteria for all participants:

1. **Aged 4 years or older:** Participants had to be aged four years or older. The clinical protocol to measure autism behaviours and the eye tracking task used to look at preference for social stimuli were originally designed and tested in participants over the age of four years (Loth et al., 2017). This age criterion was selected in the present thesis to ensure the study protocol was developmentally appropriate and accessible for all participants.
2. **Capability for Clinical Research Assessments and Eye Tracking Protocols:** Participants had to have the ability to complete both the clinical research assessments and eye tracking protocols. This criterion was deemed necessary for comprehensive data collection.
3. **Normal or Corrected-to-Normal Vision:** Participants were required to have normal or corrected-to-normal vision. Eye tracking relies on accurately capturing and recording eye movements. Individuals with visual impairments or uncorrected visual problems may exhibit atypical eye movement patterns or struggle to focus on stimuli.
4. **Fasting Ability:** Participants needed to have the ability to fast without any underlying medical conditions that could pose a risk to their health during fasting. Ability to fast

was an inclusion criterion as a key question of this thesis was to see if differences exist in attention bias to food stimuli across conditions of hunger and satiety.

Additional inclusion criteria for PWS participants:

5. **Genetic Diagnosis of PWS:** For participants with PWS, a confirmed genetic diagnosis of PWS was required to ensure each participant's diagnosis was accurate as there are conditions that can overlap clinically with PWS but have a different genetic cause e.g. Schaaf-Yang syndrome (McCarthy et al., 2018).

Additional inclusion criteria for comparison participants:

6. **Age and Sex Matching:** Participants in the comparison group had to be matched in age and sex to a PWS participant. This matching strategy was employed to account for sex and age across the two participant groups.

Exclusion Criteria for Comparison Participants:

7. **Neurodevelopmental or Psychiatric History:** Exclusion criteria included a personal or familial history (first degree) of neurodevelopmental conditions or psychiatric illnesses. Social cognition has been reported to be altered in certain neurodevelopmental and psychiatric conditions (e.g. autism, anxiety, and psychosis ((Besag et al., 2019))). This criterion was introduced to eliminate potential confounding variables when investigating differences in social functioning and social cognition between the PWS group and comparison groups.
8. **Eating Disorder:** Participants with the presence of an eating disorder were excluded from the comparison group as there is evidence to suggest that attentional bias to food stimuli is altered in individuals with eating disorder (Stott et al., 2021).
9. **Special Diet Adherence:** Participants with explicit dietary restrictions were excluded from the study as previous studies have shown individuals with specific dietary restrictions can show altered visual patterns towards food stimuli that they are unable to eat. Approach-avoidance is when a participant switches between being drawn towards a food they do not eat (approach) and being inclined to look away from it (avoidance) (Hollitt et al., 2010; Werthmann et al., 2011). To ensure this was not a confounding factor in analysis, participants who had potential negative associations with specific food stimuli (e.g., meat stimuli for vegetarians) were excluded.

2.5. Clinical Research Assessments

The clinical protocols for the studies described in Chapters 4, 5 and 7 included demographic, cognitive, adaptive, behavioural and mental health and physical assessments. The specific clinical protocol for each experimental chapter is outlined in Appendix 2.

2.5.1. Clinical Protocol for Chapter 4 and Chapter 7

2.5.1.1. Demographic Questionnaire

Demographic information (age, sex, height and weight) was collected from each participant by the researcher on the day of their research visit.

2.5.1.2. Assessment of height, weight and BMI:

Participants were weighed without shoes and heavy clothing using a digital scale in the research lab, and weight in kilograms (KG) was recorded. To check the accuracy of the weighing scales, self-reported or caregiver-reported weight was compared with the weight measurement obtained from the lab scales and discrepancies were flagged. Height was measured using a wall-mounted height chart. Each participant was asked to remove their shoes, stand with their feet flat on the ground, heels against the wall, and head facing straight ahead and height was recorded in centimetres (CM). Body Mass Index (BMI) was calculated by dividing the participant's weight in kilograms by the square of their height in meters (BMI = weight (kg) / height^2 (m^2)). BMI provided a numerical indicator of the participant's body mass relative to their height, a widely employed method to assess body composition.

2.5.1.3. The Wechsler Abbreviated Scale of Intelligence Second Edition (WASI-II)

The Wechsler Abbreviated Scale of Intelligence Second Edition (WASI-II; Weschler, 2011) is a standardised test used to measure cognitive abilities and assess intellectual functioning in participants aged six years and above. The WASI-II comprises four subtests: Vocabulary, Similarities, Matrix Reasoning, and Block Design. The Vocabulary subtest measures verbal knowledge, the Similarities subtest measures verbal reasoning, and the Matrix Reasoning and Block Design subtests measure nonverbal reasoning and spatial ability, respectively. The subtests are administered and scored according to standardised procedures and guidelines in the test manual. The test requires a trained examiner to administer and a quiet environment. I received training and supervision in administering the WASI-II from members and carried out all WASI assessments for both PWS and comparison participants. Participants respond to prompts, and answers are scored using standardised criteria. The WASI-II provides a composite score, the Full Scale Intelligence Quotient (FSIQ), representing overall intellectual functioning based on the four subtest scores. It also provides a Verbal Comprehension Index (VCI) based on the vocabulary and similarities subtest and the Perceptual Reasoning Index (PRI) based on the matrix reasoning and block designing subtests. The WASI-II has excellent internal and interrater reliability and acceptable-excellent test-rest reliability (McCrimmon & Smith, 2013).

2.5.1.4. The Mullen Scales of Early Learning (MSEL):

The Mullen Scales of Early Learning (MSEL; Mullen, 1995) is a standardised developmental test that measures cognitive function in young children from birth to 68 months. The MSEL comprises five scales: Gross Motor, Fine Motor, Visual Reception, Receptive Language, and Expressive Language. Each scale consists of several subtests that are scored according to established criteria provided in the test manual. Subscales yield t-scores and age equivalents, which are combined to estimate overall developmental functioning. The MESL has good internal, test-retest, and interrater reliabilities (Mullen, 1995). The MSEL is administered individually by a trained examiner and takes approximately 30 to 60 minutes to complete. The test requires a quiet, distraction-free environment and involves presenting the child with tasks and questions to assess their cognitive and motor skills. The MSEL can identify developmental delays and strengths, monitor progress, and inform intervention and treatment planning for young children with developmental concerns. The MSEL was administered to all participants under the age of 6 years. The MESL was administered to a small subgroup of participants with

PWS (n=2) who were over the age of six years but were non-verbal or had minimal verbal communication abilities and were unable to complete the WASI-II. Test-retest reliability for the MSEL was rated as good, interrater reliability as adequate, and internal reliability as excellent (Colbert et al., 2020).

2.5.1.5. Vineland Adaptive Behaviour Scales-II (VABS-II)

The Vineland Adaptive Behaviour Scales-II is a standardised measure of adaptive behaviour used to assess individuals from birth through adulthood (VABS-II; Sparrow et al., 2005). The assessment was completed through a semi-structured interview with the caregiver/parent. The VABS-II assesses adaptive behaviours across three domains: Communication, Daily Living Skills, and Socialisation. It provides standard scores, and percentile ranks for each domain, and an overall Adaptive Behaviour Composite score. Test-retest reliability for the VABS-II ranged from good to very good, interrater reliability was rated as good to very good, and internal reliability as good (Sparrow and Cicchetti, 1989; Bildt et al., 2005).

2.5.1.6. Social Communication Questionnaire (SCQ)

The Social Communication Questionnaire (SCQ; Rutter et al., 2003) is a 40-item, parent-report screening measure that focuses on items relating to autism behaviours likely to be observed by a primary caregiver. Each item in the SCQ requires a "yes"/"no" response, and each scored item receives a value of 1 point for autism-like behaviour and 0 points for the absence of an autism-like behaviour. There are two different versions of the SCQ. The SCQ Current component requires participants to report whether certain behaviours have occurred within the past three months. The SCQ Lifetime relates to the individual's developmental history and requires respondents to report whether the behaviours in question have ever occurred and whether they were present at the age of 4 years. The SCQ lifetime was used in this study and takes approximately 10 to 15 minutes to complete. The SCQ has shown promise as a screening measure for autism in a research-referred older sample, though recent studies with younger children reported lower sensitivities when using the suggested cut-off of ≥15 to differentiate autism from children with non-spectrum conditions (Corsello et al., 2007). The SCQ lifetime was collected from parents/caregivers for all participants. The SCQ has demonstrated good test-retest reliability, good interrater reliability, and good internal reliability (Liu et al., 2022).

2.5.1.7. Food Related Problems Questionnaire (FRPQ)

The Food Related Problem Questionnaire (FRPQ) is a 16-item questionnaire designed to measure eating behaviour in PWS. Subscales of the FRPQ include preoccupation with food, impairment of satiety and a composite score of negative food-related behaviours (Russell & Oliver, 2003). The FRPQ has acceptable test-retest reliability and good interrater and internal reliability (Russell & Oliver, 2003). Caregivers of all participants completed the FRPQ.

2.5.1.8. Hyperphagia Questionnaire (HQ)

The Hyperphagia Questionnaire (HQ) is an 11-item informant report questionnaire designed to measure food-related preoccupations and difficulties, specifically in PWS (Dykens et al., 2007). The HQ uses a Likert-type scale with three subscales (hyperphagic behaviours, drive, and severity). Higher scores are indicative of more severe hyperphagia. Caregivers of participants in

each group completed the FRPQ. Test-retest and interrater reliability for the HQ have not been reported, but the internal reliability was rated as acceptable (Dykens et al., 2007).

2.5.2. Additional PWS Assessments for Chapters 4 and 7

Additional assessments were collected in the PWS cohort to characterise autism features comprehensively. As a diagnosis of autism or a first-degree relative diagnosed with autism was an exclusion criterion for the comparison group, autism assessments were only required in the PWS.

2.5.2.1. Autism Diagnostic Observation Schedule – Second Edition (ADOS-II)

The Autism Diagnostic Observation Schedule – second edition (ADOS-II; (Lord et al., 2012)) is a semi-structured, play-based diagnostic measure of the core features of autism. The ADOS assessment includes four modules, namely Modules 1, 2, 3, and 4. The appropriate module selection is determined by considering the participant's age and language abilities (Table 2.1). Exceptions to the guidelines are specified in the manual. For example, Module 3 is suited to individuals under the age of 16 years, but there is flexibility to also consider the relevance of tasks to the examinee's interests and abilities when determining the appropriate module. For this thesis, 7 participants over 16 years were administered Module 3 based on the suitability of the tasks to the participants' interests. To administer the ADOS-2, an examiner must have certified ADOS-II research training and prior administration experience to ensure their research reliability. I completed training and reliability training and administered the ADOS-II to all participants in the PWS group on the day of their research visit.

The ADOS-II scores for all participants were converted to calibrated severity scores (CSS), where ratings 1–3 represented non-spectrum cases, 4–5 non-autism, and 6–10 autism. The CSS score enables comparison across different modules within analyses (Gotham et al., 2008; Hus et al., 2014). In contrast, the total score on the ADOS-II is influenced by factors such as chronological age and language aptitude, making it difficult to compare autism severity across different groups and over time. To overcome these limitations, Gotham et al. 2008 implemented the CSS, which allowed for the comparison of diagnostic features of autism independent of a participant's age and related conditions. A sample of children with autism (n = 1118) was categorised into specific age and language groups, and ADOS CSS values were generated within each group based on percentiles of total raw scores. The ADOS CSS demonstrated less variance explained by factors like expressive language ability and maternal education than the ADOS total score. This indicated that the ADOS CSS was less influenced by developmental functioning and demographic factors. The ADOS CSS score has strong test-retest reliability (Janvier et al., 2021), moderate to good interrater reliability (Zander et al., 2016), and good internal reliability (Hus et al., 2014)

In response to the challenges posed by the COVID-19 pandemic, adaptations were made to the administration of the ADOS-2. These adaptations were made to reduce the risk of COVID-19 transmission while striving to maintain alignment with the established procedures outlined in the ADOS-2 manual. The guidelines used were taken from "Face-to-Face ADOS-2 Informed Assessments During Covid-19 Guidelines" prepared by Emma Woodhouse, ADOS 2 Trainer for the AIMS-2-TRIALS, European Autism Research Consortium (Loth et al., 2017). The adjustments

were designed to ensure the safety and well-being of both the assessor and participants while conducting face-to-face assessments.

- The examiner wore a mask for the assessment and the participant did not. In the **Construction Task,** blocks were positioned at a distance to adhere to social distancing protocols, altering the usual procedure of placing them within reach and creating a barrier with the arm. The same instructions were maintained, emphasizing communication regarding the need for more blocks.
- In the **Make Believe Play & Joint Interactive Play Task,** selected items were provided to the child, ensuring engagement while considering contamination concerns. The assessor retained one action figure and a few items, creatively incorporating the distancing element into play scenarios.
- In the **Demonstration Task**, the tooth brushing and handwashing tasks were administered as per the manual, with modifications due to the absence of 'real' objects (towel and soap) because of contamination concerns.
- In the **Description of a Picture Task**, the standard instructions were followed as outlined in the manual, with the only difference being the assessor and participant did not view the picture jointly.
- For the **Telling a Story From a Book Task**, procedures were aligned with the manual, emphasising joint storytelling and coordinated tasks while maintaining appropriate distancing measures.
- For the **Break Task**, non-plastic items were removed due to contamination concerns, with the rest of the administration following the manual's guidelines.
- For the **Creating a Story** Task, specific items were kept aside for the child, allowing for creative storytelling while ensuring safety and adhering to manual guidelines.

This administration deviated from the standardised administration of the ADOS and therefore the scores were not considered clinically valid but were considered informative and therefore were included as part of the analyses in this thesis.

Table 2.1: Age ranges and language requirements associated with each ADOS module.

Module	Age Range	Language Requirement
Module 1	At least 31 months; may not be appropriate past school age	No speech to simple phrases
Module 2	It may not be appropriate past school age	Three-word phrases/not yet verbally fluent
Module 3	Child or adolescent (= 16 years and younger)	Regular use of complex phrases

2.5.2.2. Autism Diagnostic Interview (ADI-R)

The Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994) is a standardised, semi-structured interview conducted by trained clinical or research professionals with parents or caregivers of individuals assessed for autism. It is validated for individuals with a mental age of at least 24 months up to adulthood who are suspected to have autism. The ADI-R typically takes approximately 90 to 180 minutes to administer. It consists of 93 items and 153 ratings, organised into six sections: early development, language/other skill acquisition and loss, language and communication functioning, social development and play, interests and behaviours, and general behaviours. The items in the ADI-R are scored for "current" as well as "ever" or "most abnormal period (4-5 years)" behaviour. Most questions focus on specific behaviours associated with autism spectrum condition (ASC) and align with the diagnostic criteria for autistic disorder/childhood autism according to DSM-IV/ICD-10. The standard algorithms in the ADI-R are organised into four sections that correspond to the diagnostic definition of autism in the DSM-IV/ICD-10. Each section has cut-offs provided for diagnostic criteria, including qualitative abnormalities in reciprocal social interaction (RSI), qualitative abnormalities in communication (COMM), restricted and repetitive patterns of behaviour (RRB), and abnormality of development evident before or at 36 months. Studies have reported the diagnostic validity of the ADI-R when administered by trained researchers, with sensitivities and specificities ranging from 80% to over 90% (Le Couteur et al., 2007; Lord et al., 1994). The ADI has very good test-retest reliability, very high interrater reliability (Zander et al., 2017), and excellent internal reliability (Saemundsen et al., 2003).The ADI-R requires certified training to administer and code the interview. I completed a training course and was responsible for administering the ADI-R to all PWS participants.

2.5.3. Additional questionnaires for Chapter 5

Additional measures were collected in Chapter 5 to assess eating behaviours concerning the premeal and postmeal conditions of the eye tracking data collection.

2.5.3.1. Satiety labelled intensity magnitude scale (SLIM)

The satiety labelled intensity magnitude scale (SLIM; Cardello et al., 2005) assesses the overall perceived satiety produced by a person when in a certain hunger/ satiety state. The SLIM includes phrases that define satiety and hunger on a rating scale of -100 to 100. Participants are instructed to circle the phrase that closest describes the level of hunger/satiety they are currently experiencing. Each phrase corresponds with a score; 100 (Greatest Imaginable Fullness), 80 (Extremely Full), 60 (Very Full), 40 (Moderately Full), 20 (Slightly Full), 0 (Neither Full nor Hungry), -20 (Slightly Hungry), -40 (Moderately Hungry), -60 (Very Hungry), -80 (Extremely Hungry), -100 (Greatest Imaginable Hunger). Higher scores correspond to higher levels of satiety. A comparison between the SLIM scale and a Visual Analogue Scale found that

the SLIM scale had a reliability coefficient of 0.90 and a greater level of discriminative sensitivity (Gliga et al., 2009). Participants completed this scale before beginning the eye tracking task in both the premeal and postmeal conditions (Chapter 5, section 2). Test-retest reliability of the SLIM is adequate while interrater and internal reliability have not been reported (Cardello et al., 2005).

2.5.3.2. Food Stimuli Rating Scale

A food stimuli rating scale was designed and administered to each participant on completion of eye tracking to assess if the food stimuli used in the paradigm were food that participants would like to eat. The rating scale consisted of images of the 20 food stimuli used in the paradigm. Each image was paired with a Likert scale ranging from 1-5. Participants were asked to rate each food stimulus on a scale of 1 to 5. A rating of 1 ('I would hate to eat this'), 2 ('I would not eat this'), 3 ('neutral'), 4 ('I would eat this'), 5 ('I would love to eat this'). Participants were excluded if they scored lower than a three on more than 50% of the food stimuli)(Figure 2.1).

Figure 2.1: A sample item from the food stimuli rating scale

2.5.3.3. Honesty Questionnaire

All participants in the study were requested to complete an Honesty Questionnaire after the research. The primary aim of this questionnaire was to ensure the integrity and validity of the study's measurements. Participants were instructed to provide truthful responses using a binary "Yes" or "No" format. The questionnaire consisted of three items designed to gather specific information: 1) participants were asked about the consumption of food during the four-hour fasting period preceding the initial eye tracking task, 2) participants were asked whether they had formed any speculations regarding the purpose of the study, and 3) participants were asked to evaluate whether they had altered their behaviour in accordance with their understanding of the study. Additionally, the questionnaire concluded with an open-ended question, allowing participants to elaborate on any additional behavioural changes they might have made. Participants that answered yes to any of the questions on the honesty questionnaire were excluded from the analysis.

2.6. Adaptations to clinical procedures due to the COVID-19 pandemic:

During the COVID-19 pandemic, the Irish government implemented a range of measures to slow the spread of the virus and protect public health. These measures included restrictions on travel, social gatherings, and businesses, as well as the closure of schools and universities (Government of Ireland, 2019, https://www.gov.ie/en/publication/472f64-covid-19-coronavirus-guidance-and-advice/). These restrictions significantly impacted the clinical

recruitment process as research visits could not occur for 22 months (March 2020 – May 2022). Before the pandemic, all data collection activities were conducted in person through 1-3 visits to the research lab. However, certain protocol modifications were implemented to comply with COVID restrictions and reduce face-to-face interactions. Specifically, remote testing was introduced, allowing participants to complete assessments from home. The ADI-R and VABS-II were collected via the Zoom conferencing platform - a substantial portion of the protocol still required face-to-face testing. To ensure the safety of research participants during face-to-face testing, specific procedures were established following the safety protocol developed by the School of Medicine, Trinity College Dublin. The full list of procedures added to the study protocols to minimise the risk of COVID-19 transmission is outlined in Appendix 3.

2.7. Eye Tracking

Eye tracking has become an increasingly common methodology in developmental research due to the introduction of corneal-reflection eye tracking, which allows for remote eye movement tracking without head restraints (Holmqvist et al., 2011). This technique enables the mapping of eye movements in a 3D space. An eye tracking system consists of micro projectors that emit near-infrared light towards the eyes of the participant. The eye's cornea and pupil reflect this infrared light, creating distinct patterns captured by two cameras. Specialised algorithms are used to analyse the captured images and track the position of the pupil and the corneal reflection in real-time. By triangulating the position of the pupil and corneal reflection, the system can accurately determine the gaze location on the screen (Holmqvist et al., 2011). Recent advancements in computer capacities and eye tracking algorithms have facilitated the development of user-friendly eye tracking systems (Holmqvist et al., 2011).

One of the primary reasons for the widespread use of eye tracking in neurodevelopmental research is its effectiveness in measuring attention. The neural networks associated with visual attention and eye movement execution overlap, making eye movements an effective tool for studying attention (Kowler, 2011). Eye movements provide valuable insights into attention by harnessing knowledge about the visual system. Visual acuity is a measure of the ability of the eye to distinguish shapes and the details of objects at a given distance. Visual acuity decreases as objects move away from the line of sight due to the distribution of photoreceptors in the retina, with high acuity vision limited to the fovea (Purves et al., 2014)

Consequently, our eyes constantly make small movements to explore the visual world (Purves et al., 2014). Perception is guided by sequences of fixations and saccades (Kowler, 2011). Saccades are eye movements that move the fovea rapidly from one point of interest to another. Fixations are when the eyes stop scanning and hold the foveal area of our field of vision in one place, allowing the visual system to process information in more detail (Kowler, 2011). Due to the fast movement during a saccade, the image on the retina is of poor quality and information intake usually happens mostly during the fixation period. The duration of a fixation reflects the effort used to process visual information and is used to make inferences about attention.

In eye tracking studies, the aim is typically to assess the duration and frequency of participants' fixations on specific elements within a stimulus, such as a specific object or a specified facial feature, e.g. the eyes. These areas of interest (AOIs) are predefined regions of a stimulus. After data collection, the data is processed to provide variables related to a participant's engagement

with these AOIs. These variables include the duration required to initially fixate on an AOI (referred to as time to first fixation within an AOI), the cumulative duration of fixations within an AOI (total duration of fixations), and the count of fixations made within an AOI (number of fixations) (Hessels et al., 2016). In Chapter 5, these variables are described in more detail and were used to analyse the eye tracking data as the cohorts consisted of typically developing adults.

When analysing eye tracking data from a cohort that includes children or individuals with neurodevelopmental conditions, factors such as attention span, processing speed, or cognitive abilities may impact visual processing (Holmqvist et al., 2011). Children and individuals with intellectual disability often exhibit variable levels of attention and engagement during eye tracking tasks, resulting in higher rates of missing data. Using proportional measures such as proportional dwell time (the total duration of fixations with an AOI relative to the total duration of fixations for the entire stimulus) makes it possible to capture the relative importance or salience of different AOIs despite differences in overall gaze duration. This compensation for variable attention and engagement enhances the accuracy and reliability of the eye tracking analysis, particularly in populations with such characteristics. In Chapters 4 and 7, proportional eye tracking measures were used as the participants had a wide age range (5 – 42 years) and intellectual ability.

2.7.1. Eye tracking data acquisition

Eye movements were captured using a Tobii X2-60 screen-mounted eye tracker (Tobii, Danderyd, Sweden), which uses a corneal-reflection system (as described above in section 2.3). Participants' eye movements were recorded at the rate of 60 Hz meaning eye position was captured 60 times per second. Using Tobii Studio, the stimuli were displayed as full-screen on a 25” monitor with a 1024 x 1280 pixel resolution. The Tobii X2-60 eye tracker has a spatial resolution of approximately 0.5 degrees of visual angle, meaning it can accurately measure eye movements and gaze positions with a precision of approximately 0.5 visual degrees. A visual degree is 1/360th of an imaginary circle around the head (Purves et al., 2014). For reference, when a participant’s arm is fully extended and their thumb is raised, the width of their thumb is approximately two visual degrees.

2.7.2. Calibration Process

Calibration establishes a relationship between eye movements and corresponding positions on the eye tracking system (Holmqvist et al., 2011). By aligning the eye tracking system with the participant’s gaze accurately, calibration ensures that the recorded eye movements correspond closely to the participant’s actual gaze positions, ensuring accurate and reliable measurement. Calibrating a person before starting an eye tracking paradigm is critical to ensure calibration accuracy and precision. Calibration accuracy refers to the closeness of the calibration points to the actual gaze positions, while calibration precision relates to the consistency and repeatability of the calibration procedure.

The calibration consisted of an automatic five-point calibration sequence (one point in the centre and one point in each of the screen’s four corners). Both participants’ eyes needed to be

visible and centred on the screen and positioned 55cm from the screen before beginning the calibration run. After the eye-tracker had computed a calibration with sufficient samples, the experimenter visualised the results on the screen (as five average gaze points and 5 target gaze points at the centre of the screen, bottom left and right, top left and right corners, see Figure 2.2.) and had to indicate whether the calibration was satisfactory (i.e., all 5 calibration points sampled with right and left eye had a close overlap of average gaze point and target gaze point). Otherwise, it would restart. At both checkpoints, the experimenter could adjust the sitting position of the participant. After 3 calibration attempts, the experimenter could skip the battery, e.g., if the participant was non-compliant/restless.

Figure 2.2: Screenshot of the type of plot that appeared after calibration for the face-pop task in Chapter 4.

2.7.3. Paradigms

2.7.3.1. Face Pop Task (Gliga et al., 2009)

The Face Pop Task (Gliga et al., 2009) is a free viewing paradigm that tests whether participants automatically orient to faces and prefer to look at faces compared to other stimuli (i.e. look disproportionally longer to faces). The task is part of a comprehensive battery of eye tracking tasks used within the framework of EU-AIMS and AIMS-2-TRIALS, European research initiatives devoted to investigating the biological mechanisms underlying autism (Elsabbagh et al., 2013; Gliga et al., 2009; Loth et al., 2017). The EU-AIMS and follow-up AIMS-2-TRIALS project incorporate contemporary paradigms with enhanced ecological validity, ensuring their applicability across diverse age groups and cognitive abilities. The Face Pop task consists of eight different arrays, each with five stimuli controlled for visual saliency and counterbalanced for location was presented (see Figure 2.3). Each array consisted of five stimuli: a face image, a mobile phone, a bird, a car, and a visual “noise” stimulus. The noise stimulus was created by

randomising the pixels from the face image used in the array. It served as a control for the face stimulus, as it was matched in amplitude and colour spectra. Each slide was presented for 20 seconds and accompanied by music to maintain the participant's attention.

In Chapter 4, the following outcome variables were assessed in the Face Pop Task to analyse participants' attention allocation; Proportion of first looks, proportional dwell time and average look duration. The proportion of first looks measures the relative frequency with which a participant initially fixated on a specific AOI category. Proportional dwell time measures the relative total duration of fixation to an AOI category, offering insight into the stimuli that were preferentially attended to. Given that individuals with autism often demonstrate shorter sustained attention to faces compared to typically developing controls (Major et al., 2022), average look duration was also considered as an outcome variable. Average look duration measures the average length of fixations within an AOI."

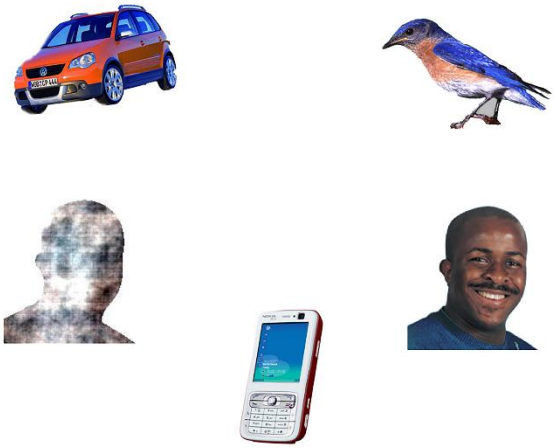

Figure 2.3: A sample stimulus array from the Food Pop Task (Gliga et al., 2009)

2.7.3.2. Food Attentional Bias Task

The *Food Attentional Bias (FAB) Task* was adapted from the "Face Pop-Out Task" (Gliga et al., 2009) for this thesis and was designed to test whether food stimuli captured and maintained attention longer than other stimuli categories. Twenty stimulus arrays were designed, each containing one food stimulus and four distractor non-food stimuli (clothing, household objects, instruments, and vehicles). Stimuli were selected from the Food-Pics Database (Blechert et al., 2019) and Google image search when specific distractor images were required. Stimuli were matched for colour for each stimulus array. Stimuli were pseudo-randomly assigned to array locations, ensuring that each stimulus category occurred equally in each location to control for location effects. Saliency for each stimulus was ranked and matched across categories using The Saliency Toolbox for MATLAB (Walther & Koch, 2006). Each image was 6.67cm in height and 4.7 cm in width. The sequence in which stimulus arrays were presented was randomised to account

for order effects. Stimulus arrays were divided into three blocks of three trials (with four in the last block). Participants were shown a 30-second rest video clip before each block started, consisting of natural scenes (obtained from youtube.com). The three blocks and three rest videos lasted 4 minutes and 36 seconds (see Figure 2.4). The outcome variables for the FAB task in Chapter 5 include the duration required to initially fixate on an AOI (referred to as time to first fixation within an AOI), the cumulative duration of fixations within an AOI (total duration of fixations), and the count of fixations made within an AOI (number of fixations). Proportional eye tracking measures were used in Chapter 7 to analyse the adapted version of the FAB for PWS as the participants had a wide age range (5 – 42 years) and intellectual ability. Proportion of first looks proportion dwell time and proportional fixation count were selected as outcome variables. Proportion of first looks measures the relative frequency with which a participant initially fixated on a specific AOI category. Proportional dwell time measures the relative total duration of fixation to an AOI category and proportional fixation count measures the relative number of fixations to an AOI category.

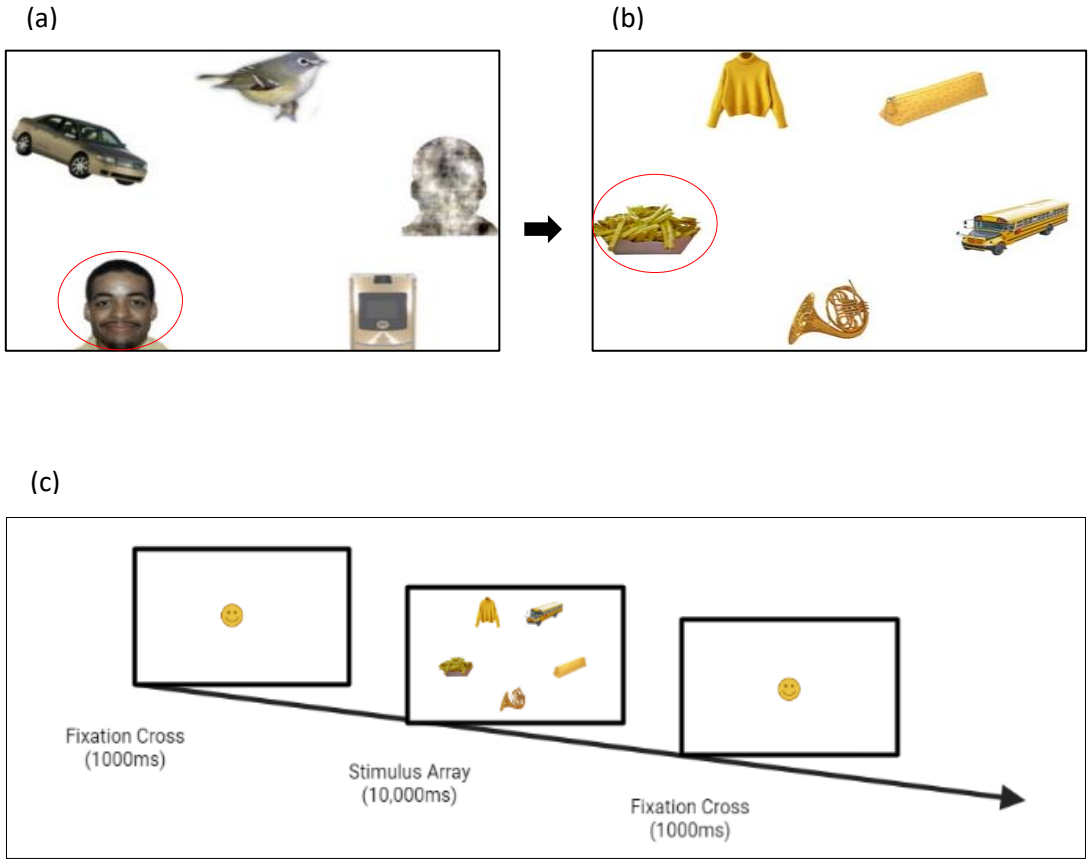

Figure 2.4: (a) A sample stimulus array from the Face Pop Task (Gliga et al., 2009) and (b) a sample of stimulus array from the FAB task. The structure of the FAB task stimulus array was adapted from the Food Pop task (c) the sequence of the FAB task - a smiley face appeared as a fixation point for 1 second, which was then followed by the stimulus array for 10 seconds, followed by a fixation smiley again for 1 second.

2.7.4. Eye tracking Procedure

Eye tracking data were collected in the neurodevelopmental research lab or remotely in the participant's home. The same equipment and setup were used for both settings. Participants were seated approximately 55 cm from the monitor for stimulus presentation in a quiet, dimly lit room (see Figure 2.5). The height and distance of the screen were adjusted for each participant to obtain optimal positioning of the eyes. Younger participants could sit on their caregiver's lap for the session. The caregiver was asked to wear sunglasses to mitigate any potential tracking inaccuracies. The purpose of having the caregiver wear sunglasses was to prevent the eye tracker from mistakenly tracking the caregiver's eyes instead of the child's. Instructions were "to relax, sit back and look at the picture and movies as if watching tv". A research team member was seated near the participant during the session and provided verbal redirections toward the screen when necessary. Another researcher controlled the eye tracking and stimulus presentation computer.

(a) (b)

Figure 2.5: (a) shows the eye tracking setup for a participant in the lab, and (b) shows the remote eye tracking setup in a participant's home.

2.7.5. Data processing

Fixations and saccades were identified using the Tobii I-VT Fixation filter (Olsen & Matos, 2012), which categorises saccades and fixations using a velocity threshold of 30°/sec. Fixations shorter than 60 ms were discarded, and adjacent fixations within 75 ms and a maximum of 0.5° were merged. In both eye tracking paradigms, areas of interest (AOIs) were drawn around each stimulus within the array. For the Face Pop Task, areas of interest (AOIs) masks were placed around each stimulus using MATLAB R2014b and dilated by 2° to account for calibration error. For the FAB task, AOIs were created using the Tobii Pro Lab software (Tobii Pro Lab, 2014). Each AOI was rectangular shaped and extended the stimuli by 2cm. The arrays were purposely designed with a 5cm separation between stimuli. This ensured no overlap in AOIs when participants' calibrations reached the calibration criteria's maximum outer precision and accuracy boundaries.

In both paradigms, gaze data were extracted for each AOI and total (the entire slide). Data were excluded for participants with fewer than three valid trials. Trials were considered valid based

on two criteria: firstly, the participant directed their gaze towards the stimulus array for more than 5000ms out of the 10000ms duration, and secondly, the proportion of valid samples exceeded 50%. In other words, the eye tracker successfully captured and recorded the participant's eye location more than 50% of the time the eyes were sampled. The Tobi X2-60 eye tracker used in the study samples at a rate of 60 times per second.

2.7.6. Procedure for Chapters 4 and 7: Using eye tracking to characterise social cognition and hyperphagia in PWS

The study visit protocol is shown in Figure 2.6. The study protocol was designed based on the recommendations of PWS caregivers and professionals (Chapter 6). Participants were asked to fast before the visit (3 hours for participants under 12 years and 4 hours for participants over 12 years). The study was scheduled to begin 1 hour before the participant's usual lunch time, coinciding with the end of their fast. A cognitive assessment was completed within the first hour. Participants then completed the first run of the FAB task (premeal condition). Participants were then given a standardised meal consisting of a piece of fruit, a sandwich, and a bottle of water. When the participants finished eating, a timer was set for 30 minutes. At this time, the participant's height and weight were measured. After 30 minutes, the FAB task paradigm was presented again to participants (postmeal condition). Participants were permitted a movement break and then began the Face Pop task. The PWS participants were administered the ADOS-II. Each participant was thanked and presented with their certificate award and token of gratitude.

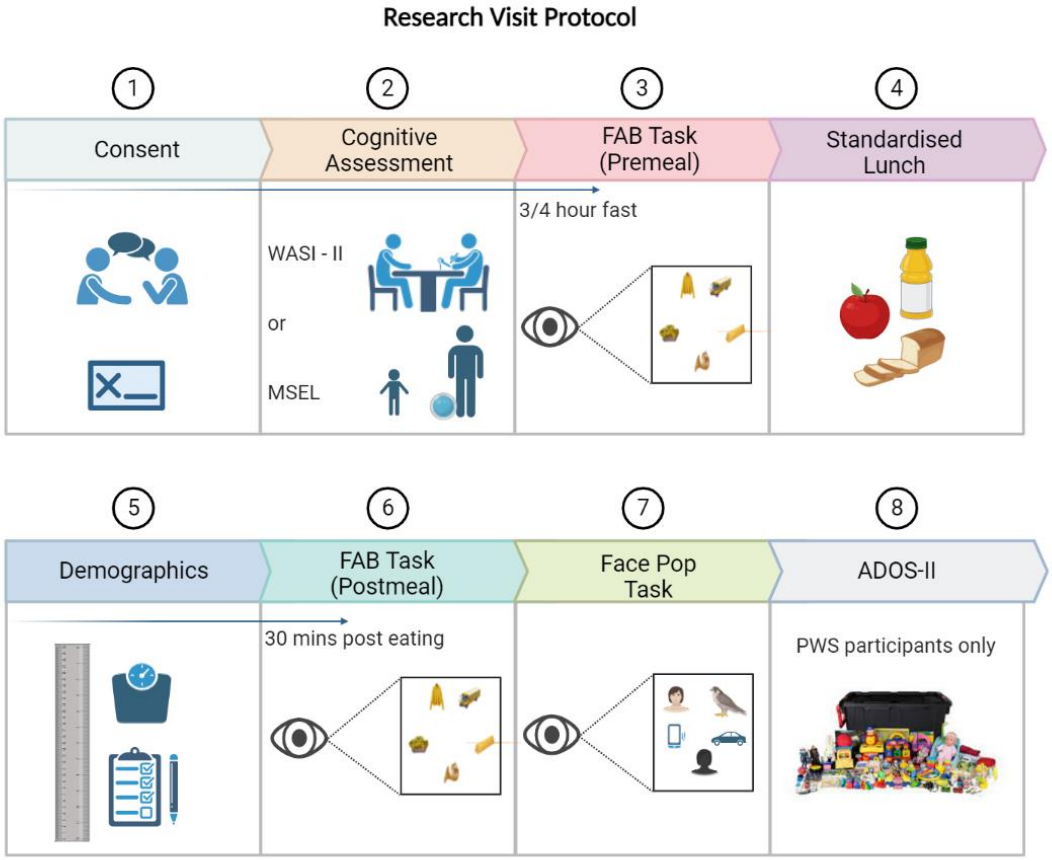

Figure 2.6: Research protocol for lab visit for PWS and comparison participants in Chapters 4 and 7.

2.7.7. Procedure for Chapter 5 – Piloting the Food Attentional Bias task

A within-subjects design with two conditions, “premeal” and “postmeal”, was used to collect the FAB task in typically developing adults in Chapter 5. Recruitment took place across two separate testing periods. During the initial recruitment phase, participants completed the FAB task in a specific sequence, with the premeal condition administered first, followed by the postmeal condition. In the subsequent recruitment phase, the order was reversed, and participants underwent the postmeal condition before the premeal condition (see Figure 2.7). Participants who completed the premeal condition first were asked to fast for 4 hours before their lab visit. Visits were scheduled between 12 pm and 2 pm. On arrival, participants were asked to complete the SLIM to determine their perceived level of satiety. To reduce the emphasis on hunger/satiety, the researchers designed replica scales of the SLIM in which participants were asked to rate their energy and happiness levels. Participants were also asked questions related to all five stimuli categories in the task so that equal emphasis was placed on all categories. Participants then completed the first run of the FAB task. Participants were then given a standardised meal consisting of a piece of fruit, a sandwich, and a bottle of water. Thirty minutes after consuming the standard meal, participants completed the postmeal condition. The SLIM and emotion/energy level rating scales were repeated, followed by the second administration of the FAB task. Participants completed the food stimuli appealingness scale and the honesty questionnaire when finished. The study visit lasted approximately 1.5 hours.

Participants who completed the postmeal condition first were instructed not to eat breakfast before attending the visit, as a standardised meal would be provided on arrival. Visits were scheduled between 8 am and 10 am. On arrival, participants were presented with a standardised meal consisting of a toast/bagel, a piece of fruit, and water. Thirty minutes after consuming the standardised meal, participants completed the postmeal condition. The SLIM and emotion/energy level rating scales were administered, followed by the first run of the FAB task. Participants were instructed to return to the lab in four hours and fast for this time. After four hours, participants completed the premeal condition. The SLIM and emotion/energy level rating scales were repeated, followed by the second run of the FAB task. Participants completed the food stimuli appealingness scale and the honesty questionnaire when finished. The study visit lasted 1 hour, followed by a four-hour wait period and 30 minutes.

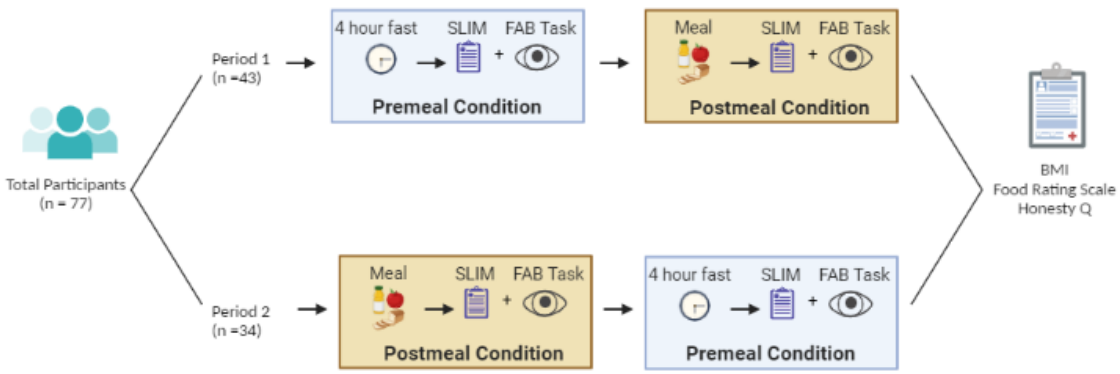

Figure 2.7: Research protocol for the pilot studies of the Food Attentional Bias (FAB) task. The participants were assessed in pre and postmeal conditions in the first recruitment period. This order was reversed in the second recruitment period to investigate any order effects on meal conditions.

2.8. Statistical Analysis

2.8.1. Analytic approach for Chapter 5: Piloting the Food Attentional Bias (FAB) task

A repeated measures analysis of variance (ANOVA) was used to compare the FAB task across the premeal and postmeal conditions in typically developing adults. Repeated measures ANOVA is a widely used method in research designs involving within-subject factors, i.e. where participants are measured under different conditions or at multiple time points (Field et al., 2016). This approach allowed for examining the effects of the repeated factor (premeal vs postmeal) on the outcome variable (total duration of fixations, number of fixations and time to first fixation) while accounting for the dependency between measurements within the same subject. This method estimates the main effects and interactions and allows for hypothesis testing and interpretation regarding the significance of these effects. To run a repeated measures ANOVA, data were collapsed into averages for each AOI category, participant, and meal condition. Repeated measures analysis of variance was conducted on each primary variable, with meal condition (premeal vs postmeal) as the within-subjects variable and AOI (Food, clothes, household items, instruments/ vehicle) as the between-groups variable. A significant interaction for any dependent variable would imply that one AOI category type disproportionately influences one of the meal conditions. All significant interactions were followed up with post hoc analyses to identify the direction of the effect.

2.8.2. Analytic approach for Chapters 4 and 7: Using eye tracking to measure social cognition and hyperphagia in PWS and comparison group

Linear mixed models (LMM) were used to compare the PWS and COM participants in eye tracking studies. LMMs extend traditional linear regression models by accounting for both fixed factors and random factors in the model (Silva et al., 2022). Fixed factors represent variables of interest whose effects are assumed to be constant, e.g. for Chapter 5 meal condition (pre vs post), AOI category (Food vs non-food) and participant group (PWS vs COM). Random factors help to account for unexplained variance in the model capture, such as individual differences in baseline eye movements. For example, some participants may have larger individual variations in fixation durations (Holmqvist et al., 2011). The intercept in random effects represents each participant's average eye movement behaviour, while the slope represents the individual-specific deviations from this average behaviour. Considering these random effects makes it easier to understand how the differences in eye movement responses among participants are influenced by the experimental conditions as opposed to participant-specific variation (Holmqvist et al., 2011).

LMMs were used to analyse group differences between PWS and comparison participants on eye tracking tasks. This approach was chosen to effectively handle the issue of increased levels

of missing eye tracking data commonly observed in the PWS cohort compared to the comparison group due to reduced levels of attention and engagement during the tasks. LMMs can accommodate unbalanced data by allowing for different numbers of observations per participant and therefore use all available data points from participants even if they have some missing observations (Silva et al., 2022). They are often considered a better analysis approach than repeated measures ANOVA in cohorts where the levels of missing data may not be balanced across participant groups. LMMs differ from ANOVA, where data points are aggregated into one data point per participant. This leads to more efficient use of the available data and reduces potential bias by excluding participants with missing data.

How LMMs should be created, or fit is debated (Meteyard & Davies, 2020). In Chapters 4 and 7, the LMMs are fitted with a minimum-maximum fit, also known as restricted maximum likelihood (REML) estimation. The minimal to maximal-that-improves fit approach to model building is usually recommended for exploratory analysis and therefore was selected for the analyses in this thesis since the face pop paradigm (Chapter 4) and FAB task paradigm were administered to PWS participants (Chapter 7) for the first time (Baayen et al., 2008; Meteyard & Davies, 2020). Akaike's Information Criterion (AIC) was used to determine the best-fitting model to compare the different LMMs. A lower AIC value indicates a better balance between model fit and complexity. The model with the lowest AIC was determined as the most parsimonious, and it was selected as the final model (Meteyard & Davies, 2020).

To build the model, random effects were fitted in a stepwise fashion, starting with the participants' intercepts and slopes. Effects that did not improve the fit (i.e., a considerable decrease in AIC), had estimates that approximated zero, or caused errors in computation (e.g., failure to converge), were eliminated from the model. Fixed effects were added once the random effects models were established. Factors and covariates were both included as fixed effects in the model. The final model selected had the lowest AIC that returned no error. Fixed effects deemed essential to the model were not removed, even if non-significant (Baayen et al., 2008).

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Chapter 3: A profile of mental health and behaviour in Prader-Willi Syndrome

This chapter has been adapted from the following article:

Feighan, S. M., Hughes, M., Maunder, K., Roche, E., & Gallagher, L. (2020). A profile of mental health and behaviour in Prader–Willi syndrome. *Journal of Intellectual Disability Research*, *64*(2), 158–169.

Published Work List

3.1 Introduction

PWS is a neurogenetic syndrome with a characteristic behavioural phenotype, a high incidence of maladaptive behaviours and psychiatric comorbidities. PWS has a birth incidence rate of about 1:25,000 (Smith et al., 2003; Annick Vogels et al., 2004; Whittington et al., 2001) and a population prevalence in the UK of about 1:50,000 (Whittington et al., 2001). It is the first recognised disorder related to genomic imprinting in humans, a process whereby genes are programmed to be silent or expressed depending on parental origin of the chromosome. PWS is due to a failure of paternal expression of maternally imprinted genes at the 15q11-13 region, due to 1. deletion of the 15q11-13 region on the paternal chromosome (DEL) 2. maternal uniparental disomy of chromosome 15, (mUPD) or 3. imprinting centre defects or translocations (IC) (Cassidy et al., 2012). In the literature on PWS, the proportion of cases in each of the genetic subtypes are usually given as (approximately) 70% DEL, 25-40% mUPD and 3-5% other (IC or translocations) (Cassidy et al., 2012). However, recent studies have shown an increase of a greater proportion (50%) of those with the mUPD subtype in younger children and have suggested that an increase in maternal age may be driving this changing proportion (Lionti et al., 2015; Whittington et al., 2007).

PWS presents with a complex and changing developmental profile. Infants are born with hypotonia, poor suck, feeding problems, failure to thrive and developmental delay. Motor milestones and language development are delayed, and all individuals have some degree of cognitive disability. Children with PWS can experience a range of endocrinological problems affecting the thyroid, adrenal and gonadal axes. Growth hormone insufficiency or dysfunction is common, leading to short stature. Obesity occurs after a characteristic period of failure to thrive and is associated with extreme food-seeking behaviour and hyperphagia in early childhood (Cassidy et al., 2012). Hyperphagia is related to an impaired satiety response and a high reward value of food in PWS (Hinton et al., 2006; Miller et al., 2007). This extreme drive for food is a life-long stressor for affected individuals and their carers due to the necessity to limit overeating significantly and impacts significantly on their health and well-being.

Individuals with PWS have an increased risk for specific comorbid behavioural and psychiatric difficulties (Whittington and Holland, 2018). A recent study found that 89% of patients with PWS over 12 years of age had at least one psychiatric disorder (Shriki-Tal et al., 2017). The core behavioural phenotype of PWS is characterised by temper tantrums, mood lability, repetitive and ritualistic behaviours and severe skin picking, seen in all genetic subtypes. However, the mUPD genetic subtype has a strikingly higher prevalence of psychosis, which has been estimated at a 60-100% lifetime prevalence, compared to the deletion subtype, which has a similar prevalence to adults with intellectual disabilities in the wider population (Boer et al., 2002; Hinton et al., 2006; Soni et al., 2007, 2008; Verhoeven et al., 2003; A. Vogels et al., 2004). Autism spectrum condition (ASC) diagnosis occurs in 12-25% of individuals with PWS, with the mUPD genetic subtype having a significantly increased risk of autism (Bennett et al., 2015). Additionally, clinically impairing autism traits occur in both the mUPD and DEL genetic subtypes (Bennett et al., 2015; Dykens et al., 2017). Psychiatric comorbidities rank highly as factors negatively affecting the quality of life of individuals with PWS and are reported as the most difficult aspect of the condition to manage by their caregivers (Lanfranchi and Vianello, 2012). In a survey study of children with neurogenetic syndromes, including PWS, fragile X, Williams

Syndrome and 22q11.2 deletion syndrome, having a behavioural/psychiatric condition was a significant predictor of negative family outcomes across all syndromes (Reilly et al., 2015)

Due to the range of problems and variability of symptom severity across individuals with PWS, clinical management is age-dependent, multidisciplinary, targeted at symptoms and tailored to the individual. As such, there is no specific treatment for PWS-specific behavioural disturbances. Applied behaviour analysis (ABA) interventions, a treatment approach that is commonly used in autism, showed efficacy in some small case studies for treating skin-picking and food-related behaviours, but research is limited (Maglieri et al., 2000; Page et al., 1983; Stokes and Luiselli, 2009). Treatment with numerous psychotropic medications such as antidepressants, antipsychotics and appetite suppressants has shown very little effectiveness in controlling hyperphagia and behaviours related to the phenotype, although these may also be used to treat psychiatric comorbidity (Bonnot et al., 2016a).

The combination of severe hyperphagia, psychiatric comorbidities, challenging behaviours, and lack of effective treatments create unique challenges in caring for persons with PWS. Managing and treating these features has become a critical issue in the clinical care of people with PWS (Schwartz et al., 2016). However, there have been limited Irish studies exploring the clinical needs of individuals with PWS and their families (Skokauskas et al., 2012; Reilly et al., 2015). Conducting research at the country-specific level is pivotal for gaining a deeper understanding of the unique needs and challenges faced by PWS patients, especially within the context of a specific healthcare system, such as the Health Service Executive (HSE). Notably, no studies to date have investigated crucial factors, including access to services, school functioning, psychiatric and behavioural profiles, medical appointments, and data concerning young adults' employment and respite care for individuals with PWS in Ireland. These gaps are particularly significant for Irish families, influencing their planning and provision for their loved ones with PWS.

The primary objective of our research was to develop a comprehensive survey that could map the support needs of families with a member affected by PWS across different life stages. The goal was to provide recommendations for support provisions specific to the Irish context. The survey delved into critical areas of need for individuals with PWS and their families, including early life and development, physical health, mental health and behaviour, education and employment, residential and respite support requirements, and the impact of our findings. In this paper, our focus is on the mental health and behavioural aspects, as well as the impact of PWS on families. Given the high prevalence of mental health and behavioural issues in PWS, we hypothesised the following; 1/There would be a high prevalence of psychiatric disorders and complex behaviours in this population similar to previously reported prevalence's from datasets collected UK, the USA, Australia and Italy, 2/The use of psychotropic medications would be prevalent among older participants with PWS and 3/ Caregivers would report a significant negative impact of caring for a person with PWS.

3.2 Method

This study was the result of a collaboration between the Prader-Willi Syndrome Association of Ireland (PWSAI), Trinity College Dublin and Tallaght University Hospital Dublin to undertake a

national survey in Ireland to identify the physical, mental health/ behavioural and service needs of individuals with PWS and their families to inform policy developments. Community and participant engagement was an important component of the development of this research. A panel of clinicians, researchers and parent advocates were involved in the survey design, which was informed by a literature review and clinical consensus. The survey was informant-based and targeted at caregivers, with quantitative and qualitative elements, and focused on: early life and development; physical health; mental health and behaviour; education and employment; residential and respite support needs; and caregiver impact. The survey was revised based on feedback from the PWSAI committee and expert medical and behavioural clinicians. The revised survey was piloted with parents of individuals with PWS, which informed further minor revisions. The final version was approved by the PWSAI. Ethics approval for the survey was provided by the Tallaght University Hospital / St. James's Hospital Joint research ethics committee.

The mental health and behaviour section included parent-reported diagnoses of psychiatric disorders, the date of first diagnosis, current and past psychotropic medication, dates commenced and duration of treatment. For the purpose of this study, the term psychiatric disorder encompassed "Anxiety Disorder", "Bipolar Disorder", "Depression", "Obsessive Compulsive Disorder", and "Psychosis". There was also an "Other" option where participants could name any other psychiatric diagnosis. In this section, participants were also asked to report if they had a diagnosis of "Autism Spectrum Condition". Two subscales of the behaviour problems inventory - short form (BPI-S) (Rojahn et al., 2012) - provided measures of self-injurious behaviours (SIB) (8 items) and aggressive-destructive behaviour (ADB) (10 items). Caregivers were asked to rate frequency ("Never", "Monthly", "Weekly"," Daily", "hourly") and severity ("No Problem", "Mild", "Moderate", "Severe) and subtotals for each were calculated. In consultation with the family association, we decided that only caregivers of individuals with PWS over four years would be asked to complete the questionnaire's mental health and behaviour sections.

We incorporated the Hyperphagia Questionnaire (Dykens et al., 2007), a 13-item instrument that measures the presence and severity of food-related preoccupations and problems in PWS on a five-point scale (1 = not a problem to 5= severe and/or frequent problem). The scale provides a total score and three subscores: behaviour, drive, and severity. We assessed caregiver impact using the Brief Family Distress Scale (Weiss and Lunsky, 2011) to evaluate the current level of crisis experienced by the caregiver/ family on a 10-point scale. Each point was grounded in a statement describing a point along a scale from no stress ("0") to complete crisis ("9"). We also asked caregivers to rate the impact of caring for an individual with PWS on the family on a scale of 1-4 (1 = "no impact", 2 = "small negative impact", 3 = significant negative impact", and 4 = "extreme negative impact"). Study information was emailed to PWSAI members, and hard copies were given to patients and carers attending the PWS Specialist Medical Clinic at Tallaght University Hospital, who contacted the study team if they wished to participate. Further information regarding the study was discussed by telephone. Information sheets, consent forms and surveys were sent via post. On the advice from PWSAI, caregivers of children less than four years of age (n=8) did not complete the behaviour and psychiatric sections, as it was thought that this might burden them unduly.

Seventy-one participants provided their contact details to the research team, of whom 65 were successfully contacted, and 61 returned completed surveys (Response rate: 94%). We estimate that the respondents represented approximately 60% of the total known PWS population in Ireland, based on estimates of diagnoses from the National Centre for Medical Genetics (NCMG), Our Lady's Hospital for Sick Children Crumlin. However, this does not include individuals with PWS undiagnosed in Ireland or who received a diagnosis abroad. The following section presents demographic, mental health and behaviour, and caregiver impact data analysis.

3.3 Results

Sixty-one caregivers of individuals with PWS participated in this study; 82% of respondents were the biological mother of the person with PWS, 13% were the biological father, and 5% were siblings. The age of the individuals with PWS ranged from 11 months to 52 years, with a mean age of 16.3 years (SD = 11.3). Within the sample, 58% were female (n =35), and 42% were male (n = 26). Based on caregiver reporting, 43% (n=25) had the deletion subtype, 26% had the mUPD subtype (n=17), 3% had an imprinting centre defect (n=2), and 28% were unsure of the genetic subtype (n=18). Recognising that developmental and behavioural needs change across the lifespan, we subset the results into three age groups based on education stage: children (primary school, aged 4-12 years); adolescents (secondary school, 12 – 18 years); and adults (>18 years).

3.3.1 Psychiatric Disorders

Table 3.1 shows the prevalence of psychiatric disorders in adolescent and adult participants (n=38). No participants under the age of 12 had been diagnosed with a psychiatric disorder. Fifty percent of participants over the age of 12 years had been diagnosed with a psychiatric disorder (see Table 3.1). Anxiety was the most common diagnosis in adolescents, followed by OCD. Anxiety was also the commonest diagnosis in adults. Adults had more diagnoses of depression, psychosis and bipolar disorder compared with adolescents (see Table 3.2). The average onset of a psychiatric disorder was 16 years (SD +4.9, range 6-23). The commonest comorbid diagnoses were anxiety with OCD, followed by anxiety with depression.

Table 3.1: Number of psychiatric diagnoses received by participants over the age of 12 years

Number of Diagnoses	Participants ≥ 12 years	
	N	%
0	19	50
1	8	21
2	6	16
≥3	5	13
Total	38	100

This table has been published in my paper, Feighan et al. 2020 (Appendix 4).

Table 3.2: Prevalence of psychiatric diagnoses

		Age Group		Gender		Genetic Subtype			
	Total	12-17 years	≥18 years	Male	Female	DEL	mUPD	IC	NK
n	38	17	21	14	24	17	5	2	14
Anxiety Disorder %	37	29	43	36	38	29	80	100	21
ASC %	5	12	0	7	4	6	0	0	0
Bipolar Disorder %	8	0	14	7	8	6	20	50	0
Depression %	24	6	38	14	29	18	20	100	21
OCD %	16	18	14	14	17	6	20	100	14
Psychosis %	16	6	19	14	17	18	0	100	7

Prevalence of psychiatric diagnoses across age groups (adolescents and adults), gender and genetic subtype % is prevalence within each group. ASC = Autism Spectrum Condition, OCD = Obsessive Compulsive Disorder, DEL = Deletion subtype, mUPD = maternal uniparental disomy, IC = imprinting centre defect, NK = not known. This table has been published in my paper, Feighan et al. 2020 (Appendix 4).

3.3.2 Psychotropic Medication

No participants less than 12 years old were prescribed psychotropic medication. Forty-two percent (n=16/38) of participants over 12 years old were currently prescribed psychotropic medication (Table 3.3). Selective Serotonin Reuptakes Inhibitors (SSRIs) were the commonest prescribed medications in adolescents, and antipsychotics were the commonest in adults (Table 3.4). Antipsychotic medication was significantly more likely to be prescribed in the mUPD subtype than the DEL subtype (Fisher's Exact Test: $p < .01$, odds ratio=23.1, CI 95% = 2.0, 768.9). Although 80% (n = 4/5) of mUPD participants were prescribed antipsychotic medication, none were reported as having a clinical diagnosis of psychosis.

Table 3.3: Number of psychotropic medications prescribed

Number of medications	Participants ≥ 12 years	
	N	%
0	22	58
1	6	16
2	4	10
≥3	6	16
Total	38	100

The number of psychotropic medications prescribed for participants over the age of 12 years. This table has been published in my paper, Feighan et al. 2020 (Appendix 4).

Table 3.4: Prevalence of psychotropic medication

		Age Group		Gender		Genetic Subtype			
	Total	12-17 years	≥18 years	Male	Female	DEL	mUPD	IC	NK
n	38	17	21	14	24	17	5	2	14
On medication									
%	42	35	48	36	46	35	100	100	21
Antipsychotic									
%	24	6	43	14	29	12	80	100	7
Mood Stabiliser									
%	11	6	14	14	8	6	20	50	7
SSRI									
%	26	29	24	21	29	29	40	0	21
Other									
%	8	6	10	7	8	6	0	100	0

Prevalence of psychotropic medication usage across age groups (adolescents and adults), gender and genetic subtype. % is prevalence within each group, SSRI = Selective Serotonin Reuptake Inhibitor, Other = Alprazolam, Biperiden, Lorazepam, Sodium Valpraote. This table has been published in my paper, Feighan et al. 2020 (Appendix 4).

3.3.3 Behaviours

Skin picking was the most prevalent reported behaviour on the self-injury scale in children, adolescents and adults, reported in 76% of cases (n = 40/53). Skin picking was particularly common in adolescents (93%, n = 15/17) (Table 3.5). Teeth grinding was the second most prevalent self-injurious behaviour, highest in the adolescent group (Table 3.5). Aggressive

behaviours differed across age groups. "Hitting others" was the most endorsed item in children, whereas "verbal abuse" was the commonest in adolescents and adults. On the PWS-specific subscale, repetitive questioning was a highly prevalent reported behaviour in all three age groups, reported by 100% (n = 32/32) of caregivers of children and adolescent and 67% (n = 21/32) of adults (see Table 3.5). Stealing food, money, and lying, associated with the core hyperphagia phenotype, were prevalent behaviours. However, caregivers across all age groups reported non-compliance, difficulty transitioning and obsessions/compulsions more frequently.

Hyperphagia occurred in 81% (n = 43/53) of participants over the age of four. The average age of onset of hyperphagia was 3.8 years (± 1.6 months, range 1-7 years). Hyperphagia drive (HD), hyperphagic behaviour (HB), and hyperphagic severity (HS) did not differ based on gender, genetic subtypes or age group (Table 3.6). A small correlation was observed between age and hyperphagic behaviour (r = .28, p<.07) in children/adolescents. Thirty-six percent reported little variability in food preoccupation, 49% reported occasional variability, and 14% showed high variability.

Table 3.5: Prevalence of challenging behaviours

		Total	Age Categories		
Subscale	Item	(4-52 yrs) N=53	Children (4-11yrs) N=15	Adolescents (12-17yrs) N=17	Adults (≥18 yrs) N=21
Self-Injurious Behaviours	Self-biting %	11	21	13	6
	Head hitting %	4	7	7	0
	Body hitting %	12	14	27	0
	Pica %	11	14	12	11
	Inserting objects %	16	7	24	21
	Hair Pulling %	11	7	18	11
	Teeth grinding %	36	40	53	21
	Skin-Picking %	76	71	93	72
Aggressive Destructive Behaviours	Hitting others %	27	40	29	21
	Kicking others %	17	7	29	17
	Pushing others %	34	33	59	24
	Biting others %	7	7	13	5
	Grabbing/Pulling %	23	33	29	16
	Scratching others %	13	20	18	5
	Verbally abusive %	39	33	59	37
	Pinch others %	16	27	23	6
	Destroying things %	29	20	41	26
	Bullying %	20	20	35	11
PWS Specific Behaviours	Stealing food %	45	53	50	47
	Stealing money %	16	7	23	24
	Lying %	53	40	71	40
	Repetitive Questions %	78	100	100	67

Obsessions/Compulsions %	44	67	64	22
Non-compliance %	62	80	71	56
Difficulty transitioning %	50	87	64	22

Item-level prevalence of challenging behaviours from the subscales of the BPI-S (self-injurious and aggressive/destructive) and survey (PWS specific) across the total sample and age groups. This table has been published in my paper, Feighan et al. 2020 (Appendix 4).

Table 3.6: Hyperphagia Questionnaire Scores

Subscale	Age Group	N	Mean	SD	Range
Behaviour	Children	15	14.4	3.4	10-21
	Adolescents	17	13.5	3.9	7-20
	Adults	21	12.8	5.2	0-20
Drive	Children	15	7.7	2.8	1-12
	Adolescents	17	7	1.8	3-10
	Adults	21	5.3	2.9	0-10
Severity	Children	15	4.9	1.6	1-7
	Adolescents	17	4	1.9	1-7
	Adults	21	3.6	2	0-6

Factor means, standard deviations and ranges of the hyperphagia questionnaire across age groups. This table has been published in my paper, Feighan et al. 2020 (Appendix 4).

3.3.4. Access to Services

All sixty-one participants were asked to answer questions about their access to clinical services. Ninety-two percent (n = 56/61) of participants had attended speech and language therapy (SLT). The average time participants had to wait for their first SLT session was 11 months, and at the time of this study, 4% (2/61) of participants were on waiting lists to attend speech and language therapy. A smaller proportion of participants, 75% (n=45/61) were receiving occupational therapy. The average wait time for the first occupation therapy was 12 months (see Figure 3.1). Fewer participants (67%, n = 41/61) had attended psychology services; however, there was a notable increase in wait times for this service (22-month wait for an appointment). Twenty-eight (n=17/61) percent of participants had attended a psychiatrist, with an average waiting time of 4 months for the first appointment.

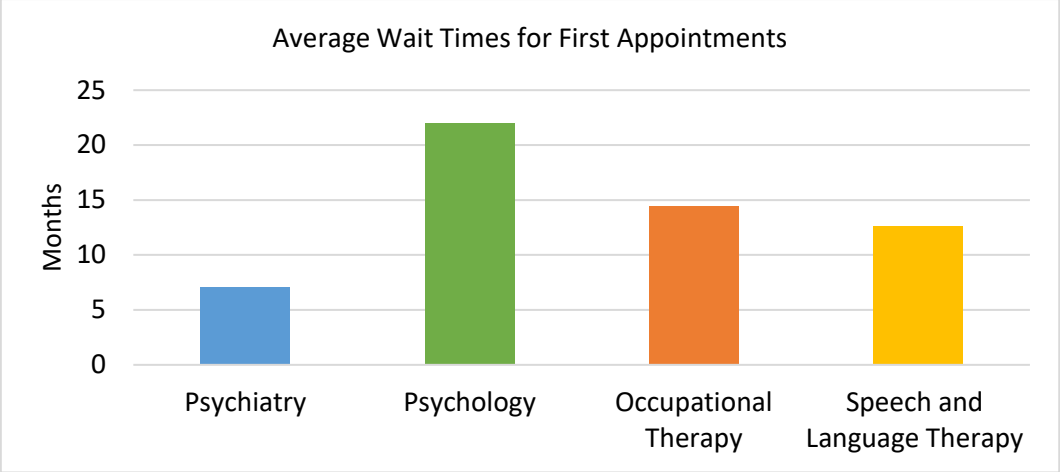

Figure 3.1: Average wait times for access to services

3.3.5 Impact on Families

A significant impact of caring for an individual with PWS on caregiver employment was reported by a large proportion of respondents. Seventy-five percent (n = 46/61) reported that either they or their partner had stopped working entirely, and 16% (n = 10/61) reported that they reduced their working hours. Only 9% (n = 5/61) reported no impact on employment. Family impact was measured on a scale of 1-4 (1 = "no impact", 2 = "small negative impact", 3 = significant negative impact", and 4 = "extreme negative impact"). Seventy-five percent (n = 46/61) reported significant or extremely negative emotional impact on the family related to caring for their relative with PWS relative. The emotional impact was reported as less severe in caregivers of individuals with PWS who lived in assisted accommodation. Negative physical impact and impact on family relationships were highest in the adolescent group, and financial impact was highest in the adult group. Caregivers were also asked to rate their perceived level of crisis. Thirty-two percent (n = 20/61) said everything is fine/sometimes a little stressful", 52% (n = 31/61) said "things are very stressful", and 16% (n = 10/61) said they are "in crisis and cannot cope".

3.4 Discussion

The main aim of this study was to profile the mental health and behavioural needs of people with PWS in Ireland. The survey results revealed a high prevalence of psychiatric disorders in this population, with anxiety being the most diagnosed condition. Psychotropic medications were prevalent among older participants, particularly antipsychotics and prescribed more frequently in individuals with the mUPD subtype of PWS. The study also found that behaviours associated with hyperphagia, and repetitive questioning were prevalent across all age groups. Caregivers reported a significant negative impact on their employment, family relationships, and emotional well-being, highlighting the challenges individuals with caregivers and families face. The findings illustrate the complex mental health and behavioural needs of individuals with PWS and emphasise the challenges these individuals face. Importantly, this study has provided a profile of these needs within an Irish context for the first time, providing insight into

gaps in service provision and resource allocation and highlighting the pressing demand for skilled professionals and specialised behaviour support services.

The Irish PWS population exhibit a similar psychiatric profile to previous studies on PWS: high anxiety levels appear in adolescence, followed by the onset of affective disorders and psychosis in adulthood (Manzardo et al., 2017; Shriki-Tal et al., 2017; Soni et al., 2007; Soni et al., 2008). The prevalence of psychiatric diagnosis in participants over the age of 12 years in our study was lower than in a recent study from Israel in which the majority (89%) had a psychiatric diagnosis. This may be explained by the reliance on parent-reported psychiatric diagnoses in the current study as opposed to the use of direct clinical assessment. Anxiety has previously been found to be the commonest diagnosis in a study looking at a neuropsychiatric diagnosis of adults with PWS in residential care (Manzardo et al., 2018). Notably, anxiety is one of the most significant predictors of psychosis in 22q11.2 deletion syndrome, a genetic syndrome associated with high rates of psychosis (Tang et al., 2017). More research is needed to investigate predictors of psychosis in PWS, especially in the mUPD genetic subtype, who are more at risk. Thirteen percent (n = 5/38) of participants had received three or more comorbid psychiatric diagnoses, emphasising the complex nature of psychiatric disorders in PWS. A recent review of the psychiatric conceptions of mental and behavioural disorders in PWS discussed how psychiatric disorders in PWS differ from those observed in the general population. However, there are overlaps in symptoms (Whittington and Holland, 2018). As psychiatric disorders in PWS are atypical, it may be difficult to classify them within existing psychiatric diagnoses, which may explain why some participants in the current study had three or more diagnoses.

In our study, 42% (n = 12/38) of the sample over the age of 12 years were prescribed at least one psychotropic medication. SSRIs were the commonest in adolescents and antipsychotics in adulthood and were highly correlated with comorbid psychiatric diagnoses. SSRIs were also the commonest prescribed psychotropic medication in a separate review of psychotropic medication usage in PWS (Bonnot et al., 2016b). In the present study, the mUPD genetic subtype was more likely to take antipsychotic medication than the deletion subtype. This probably reflects the known increased risk of psychosis in the mUPD genetic subtype (60-100% prevalence) compared to the deletion subtype, where prevalence rates are similar to individuals with intellectual disability more generally (Aman et al., 2018; Rice et al., 2016; Soni et al., 2008;Soni et al., 2007; Vogels et al., 2003). It has previously been shown that those with mUPD are more likely to have been prescribed psychotropic medication and to have tried a larger number of psychotropic medications, possibly due to a poor response to medication (Soni et al., 2008). Although 80% (n = 4/5) of mUPD participants were prescribed antipsychotic medication, none were reported as having a clinical diagnosis of psychosis. The individuals may have been prescribed antipsychotic medication for other symptoms, such as irritability (de Kuijper et al., 2021). A weakness of the current study is that we did not ask respondents to specify what exact symptom medication was being used to target. We also relied on caregiver reports of psychiatric diagnosis which may explain the discrepancy between medication usage and psychiatric diagnosis.

The current study identified a pattern of aggressive behaviours in PWS using the aggressive destructive behaviour subscale (ADB) of the behavioural problems inventory (BPI-S). This is the first study in PWS to characterise aggression at an item level. "Hitting others" was the most

frequently rated aggressive, destructive behaviour in children and "verbal abuse" was the most frequent and severe behaviour in adolescents and adults. Although research characterising aggression in PWS is limited, recent studies have highlighted the prevalence of disruptive behaviour disorders (DBD), such as oppositional defiant disorder (ODD) and conduct disorder (CD) (Shriki-Tal et al., 2017; Lo et al., 2015). A two-year longitudinal follow-up study of psychiatric disorders in children and adolescents with PWS identified ODD in 20% of participants and identified "arguing with parents" as a common feature (Lo et al., 2015). DBDs in the Israel national cohort were also commonly reported in individuals with PWS over 12 years with fifty percent receiving a diagnosis of ODD and 17% receiving a diagnosis of CD (Shriki-Tal et al., 2017a). A very small minority of caregivers in the present study reported that their child had a diagnosis of DBD. The very low prevalence rates of DBDs in the current study may be related to the self-report nature of the study or the underuse of these diagnostic labels in Ireland. Further research is needed to understand how specific features of the PWS behavioural phenotype, such as hyperphagia, may explain the high prevalence of DBDs. For example, lying is a criterion for diagnosing CD; however, this may only be relevant in the context of food in PWS. It would be interesting to see how direct clinical assessment would impact the prevalence of DBDs in the current study.

Skin picking was extensively reported by caregivers in all age groups, most notably in 93% (n = 15/17) of adolescents. Skin-picking is a widely recognised feature of the PWS behavioural phenotype (Morgan et al., 2010). It is a matter of debate if skin picking is characteristic of self-harm, obsessive-compulsive behaviour or a direct consequence of loss of the Necdin gene (Whittington and Holland, 2018). The latter association is suggested by observing a skin picking phenotype in Necdin knockout mice (Muscatelli et al., 2000). It was previously found that skin picking was related to disruptive behaviour disorders but not OCD in PWS, suggesting skin-picking is not representative of obsessive-compulsive behaviour (Shriki-Tal et al., 2017b). This is further supported by a factor analysis study of behaviour in PWS, which found skin picking did not load onto the same factor as compulsions (Holland et al., 2003). Further research is needed to understand better the processes driving skin-picking behaviour in PWS to develop better approaches to address this behaviour.

Other commonly reported behaviours in the present study included repetitive questioning, difficulty transitioning, non-compliance, food stealing and obsessive/compulsive behaviours. Repetitive questioning, difficulty transitioning, and obsessive-compulsive behaviours overlap with behaviours seen in autism. In addition to the occurrence of autism traits in PWS, there are also potentially overlapping genetic susceptibilities with the PWS critical region 15q11-13 being referred to as an epigenetic "hotspot" for autism susceptibility genes (Dykens et al., 2011). "Insistence on sameness" in autism has been associated with one of several $GABA_a$ receptors within the PWS critical region (Shao et al., 2003). Autism diagnosis occurs in 12.3-25% of individuals with PWS; mUPD carriers are particularly at an increased risk (Bennett et al., 2015; Dykens et al., 2017). Some have argued that autism symptoms become more prevalent throughout childhood in PWS, although the reasons for this are unclear (Lo et al., 2013; Song et al., 2015). Further research is needed to improve our understanding of the clinical relationships between PWS and autism and possible shared genetic pathways.

In this study, 97% of participants attended speech and language therapy and had timely access to their first appointments. In contrast, participants experienced longer waiting times for psychological services for behavioural interventions, on average 22 months. Management of behavioural problems is most effective if detected early, as multiple studies have shown difficulties tend to increase with age in PWS. While there is limited research on PWS, targeted behavioural interventions effectively treat anxiety/obsessive-compulsive symptoms (Ung et al., 2015) and self-injury (Peters-Scheffer et al., 2011) in children and adolescents with other neurodevelopmental disabilities. Therefore timely access to behavioural management supports an important clinical need. Further research is needed to modify and test the efficacy of behavioural interventions for anxiety, temper outbursts and social challenges in PWS.

Finally, our study highlighted negative impacts on the caregiver and the family regarding their financial circumstances, emotional and physical well-being. A high proportion (75%) gave up or reduced their work, and a small but significant proportion of participants reported a severe negative financial impact. Change in employment status underscores the loss of income to families directly attributed to caring for PWS individuals/children. Significant emotional and physical impacts were most notable in the adolescent group, which has been related to higher levels of caregiver burden in carers of adolescents and young adults with PWS compared with older adults and younger children (Kayadjanian et al., 2018). The findings of this study are in line with prior research indicating heightened levels of parenting stress among caregivers of individuals with Prader-Willi Syndrome (PWS). Notably, 56% (80 out of 142) of families caring for individuals with PWS reported a high level of care burden, as assessed by the Zarit Burden Interview (Kayadjanian et al., 2018). Additionally, heightened familial stress, measured through the Stress and Resources Questionnaire (Salisbury et al., 1989), was observed in PWS caregivers compared to those caring for individuals with unknown causes of developmental delay (Hodapp et al., 1997).

More broadly, parents of children with developmental disabilities are reported to experience greater stress and psychological challenges, such as anxiety and depression, in comparison to those without developmental disabilities or the general population (Patton et al., 2018; Seymour et al., 2013; Totsika et al., 2011; Zablotsky et al., 2013b). A recent scoping review highlighted that parents of children diagnosed with rare genetic syndromes endure even higher levels of distress than parents of children with intellectual disabilities of unknown origin (Fitzgerald and Gallagher, 2021). Notably, within this review, maladaptive behaviour and emotional difficulties in the child consistently predicted poorer parental outcomes. The majority of reviewed studies within the review indicated that the level of intellectual functioning was not associated with parental outcomes. These findings suggest that the impact on parental well-being is primarily influenced by behavioural challenges rather than the level of intellectual disability. While it's plausible that the heightened parental stress observed in this study may be linked to the behavioural challenges associated with the PWS phenotype, further investigation is essential to fully comprehend the contributing factors. Our study highlights that mental health monitoring and treatment in PWS as well as caregiver well-being should be priorities for care. Additional social and respite supports are likely required during more challenging periods of the individual's life to protect family relationships and prevent caregiver burnout.

A limitation of the present study is relying on a single source for clinical and genetic information, namely the primary caregiver. We believe that the data are representative of the PWS population in Ireland, which is estimated to be 100 cases. However, ascertainment bias cannot be excluded as we may not have detected those with the greatest difficulties. Finally, it would be preferable to confirm genetic diagnosis through clinical genetics services in future studies.

3.5 Conclusion

The mental health and behavioural needs of individuals with PWS in Ireland are significant and illustrate the challenges faced by individuals with PWS and those caring for them. The complexity of the mental health and behavioural needs of PWS individuals requires skilled multidisciplinary professionals who can provide appropriate assessment and individual-centred interventions. The development of specialist behaviour support services in Ireland is urgently needed to help manage the complex behavioural phenotype of PWS and help reduce caregiver burden so we do not rely solely on a medication-based approach to mental health management for this medically complex group.

Another crucial step in enhancing behavioural and mental health outcomes for individuals with PWS involves conducting further research to deepen our understanding. While PWS serves as a robust genetic predictor of complex mental health conditions, the neurobiological mechanisms connecting PWS to these conditions remain largely elusive. Undertaking comprehensive clinical and cognitive phenotyping of PWS is vital for unravelling the pathophysiology associated with mental health and behaviour in PWS, paving the way for the development of comprehensive treatments."

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Chapter 4: The role of social cognition in autism behaviours within PWS

4.1. Introduction

In Chapter 3, the survey results illuminated the pronounced mental health and behavioural needs among individuals with PWS. To enable more precise and effective treatments, additional research is necessary to uncover potential neural and psychological mechanisms linking genotype to phenotype. This chapter will focus on one aspect of the PWS behaviour phenotype—autism behaviours —and aim to investigate the underlying neurocognitive processes in PWS. Presently, the nature of autism behaviours within the context of PWS remains inadequately understood. Overlap In autism, reduced social cognitive ability is theorised as a potential mechanism of poor social functioning and differences in social skills (Bishop-Fitzpatrick et al., 2017; Keifer et al., 2021; Sasson et al., 2020). However, the link between social cognition and social functioning in individuals with PWS has not been thoroughly investigated. This chapter aims to investigate a critical aspect of social cognition, social motivation. Social motivation pertains to a set of psychological tendencies and biological processes that influence individuals to demonstrate a preference for attending to the social aspects of their surroundings (social orienting) and derive pleasure from participating in social interactions (social reward) (Chevallier et al., 2012). This study aims to assess social motivation in individuals with PWS and explore its relationship with autism behaviours using a well-established eye tracking paradigm that has been used in the context of autism.

4.1.1 Autism within Prader-Willi Syndrome

Certain behavioural similarities exist between individuals with PWS and those with idiopathic autism, although they can differ qualitatively (Bennett et al., 2015; Dykens et al., 2011). For instance, in Chapter 3, repetitive questioning emerged as the most frequently reported challenging behaviour in PWS. Repetitive behaviours are a defining characteristic of autism and involve both stereotypies (i.e. repetitive and seemingly purposeless movements, gestures, or vocalizations) and restrictive, repetitive behaviours relating to interests or activities (Lam and Aman, 2007). However, individuals with PWS exhibit distinct repetitive and compulsive behaviours (Dykens et al., 1996; Clarke et al., 2002), like repetitive questioning, as opposed to the stereotypies commonly associated with autism. Although there is an overlap between traits seen in PWS and those observed in autism, the prevalence and nature of autism in PWS have been the subject of some debate among clinicians and researchers (Schwartz et al., 2021). Distinguishing autism specific behaviours in genetic syndromes like PWS is challenging as characteristics may be attributable to a person's intellectual disability (Jenner et el., 2023). The prevalence of autism within PWS has been reported to range widely, with estimates varying from 12% to 70%, depending on the specific approach used to assess autism (Bennett et al., 2015; Dykens et al., 2017).

Approaches to measuring autism in PWS studies vary widely (Bennett et al., 2015). The gold standard diagnostic assessment for autism in autism research involves the use of two instruments, the Autism Diagnostic Observation Schedule - 2, which involves direct observation of the participant and the Autism Diagnostic Interview–Revised, a structured diagnostic interview conducted with the participant's caregiver (Le Couteur et al., 2007; see Chapter 2 for a full description of instruments). The most comprehensive study of autism prevalence in PWS

to date was conducted by Dykens et al. (2017) in 146 children and reported that 18 individuals (12.3%) met the criteria for an autism diagnosis when assessed using the ADOS-2 in combination with thorough clinical reviews by an expert clinical team. The clinical reviews included a revision of ADOS-2 videotapes, calibrated severity scores, developmental histories, indices of current functioning, IQ scores, and assessments using the Repetitive Behaviour Scale-Revised and Vineland Adaptive Behaviour Scales, Second Edition (VABS-II). Fourteen of the 18 participants who met the criteria for an autism diagnosis within the PWS cohort had the maternal uniparental disomy (mUPD) genetic subtype of PWS (Dykens et al., 2017). Consistent with previous findings, the mUPD genetic subtype has been associated with a higher occurrence of autism behaviours (Bennett et al., 2015).

Although the prevalence rate of 12.3% is lower than the initial reports from other studies using screening questionnaires, it remains significantly higher compared to the general population, estimated at around 1-2% (Zeidan et al., 2022).

4.1.2 Genetic overlap between PWS and autism

Multiple factors may influence the increased prevalence of autism within PWS. For instance, the PWS critical region, a specific genomic region located on chromosome 15q11-13, contains several genes associated with autism, including UBE3A, ATP10a, MKRN3, ZNF, MAGEL2, Necdin, and SNURF-SNRPN (Dykens et al., 2011; Guffanti et al., 2010; Hogart et al., 2010; Mishra et al., 2022; Vatsa & Jana, 2018). Individuals with the mUPD genetic subtype, characterised by a duplication of the maternal chromosome in the 15q11-13 region, have shown higher prevalence rates of autism than those with the DEL subtype. Duplications in the 15q11-13 region have also been linked to autism in general population studies, with an estimated prevalence of approximately 1 in 500 cases (Depienne et al., 2009), further highlighting the role of genetic factors in autism within PWS. Another potential factor that may contribute to increased autism behaviours in PWS is ID. The majority of individuals with PWS present with mild to moderate ID (Yang et al., 2013). Individuals with ID tend to score higher on measures of autism due to communication and social interaction challenges, which leads to difficulties in developing appropriate social and communicative skills and results in a greater reliance on routine and repetitive behaviours as coping mechanisms (Thurm et al., 2019).

4.1.3 The course of autism behaviours in PWS

Evidence suggests that the expression of autism behaviours varies across development within PWS. Notably, children with PWS generally exhibit lower rates of autism behaviours when compared to adolescents and adults in studies that have used the Social Responsiveness Scale, a questionnaire used to assess social behaviours associated with autism (Dimitropoulos et al., 2013.; Zyga et al., 2014). Similarly, studies using ADOS-2 to diagnose autism within PWS reveal a higher prevalence of autism diagnosis in adolescents than in children (Bennett et al., 2018; Zyga et al., 2014). Ogata and colleagues (2014) reported that autism scores on the Pervasive Developmental Disorders Autism Society Japan Rating Scale significantly differed between children and adolescents, with adolescents exhibiting higher scores on average. A follow-up study by Song and colleagues (2015) reported fewer autism traits in individuals with PWS aged 6-12 compared to same-age individuals with autism. Interestingly those aged 13-15 displayed

similar traits to the autism comparison group, indicating a potential shift in the expression of autism behaviours during this transitional period. Although different assessment tools have been employed in the various studies, the consistent trend of autism behaviours increasing from childhood to adolescence emerges across these studies. It is worth noting that the transition from childhood to adolescence in individuals with PWS coincides with various multisystem changes. For instance, this period marks the switch from nutritional phase 2b-3a, accompanied by the onset of hyperphagia (excessive hunger), reduced satiety (feeling full), and an increased preoccupation with food, further adding to the complexity of the developmental trajectory in PWS.

4.1.4 Social Functioning and social cognition in PWS

While prior studies may have overestimated the prevalence of autism among individuals with PWS, considerable social communication and behavioural difficulties are widely recognised in PWS (Dykens et al., 2019; Fernández-Lafitte et al., 2022). Caregivers, however, also report notable strengths in social functioning exhibited by their children with PWS, noting their displays of empathy, affection, and compassion (Downs et al., 2022). Nonetheless, individuals with PWS encounter significant challenges, encompassing social withdrawal, difficulties in forming peer relationships, and impediments in social attribution and comprehension of complex social situations (Dimitropoulos et al., 2012; Fernández-Lafitte et al., 2022; Koenig et al., 2004; Schwartz et al., 2021b).

The social functioning of individuals with PWS is likely influenced by the distinctive PWS phenotype characterised by mild to moderate intellectual disability, temper outbursts, rigidity, insistence on sameness, and repetitive, compulsive behaviours (Dykens et al., 2007; Ho & Dimitropoulos, 2010; Whittington & Holland, 2017). Additionally, individuals with PWS commonly experience executive function challenges, particularly attention and task switching (Woodcock et al., 2009). These difficulties in executive functioning may all play a role in adapting their behaviour to suit the changing or nuanced nature of social interactions. Consequently, individuals with PWS may exhibit temper outbursts, impulsivity, and inflexible thinking as responses to social situations (Woodcock et al., 2009). Such behavioural patterns can significantly impact social functioning, making it challenging for individuals with PWS to engage in flexible and adaptive social interactions. However, evidence also suggests that social cognition is impacted in the areas of emotion recognition, face processing, and social attribution - specifically, the comprehension of others' thoughts and emotions (Koenig et al., 2004; Lo et al., 2013; Whittington & Holland, 2011) with certain studies highlighting more pronounced differences in the mUPD subtype (Debladis et al., 2019; Halit et al., 2008; Key & Dykens, 2017). As noted above, this genetic region harbours genes associated with autism, prompting inquiries into potential parallels between social cognition differences in PWS and autism.

4.1.5 The Social Motivation Theory of Autism

The Social Motivation Theory of Autism has emerged as a prominent conceptual model for understanding social cognitive variations within the autism spectrum (Chevallier et al., 2012; Dawson et al., 2005). According to this theory, reduced motivation to attend to and process social information contributes to cognitive differences in individuals with autism, shaping

distinct trajectories in social development (Chevallier et al., 2012). This theory suggests that disruptions in reward processing occur early in life, potentially influencing the emergence of autism-related features. Consequently, a lack of motivation to attend to social information may result in unique patterns of social communication (Chevallier et al., 2012). While initial formulations of the theory focused on diminished reward responsiveness in the social domain of autism (Dawson et al., 2005; Schultz, 2005), more recent works indicate atypical reward processing that extends beyond the social domain, suggesting a broad reward processing deficit in individuals with autism, impacting their social development (Bottini, 2017; Clements et al., 2018; Keifer et al., 2021; Kohls et al., 2012).

There is also evidence of disruptions in reward processing in PWS. Studies have linked hyperphagic behaviours in PWS to perturbations in reward circuitry (Holsen et al., 2006; Miller et al., 2007). MRI studies of PWS patients have identified brain alterations in the frontal reward circuit and limbic system, which are associated with molecular genetics and clinical manifestations such as overwhelming eating, obsessive-compulsive behaviours, and skin picking (Brown et al., 2022; Huang & Cai, 2023). These findings suggest potential dysregulation of the reward system in the PWS population, in addition to the known dysregulation of hypothalamic appetite-regulatory pathways. Dopaminergic reward system dysregulation has also been implicated in PWS (Wieting et al., 2023). Given the importance of dopamine in reward processing and social motivation, there may be a connection between deficits in social reward and social functioning in PWS. As perturbations in reward circuitry have been implicated in both autism and PWS, it is possible that social motivation also plays a role in social functioning in PWS. It is also likely that differences in social motivation are linked to autism traits in the context of PWS.

Free-viewing paradigms are a commonly used method for investigating social motivation, providing insight into how participants attend to social information. In a free-viewing paradigm, images are presented in competition with each other, with the resulting pattern of visual orientation and attention providing insight into the relative preference or reward value of the different stimulus types (Simpson, Maylott, et al., 2019). One such paradigm is the Face Pop Task (Gliga et al., 2009), a free viewing paradigm designed to test whether participants demonstrate a "face pop" effect (i.e. automatically orient to faces) and prefer to look at faces compared to non-social stimuli. The task is part of a comprehensive battery of eye tracking tasks used within the framework of EU-AIMS and AIMS-2-TRIALS, European research initiatives devoted to investigating the biological mechanisms underlying autism (Elsabbagh et al., 2013; Gliga et al., 2009; Loth et al., 2017). In typically developing cohorts, neonates, infants and adults all show a preference for social stimuli (human faces) over non-social stimuli (Gliga et al., 2009; Johnson et al., 1991; Simpson, Maylott, et al., 2019; Valenza et al., 1996). There is even evidence of preference for faces in other species, as macaques were found to have a visual preference for their own species' faces over others as young as two months of Age (Kim et al., 1999; Simpson, Paukner, et al., 2019). Research indicates that individuals with autism exhibit reduced social motivation, as evidenced by a diminished preference for faces (Chita-Tegmark, 2016), that begins early in development (Chawarska et al., 2013) and persists into adulthood (Frazier et al., 2017).

Furthermore, individuals with autism tend to have shorter sustained attention to faces than typically developing controls (Major et al., 2022). However, the preferences of individuals with PWS regarding social versus non-social stimuli have remained unexplored, and whether they exhibit the "face pop" effect is unknown. This gap in the literature highlights the need for further investigation into social motivation and social preferences within PWS.

4.1.6 The current study

The overarching aim of this study was to investigate social motivation in PWS by measuring preference for social stimuli using a passive viewing eye tracking paradigm and to investigate if social motivation is related to autism severity within participants. The aims and hypothesis were as follows;

1. The first aim was to investigate if individuals with PWS automatically orient to social stimuli compared with an age and gender-matched comparison group using a well-established passive viewing paradigm.
2. The second aim was to compare preference for social stimuli versus non-social stimuli between individuals with PWS and the comparison group. I hypothesised that the PWS group would exhibit a reduced interest in faces compared to the comparison group.
3. The third aim was to investigate differences in preference for social stimuli based on genetic subtypes within the PWS group. I hypothesised that participants with the mUPD subtype, who are reported to have a higher prevalence of autism traits, would exhibit a reduced interest in faces compared to participants with the DEL subtype.
4. The final aim was to examine the relationship between preference for social stimuli and autism severity and social functioning in the PWS. I hypothesised that reduced interest in faces would correlate with greater autism symptom severity and poorer social functioning.

4.2 Methods

4.2.1 Participants and Clinical Assessments

Twenty-seven participants with PWS and 27 COM participants were recruited to the study (see section 2.1.3 for inclusion and exclusion criteria). Each participant underwent a clinical research assessment outlined in Chapter 2, section 2.2.1. Social functioning was assessed using the Vineland Adaptive Behaviour Scales, Second Edition (VABS-II) and the total score from the Social Communication Questionnaire (SCQ). Autism behaviours within the PWS group were quantified using calibrated severity scores from the ADOS-II, and subscales from the ADI-R, including reciprocal social interaction total, communication and language total, and restricted & repetitive behaviours. For cognitive assessment, participants under six underwent evaluation using the Mullen Scales of Early Learning (MSEL), while older participants had their IQ assessed using the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II). The Face Pop Task developed by Gliga et al. (2009) was employed to measure attention to social stimuli. This

task utilises free viewing eye tracking and examines participants' automatic orientation to faces and their preference for looking at faces compared to non-social stimuli. Detailed information about the task and data collection procedure can be found in Chapter 2, specifically sections 2.3.2 and 2.3.3.

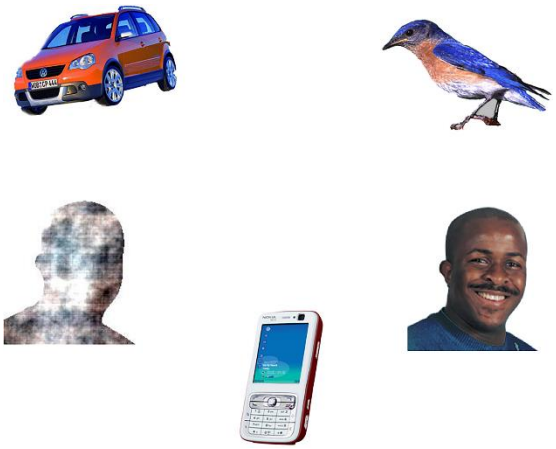

Figure 4.1: A sample stimulus array from the Food Pop Task (Gliga et al., 2009)

4.2.2 Data Analyses

Participants were required to have a minimum of three valid trials to be included in the analysis. A trial was considered valid if two conditions were met: firstly, the participant directed their gaze towards the stimulus array for more than 5000ms out of the 10000ms duration, and secondly, the proportion of valid samples exceeded 50%. In other words, the eye tracker successfully captured and recorded the participant's eye location more than 50% of the times the eyes were sampled—the Tobi X2-60 eye tracker used in the study samples at a rate of 60 times per second. To maintain group comparability, three comparison participants matched in age and gender were excluded from the analyses. For genetic subtype analyses where differences were examined between the DEL and mUPD groups, two participants were excluded as one participant had the third genetic subtype, and one participant's genetic subtype was unconfirmed.

The proportion of valid trials in which the participant orientated towards the face AOI first was calculated for each group (COM, PWS) and genetic subtype (DEL, mUPD) to investigate the "face pop effect". Four one-way t-tests with the Fisher's Least Significant Difference (LSD) correction were run to investigate if the participant orientated to the face more than expected by setting a chance level of 0.2, as there were five stimuli within each array. Two dependent variables were selected to measure preference for social stimuli. Proportional dwell time to the face AOI represents the time a participant spent looking at a face relative to non-social stimuli. Average look duration to the face indicates how faces maintain a participant's attention once they have noticed it. Investigating proportional dwell time and average look duration provides a more comprehensive understanding of attentional preferences. Proportional dwell time reveals the

relative allocation of attention, indicating which stimuli or areas are preferentially attended to, while average look duration provides insights into the temporal aspects of attention, revealing engagement with specific stimuli.

Linear mixed models were used to analyse the effect of group (PWS and COM) and AOI (face, car, phone, noise, bird) on the two dependent variables, proportional dwell time and average look duration. As previously described in Chapter 2, section 2.5, a maximal-that-improves-fit approach was used to construct the model with IQ and Age as covariates. Linear mixed models were also used to analyse the effect of genetic subtype (DEL and mUPD) and AOI (face, car, phone, noise, bird) on proportional dwell time and average look duration. Autism severity scores from the ADOS were included as an additional fixed effect in both these models to investigate how autism severity may impact proportional dwell time and average look duration for each AOI with IQ and age included as covariates. Spearman's rank correlations were used to investigate the associations among the primary variables of interest and phenotypic characteristics with and without controlling for IQ and age. Spearman's rank correlations were used to investigate the associations among the primary variables of interest and phenotypic characteristics with and without controlling for IQ and Age.

4.3 Results

4.3.1 Demographics and Clinical Characteristics

Data analysis included 24 out of the initial 27 participants with PWS. One participant with PWS did not complete the task, while two were excluded due to insufficient eye tracking data quality. The demographic and clinical characteristics of the PWS and comparison groups are presented in Table 4.1. Age and BMI did not differ statistically between the two groups. Statistically significant differences were observed in IQ and social functioning. Table 4.2 provides a more detailed breakdown of the genetic subtypes within the PWS group and additional information obtained from autism assessments (ADOS-II and ADI-R). It should be noted that the total number of participants in the PWS group decreased from 24 to 22, as one participant had a rarer subtype of PWS (imprinting centre defect), and one participant's genetic subtype was unknown. Analysis comparing the two genetic subtypes of PWS (DEL vs mUPD) did not reveal any significant differences. A detailed breakdown of participants based on their autism diagnoses as determined by the ADOS-II and ADI-R can be seen in appendix 5. On the ADOS-II, 32% of the participants (7 participants: 2 DEL and 5 mUPD) met the cut-off for an Autism diagnosis. On the ADI-R, 23% of the participants (5 participants: 2 DEL and 3 mUPD) met the criteria for an Autism diagnosis. When considering the agreement between the ADOS-II and ADI-R, 23% of the participants (5 participants: 2 DEL and 3 mUPD) met the criteria for autism on both instruments.

Table 4.1: Demographic and clinical characteristics of participants

	COM	PWS	t	*p*

	(n=24; 10:14 F:M) *Mean (SD)*	(n=24; 15:9 F:M) *Mean (SD)*		
Age	14.5 (8.8)	16.6 (9.0)	-1.6	.107
IQ[a]				
Verbal IQ	98.3(21.5)	72.2 (12.2)	4.8	<.001
Perceptual IQ	107.1 (19.2)	60.3 (14.4)	8.8	<.001
Full-Scale IQ	105.0 (12.1)	64.7 (12.3)	10.7	<.001
Body Mass Index	20.7 (7.0)	21.5 (7.1)	0.6	.556
VABS-II[b]				
Communication Std Score	101.8 (13.6)	62.9 (20.8)	7.7	<.001
Daily Living Domain	99.4 (6.9)	69.9 (13.4)	9.6	<.001
Socialisation Std Score	109.1 (14.2)	74.1 (23.5)	6.2	<.001
SCQ[c]	1.3 (1.6)	9.1 (7.2)	-5.2	<.001

Table 4.2: Characteristics of PWS participants by genetic subtype

	PWS Deletion (n=11; 7:4 F: M) *Mean (SD)*	PWS mUPD (n=11; 7:4 F: M) *Mean (SD)*	t	*p*
Age	17.7 (7.8)	18.6 (10.3)	.128	.899
IQ[a]				
Verbal	70.1 (9.6)	76.4 (14.9)	-1.24	.239
Perceptual	64.2 (13.1)	58.2 (15.8)	.935	.366
Full-Scale IQ	64.9 (10.1)	66.5 (15.1)	-.318	.756
Body Mass Index	21.8 (8.4)	21.3 (6.3)	.013	.990
VABS-II[b]				
Communication Std Score	60.8 (16.4)	66.8 (24.6)	.673	.509
Daily Living Domain	68.9 (6.7)	72.2 (19.2)	.538	.596.
Socialisation Std Score	75.3 (23.6)	74.3 (18.0)	.112	.912
SCQ[c]	10.7 (6.1)	9.2 (8.0)	.509	.616
ADOS CSS[d]	2.9 (1.3)	3.6 (2.4)	-.890	.928

ADI[e]				
Reciprocal social interaction	9.5 (5.7)	10.5 (7.9)	-.341	.737
Communication & language	9.1 (4.8)	7.7 (4.7)	.675	.507
Restricted & repetitive behaviours	2.4 (1.6)	2.6 (1.8)	.379	.709

[a]Wechlser Abbreviated Scales of Intelligence, [b] Vineland Adaptive Behaviour Scales, [c] Social Communication Questionnaire, [d]Autism Diagnostic Observation Scale, [e] Autism Diagnostic Interview

4.3.2 The proportion of first looks to the face AOI (Face Pop Effect)

To investigate if PWS participants exhibit a face pop effect, i.e., they automatically orientated to faces, the proportion of valid trials in which the participant looked towards the face AOI first was calculated for each group (COM, PWS) and genetic subtype (DEL, mUPD) (Table 4.3). One sample t-tests showed that the proportion of trials with first looks towards the face AOI was significantly above chance level (.2) for both COM and PWS groups and both DEL and mUPD genetic subtypes (all $p < 0.001$). No significant differences were found between the participant groups (PWS vs COM, $p = 0.17$) or genetic subtypes (Del vs mUPD, $p = .47$) (see Figure 4.2).

Table 4.3: Proportion of first looks for both each group and genetic subtype

		Group		Genetic Subtype	
		COM Mean (SD)	PWS Mean (SD)	DEL Mean (SD)	mUPD Mean (SD)
Area of Interest	Face	.62 (.22)	.73 (.21)	.75 (.20)	.73 (.24)
	Noise	.09 (.08)	.08 (.11)	.09 (.10)	.05 (.11)
	Bird	.11 (.11)	.05 (.10)	.04 (.06)	.06 (.13)
	Car	.12 (.15)	.07 (.11)	.06 (.11)	.08 (.12)
	Phone	.06 (.09)	.07 (.10)	.07 (.08)	.08(.12)

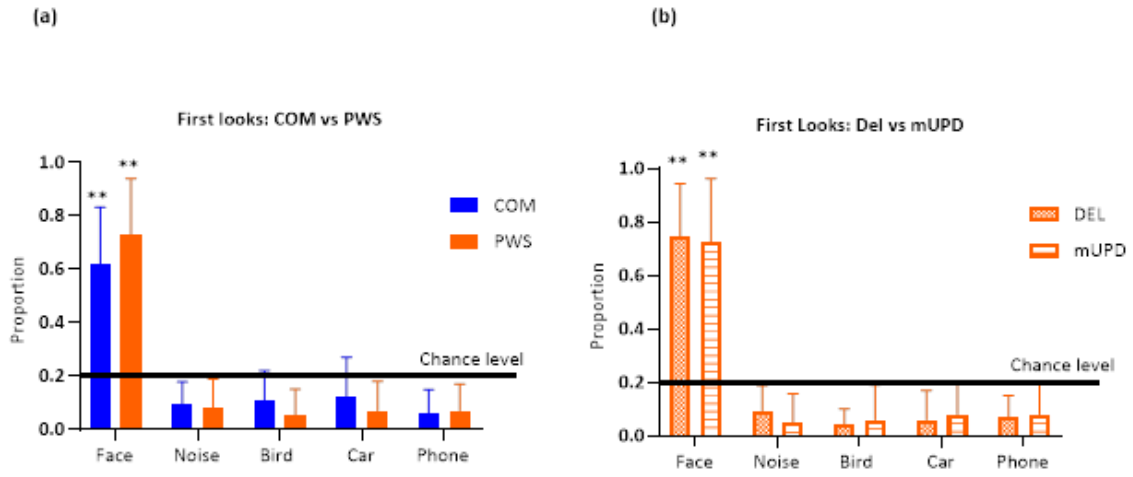

Figure 4.2: Proportion of first looks to each AOI for (a) participant groups (PWS and COM) and (b) genetic subtypes. The line in bold represents the chance level and ** p <.001 for the one-way t-test against chance level.

4.3.3 Group differences in preference for social stimuli

A linear mixed effects analysis was conducted with proportional dwell time as the dependent variable to examine if the PWS participants group looked at faces relatively less than the comparison group. Fixed factors included group (PWS vs COM) and AOI (face, car, phone, noise, and bird). Initially, the model included a random factor for participant slope, but the model failed to converge. Therefore this random factor was subsequently removed. IQ and age were included as covariates (see Table 4.4). The analysis revealed a significant main effect of Group; $F(1, 1554) = 15.4$, $p < .001$ but no significant main effect of AOI; $F(4, 1554) = 1.4$, $p = .230$. There was a significant two-way interaction between the AOI category and group, $F(4, 1554) = 47.7$, $p < .001$ (see Table 4.4). Post hoc analyses were conducted on the Group*AOI interaction to understand group differences across AOI categories, with multiple comparisons adjusted using the least significant difference (LSD) correction. The PWS group exhibited a significantly greater amount of time looking at the face AOI compared to the COM group (Mdiff = -0.12, $p < .001$, 95% CI [-.15, -.07]). The COM group spent a significantly higher proportion of time looking at all other AOIs apart from the phone AOI which showed no significant difference between the two groups: Bird AOI; Mdiff = .07, p =<.001, 95% CI [.03, .12], Car AOI; Mdiff = .07, p = <.001, 95% CI [.03, .10], Noise AOI; Mdiff = .06, p = 0.003, 95% CI [.02, .09], Phone AOI; Mdiff = 0.03, p = .08, 95% CI [-.00, 07] (see Figure 4.3a). In assessing model assumptions, linearity was confirmed by proportional response changes to different Areas of Interest. Multicollinearity was ruled out with a VIF below 10 in a linear regression model. Homoscedasticity was supported by similar variance seen in residuals' box plots for PWS and COM groups and Assessed by Levene's test for equality of variances $F(1, 1690) = .510$, $p = .475$. However, residuals did not meet the normality assumption, prompting a square root transformation which ensured model robustness. The transformed model yielded consistent conclusions, validating the retention of the original analysis while noting non-normal residual distribution.

Table 4.4: Fixed and Random Effects for proportional dwell time comparing PWS and comparison group

Fixed effects			
	Estimate (Std. error)	*95 % CI*	*p*
Intercept	.19 (.05)	[.10, .29]	<.001
Group	.08 (.02)	[.03, .12]	<.001
AOI	.01 (.01)	[-.01, .02]	.230
Group*AOI	-.03 (.00)	[-.04, -.02]	<.001
Age	-.00 (.00)	[-.00 , .00]	.204
IQ	.00 (.00)	[-.00 , .00]	.912
Random effects			
	Variance (Std. error)	*95% CI*	*p*
Residual	.02 (.00)	[.01, .02]	<.001

Number of data points = 1815; participants = 48 (24 PWS and 24 COM).
Degrees of freedom estimation: Satterwaitte

To examine if PWS participants exhibited reduced engagement with faces compared to comparison participants, a linear mixed effects analysis was conducted with average look duration as the dependent variable. Fixed factors included group (PWS vs COM) and AOI (face, car, phone, noise, and bird). The model included participant slope as a random factor, and IQ and Age were included as covariates (see Table 4.5). There was no main effect of group; $F(1, 39.38) = 3.76$, $p = .722$) or AOI; $F(1, 1401.49) = .03$, $p = .872$. The two-way interaction between the AOI category and the group was significant, $F(1, 1404.91) = 7.48$, $p = .006$. Although a positive interaction effect was found, the individual pairwise comparisons did not reach statistical significance after applying LSD correction. No significant difference in average look duration between the PWS and COM was seen for the face AOI; Mdiff = -.09, p =.451, 95% CI [-.34, .15], Bird AOI; Mdiff = .14, p =.26, 95% CI [-.11, .39], Car AOI; Mdiff = -.173, p =.162, 95% CI [-.07, .42], Noise AOI; Mdiff = .136, p =.268, 95% CI [.11, .38] and Phone AOI; Mdiff = .12, p =.122, 95% CI [-.15, .34] (see figure 4.3b).

Table 4.5: Fixed and Random Effects for average look duration comparing PWS and comparison group.

Fixed effects			
	Estimate (Std. error)	*95 % CI*	*p*
Intercept	.68 (.35)	[-.03, 1.38]	.060
Group	.04 (.12)	[-.20, .29]	.722
AOI	.00 (.02)	[-.04, .05]	.872
Group*AOI	-.04 (.02)	[-.08, -.01]	.006
Age	-.00 (.00)	[-.01, .01]	.885
IQ	.00 (.00)	[-.00 , .01]	.772
Random effects			
	Variance (Std. error)	*95% CI*	*p*
Residual	.19 (.01)	[.01, .02]	<.001
Intercept (Participant)	.02 (.01)	[.01, .04]	<.001

Number of data points = 855; participants = 48 (24 PWS and 24 COM).
Degrees of freedom estimation: Satterwaitte.

4.3.4 Genetic subtype difference in preference for social stimuli

To examine if mUPD participants exhibited reduced proportional dwell time with faces compared to DEL participants, a linear mixed effects analysis was conducted with proportional dwell time as the dependent variable. Fixed factors included genetic subtype (DEL vs mUPD), AOI (face, car, phone, bird, and noise) and to account for autism severity, autism severity scores (ADOS-CSS) were included in the model. Two interactions were added to the model: genetic subtype*AOI and ADOS-CSS*AOI (Table 4.6). Initially, the model incorporated the participant slope as a random factor, but the model failed to converge, so the participant slope was removed. Additionally, IQ and Age were included as covariates in the analysis. A significant main effect was seen for AOI, $F(1, 642) = 16.2$, $p < .001$, with both subtypes showing a longer dwell time for the face AOI on average compared to all other AOIs (see figure 4.3c). No main effect was seen for genetic subtype; $F(1, 642) = .002$, $p = .96$ or ADOS-CSS scores; $F(1, 642) = .35$, $p = 53$. No significant interaction effects were found between genetic subtype and AOI $F(1, 642) = .39$, $p = .56$ or ADOS-CSS and AOI; $F(1, 642) = 1.13$, $p = .29$ (see Figure 4.2c).

Table 4.6: Fixed and Random Effects for proportional dwell time comparing DEL and mUPD Participants.

Fixed effects			
	Estimate (Std. error)	*95 % CI*	*p*
Intercept	.41 (.07)	[.29, .56]	<.001
Genetic Subtype	.00 (.03)	[-.06, .06]	.963
AOI	-.06 (.02)	[-.09, -.03]	<.001
ADOS-CSS	-.01 (.01)	[-.02, .01]	.533
Genetic Subtype*AOI	-.01 (.00)	[-.02, .01]	.555
ADOS-CSS*AOI	-.01 (.01)	[-.00, .01]	.285
IQ	.00 (.00)	[-.00, .01]	.661
Age	-.00 (.00)	[-.00, .01]	.074
Random effects			
	Variance (Std. error)	*95% CI*	*p*
Residual	.19 (.01)	[.01, .02]	<.001

Number of data points = 1815; participants = 22 (11 DEL and 11mUPD).
Degrees of freedom estimation: Satterwaitte.

First, a linear mixed model with dependent average look duration and fixed factors genetic subtype (DEL vs mUPD), AOI (face, car, phone, bird, and noise and autism severity scores (ADOS-CSS) were included. The model added two interactions: genetic subtype*AOI and ADOS-CSS*AOI to see if these factors interacted with any AOI in particular, e.g., the face AOI. Initially, the model incorporated the participant slope as a random factor, but the model failed to converge, so the participant slope was removed. Additionally, IQ and Age were included as covariates in the analysis (Table 4.7). No main effect was seen for the genetic subtype; F(1, 538) = .1,86, p = .17. A significant main effect was seen for AOI, F(1, 538) = 6.98, p =.008, with both subtypes showing a higher average look duration for the face AOI compared to all other AOIs (see figure 4.3b). A main effect was seen for autism severity; F(1, 538) = 9.6, p = .002, with higher autism severity scores showing reduced average looking time to AOIs. No interaction effects were observed between genetic subtype and AOI F(1, 538) = .001, p = .97 or ADOS-CSS and AOI; F(1, 538) = 2.37, p = .12. It is important to note that IQ also was having an effect on average look duration; F(1, 538) = .07, p = .023, suggesting main effects were not independent of IQ. To gain further insight into the influence of IQ on the model, a new model was rerun, excluding the covariate IQ and compared to the new model using Akaike Information Criterion (AIC). In the new model, all main effects retained the same significance apart from autism severity (ADOS-CSS) which failed to retain statistical significance in the new model F(1, 661) = 3.5, p =.06. Comparing the fit of the new model, the AIC value significantly worsened. This

implies that the revised model did not provide a better fit to the data than the original model, supporting the inclusion of IQ as a covariate.

Table 4.7: Fixed and Random effects for average look duration comparing DEL and mUPD participants

Fixed effects			
	Estimate (Std. error)	*95 % CI*	*p*
Intercept	1.5 (.20)	[1.18, 1.99]	<.001
Genetic Subtype	-.09 (.03)	[-.30, .06]	.173
AOI	-.14 (.02)	[-.24, -.04]	.008
ADOS-CSS	-.09 (.03)	[-.16, - .04]	.002
Genetic Subtype*AOI	-.13 (.09)	[-.06, .05]	.971
ADOS-CSS*AOI	-.01 (.01)	[-.00, .04]	.124
IQ	.00 (.00)	[-.01, -.00]	.023
Age	-.00 (.00)	[-.01, .00]	.789
Random effects			
	Variance (Std. error)	*95% CI*	*p*
Residual	.19 (.01)	[.01, .02]	<.001

Number of data points = 855; participants = 22 (11 DEL and 11mUPD).
Degrees of freedom estimation: Satterwaitte.

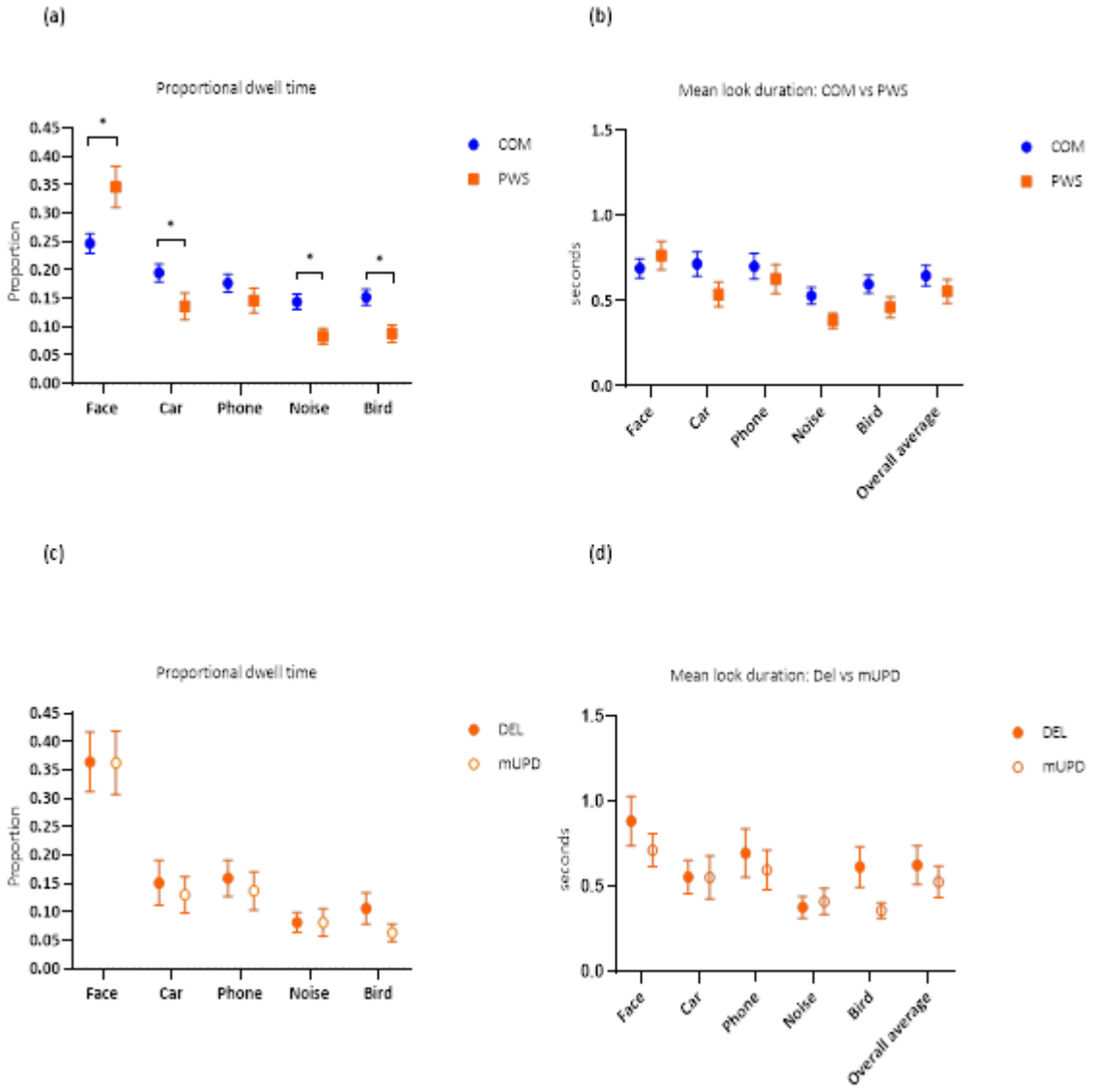

Figure 4.3: Proportional dwell time for AOI categories across (a) participant groups and (c) genetic subtypes. Average look duration for (b) participant groups and (d) genetic subtypes. Error bars correspond to 95% Cis. *p <. 05, ** p <.01

4.3.5 Association between eye tracking metrics and social profiles

The associations between the eye tracking variables (PDF for face, average look duration for face, and average look duration for all AOIs) and phenotypic characteristics (SCQ, IQ, VABS, ADOS) were analysed using Spearman's ranked correlations. SCQ or IQ scores did not correlate significantly with eye tracking measures for the PWS or COM groups (see Figure 4.4a-f). In the PWS participants, the VABS socialisation standard score significantly correlated with the average look duration for all AOIs (r_s= .58, p = .008) (see Figure 4.4i). The ADOS CSS scores were significantly negatively correlated with mean duration for face AOI (r_s= -.50 p = 0.015) and mean duration for all AOIs (r_s= -.41 p = 0.050) (see figure 4.4j-l).

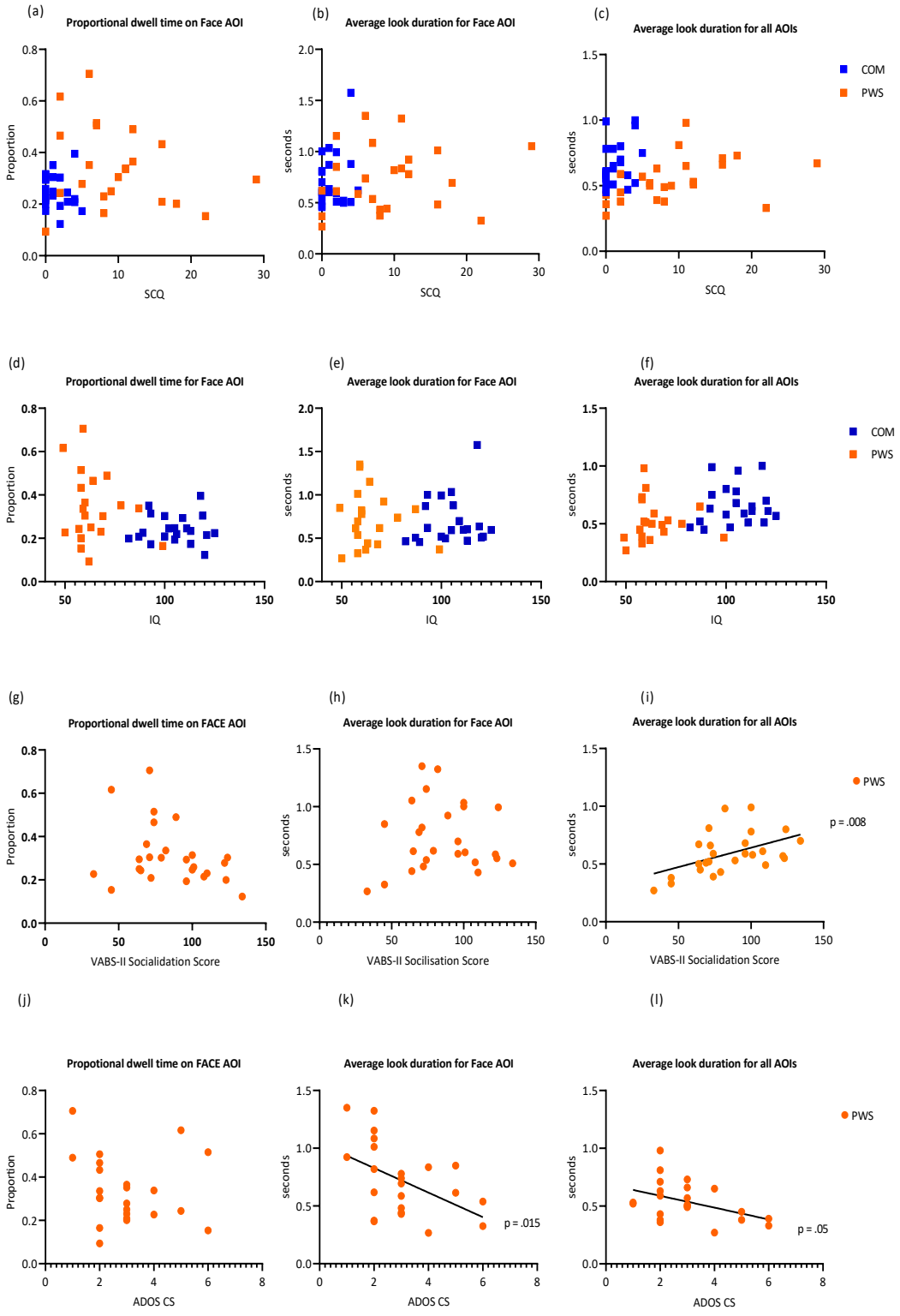

Figure 4.4: Scatterplots of eye tracking variables (PDT to face, average look duration for face and average look duration overall) plotted against SCQ (a-c), IQ (d-f), VABS-II (g-i) and ADOS CSS scores (j-l).

4.4 Discussion

This chapter investigated social motivation in PWS by examining preferences for social stimuli and their relationship with autism and social functioning using a well-established passive viewing paradigm. To the best of my knowledge, this is the first study to investigate preference for social stimuli compared to non-social stimuli in PWS. Individuals with PWS exhibited a distinct face pop response, indicating attentional capture by faces, similar to controls of the same age and gender. Unexpectedly, the PWS group showed a significantly greater preference to look at faces compared to the comparison group, as indicated by proportional dwell time, and there were no differences in face preference between the DEL and mUPD genetic subtypes. Furthermore, individuals with PWS with shorter average look durations towards faces had higher scores on the ADOS-CSS, suggesting that reduced sustained attention to faces was associated with increased autism severity. Longer average look durations across all areas of interest (AOIs) were linked to better socialisation skills (measured by VABS scores) and lower autism severity scores.

The PWS group showed a higher proportion of time looking at the face AOI, while the comparison group allocated more attention to all other AOIs except for the phone AOI. However, no significant main effects of group or AOI were found in the analysis of average look duration. The significant interaction effect between the AOI category and group indicates differences in engagement patterns between the PWS and comparison groups, although the individual pairwise comparisons did not reach statistical significance after correction. The differential findings between proportional dwell time and average look duration in the PWS and comparison groups may be explained by several factors. Individuals with PWS may have a heightened initial attentional capture or preference for faces, reflected in the proportional dwell time measure. However, the sustained attention or duration of engagement with faces, as measured by average look duration, may be similar between the two groups. Additionally, individuals with PWS may have shorter but more frequent gazes towards faces, resulting in a comparable average look duration despite allocating more overall time to faces (Holmqvist et al., 2011).

These findings contrast with studies conducted in individuals with autism, which have consistently demonstrated a reduced preference for faces compared to non-social stimuli (Elison et al., 2012; Klin et al., 2015; Sasson et al., 2008a, 2008b; Tsang et al., 2022; Del Bianco et al., 2021). Although limited research specifically examines preference for social stimuli in individuals with PWS, previous studies have demonstrated that individuals with PWS exhibit slower processing speeds in identifying facial expressions, as indicated by their performance in emotion-matching tasks (Debladis et al., 2019). The increased time spent looking at faces in the PWS group may reflect reduced speed in processing facial information. However, if participants with PWS processed faces slower, it would follow that the PWS group would have sustained attention to faces for longer. Nevertheless, average look duration did not significantly differ from comparison participants, suggesting a preference for looking at faces rather than a result of slower processing. This finding contradicts the social motivation theory in the context of PWS broadly, as the preference for social stimuli appears preserved. This suggests that challenges in social functioning in individuals with PWS are unlikely to be attributed solely to a reduced preference for social stimuli.

Surprisingly, the findings of this study did not reveal significant differences in the proportional dwell time for faces between the mUPD and DEL subtypes of PWS, which contradicts the initial hypothesis. It was anticipated that the mUPD subtype, known for a higher incidence of autism diagnosis and traits, would exhibit reduced interest in faces similar to individuals with autism (Tsang et al., 2022). Furthermore, it was hypothesised that the mUPD subtype of PWS would show decreased average look duration across all areas of interest (AOIs) based on recent investigations in autism cohorts (Tsang et al., 2022). While genetic subtype did not have a significant effect on the model, higher autism severity scores did, which is consistent with reports in the field of autism, which consistently demonstrate reduced average look duration in individuals with autism (Major et al., 2022; Tsang et al., 2022). These findings also align with Del Bianco and colleagues (2021), who also identified significant differences in temporal dynamics between the autism and comparison groups. The latter showed a prolonged engagement with faces, potentially indicating further successive components of social attention after the initial attention-grabbing effect had diminished. In contrast, the autistic group exhibited a substantial reduction in attention after the initial orientation to faces, suggesting the involvement of different attentional components influenced by factors like motivation, relevance, and experience (Orquin et al., 2017). It was observed in the present that IQ had a notable influence on average look duration, making it challenging to separate the individual contributions from IQ and autism severity and understand how they specifically contribute to the preference for social stimuli.

This is the first study to investigate preference for social stimuli compared to non-social stimuli in PWS. The current study's findings deviate from the established patterns observed in social cognition studies in individuals with PWS, which have identified genetic subtype differences. Prior studies in PWS have examined face processing using eye tracking and electrophysiological EEG approaches. Notably, (Debladis et al., 2019) reported variations in face processing, specifically in attention allocation to the eyes versus the mouth. Their findings indicated that individuals with the DEL subtype tended to focus more on the eyes, while those with the mUPD subtype directed their attention more towards the mouth, resembling patterns typically observed in individuals with autism (Chita-Tegmark, 2016; Guillon et al., 2014). In addition, two EEG studies examining the N170 component, an EEG response associated with face processing, demonstrated variations in both the amplitude and latency of the N170 among individuals with PWS. These findings suggest that there are genetic subtype differences in how facial stimuli are processed. Possible explanations for the absence of significant differences in the current study could be that social interest in faces remains preserved in both PWS subtypes, but attention to facial features differs, indicating differences in face processing. Alternatively, it is also plausible that the study may have been underpowered to detect subtle differences, given the level of heterogeneity within the cohort.

This study is the first investigation into the relationship between social cognition, specifically social motivation and its relationship to autism and social functioning in individuals with PWS. The initial hypothesis proposed was that reduced interest in faces would correspond to higher autism severity scores and impaired social functioning. However, no significant correlations were found between proportional dwell time on faces and measures of autism severity or social functioning. On the other hand, average look duration towards faces did show an association with autism severity as measured by ADOS-CSS. Using the face pop task in an autism cohort,

Tsang and colleagues (2022) also demonstrated an inverse relationship between average look duration to faces and ADOS-CSS scores. Average look duration for all AOIs in the task was positively related to autism severity and reduced social functioning as measured by ADOS-CSS scores and VABS-II. This suggests that higher autism severity and lower social functioning were associated with reduced time spent looking at social and non-social stimuli, indicating a potential overall reduction in visual attention. Recent research has highlighted the significance of average look duration as a measure of visual attention in autism, where lower average look duration for both social and non-social stimuli was linked to increased autism traits, difficulties in social communication abilities, and social withdrawal (Major et al., 2022). This finding suggests that average look duration and the ability to sustain focus on stimuli may be important in further investigations to understand the neurocognitive characteristics of autism within the context of PWS. Based on these findings, it is plausible to consider average look duration as a potential neurocognitive marker of autism within the PWS population, which could aid in distinguishing autism from the characteristic behavioural phenotype observed in PWS.

Further research is warranted to explore the utility of average look duration as a valuable assessment tool in this context. Recognition of autism-like characteristics in individuals with genetic syndromes like PWS is crucial to ensuring that individuals receive appropriate behavioural management and educational placement (Moss et al., 2009). A neurocognitive biomarker could assist in overcoming challenges in distinguishing between superficial similarities of autism and PWS, as well as in cases where a person may have both co-occurring PWS and autism.

The current study has several notable strengths. Firstly, it is the first investigation to examine preference for social stimuli in individuals with PWS and explore its association with autism using a well-validated paradigm. The chosen paradigm has been employed in large-scale, international research initiatives such as the EU-AIMS Longitudinal European Autism Project (LEAP) and the Autism Biomarker Consortium—Clinical Trials (ABCCT), lending credibility to its effectiveness (Loth et al., 2017; Tillmann et al., 2019). This enabled data collection across a wide range of ages and cognitive abilities, resulting in more representative findings encompassing the syndrome's heterogeneity. Additionally, the passive viewing nature of the paradigm promoted inclusivity, allowing individuals with varying cognitive abilities to participate in the study. Notably, the exclusion rate in the current study was only 11%, which compares favourably to previous eye tracking studies in PWS, where exclusion rates were approximately 33% (Debladis et al., 2019).

A potential limitation of the present study is using static images rather than dynamic stimuli, which may raise concerns regarding ecological validity. However, static images allowed for precise control over low-level properties of the social and non-social stimuli, such as visual angle, luminance, contrast, intensity, and orientation (Holmqvist et al., 2011). Individuals with autism have been found to exhibit distinct visual information processing patterns compared to typically developing peers, including enhanced performance on detail-oriented tasks and attention differentially driven by low-level stimulus properties (Amso et al., 2014). By matching the social and non-social stimuli on these features, the present study aimed to ensure that any observed differences in attention between groups were not solely attributed to low-level processing disparities. More complex visual stimuli may have compromised the degree of

salience matching, representing a trade-off between potential ecological validity and experimental control.

A limitation of the present study is that psychiatric symptoms, often elevated in individuals with PWS and more prevalent in the mUPD subtype, may have also contributed to atypical visual scanning patterns. Studies involving participants with psychosis, for instance, have demonstrated altered visual scanning characterised by prolonged fixation on irrelevant or non-social stimuli, reduced exploration of the visual field, and decreased attention to socially salient cues such as faces and eyes (Wolf et al., 2021). Future investigations utilising visual attention to explore neurocognition should consider incorporating measures of psychosis as covariates. This inclusion will enable a more comprehensive analysis and provide insights into the potential influence of psychosis on visual attention and its impact on neurocognitive processes. Another limitation of the current study is the relatively small sample size for the genetic subtype analysis, despite being the first to include children and adults with PWS. The power to detect differences between genetic subtypes was reduced in this study compared with the whole group comparison with controls, further supporting the importance of a larger sample to elucidate mean differences in gaze behaviour towards social stimuli between genetic subtypes. These limitations highlight potential avenues for future research to address these concerns and further advance our understanding of social motivation and visual processing of social stimuli in PWS and related conditions.

One of the primary challenges encountered in the present study is the notable heterogeneity observed within the PWS group, despite being selected based on the presence of a genetic syndrome. PWS has significant clinical and genetic heterogeneity (Schwartz et al., 2021). While it is a clinically recognisable syndrome, individuals with PWS exhibit a broad spectrum of behavioural phenotypes, as detailed in Chapter 3. Although certain characteristics such as hyperphagia, repetitive questioning, and skin picking are commonly observed, the clinical presentation within the PWS population can vary considerably, introducing additional "noise" into the data. This heterogeneity poses challenges in accurately characterising and interpreting the findings, as individual differences within the PWS group may contribute to variability in eye tracking patterns and attentional processes. During the analysis, a significant challenge arose when examining the factors impacting average look duration across different genetic subtypes. It was observed that both autism severity and IQ had a notable influence on average look duration, making it challenging to separate their individual contributions and understand how they specifically contribute to the preference for social stimuli. However, a study focusing on participants with autism revealed that average look duration was correlated with autism traits rather than IQ, indicating that autism traits may have a stronger association with average look duration for social stimuli (Major et al., 2022). Future studies will greatly benefit from collaborative efforts to increase statistical power in the analysis, enabling the investigation of heterogeneity within the PWS population and the exploration of potential subgroups or phenotypic profiles. This approach will enhance our understanding of the underlying mechanisms and implications for social cognition and visual scanning in PWS.

In conclusion, this was the first examination of social motivation in individuals with PWS and provided novel insights into the preference for social stimuli in PWS. The findings showed that individuals with PWS exhibited a distinct attentional capture by faces, similar to controls, and

had a greater preference for looking at faces than the comparison group. Reduced sustained attention to faces was associated with increased autism severity, while longer average look durations were linked to better socialisation skills and lower autism severity. Future research will benefit from increasing sample sizes and replicating these findings in larger samples to gain further insights into the role of social cognition in autism behaviours. Interventions that target social functioning and autism behaviours are needed to improve the well-being and quality of life of individuals with PWS. A deeper knowledge and understanding of the neurocognitive underpinnings of these behaviours will allow for more targeted and effective treatments.

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Chapter 5: The Food Attentional Bias Task: An eye tracking paradigm to measure the influence of food intake on attentional bias to food stimuli in a typically developing population

5.1 Introduction

In the last chapter, I demonstrated the value of examining neurocognitive factors to gain insights into autism behaviours in PWS. In this chapter, I will employ a similar methodological approach to understand the neurocognitive processes underlying hyperphagia—a prominent feature in the PWS profile that, like autism behaviours, significantly impacts well-being and quality of life in individuals with PWS.

5.1.1 Hyperphagia in PWS

Hyperphagia is an extreme drive to consume food accompanied by a lack of satiety and problematic food-related behaviours. It is observed in people with PWS. Constant vigilance is required regarding food exposure in those with hyperphagia, as uncontrolled or unsupervised access to food may result in obesity or even gastric rupture and death (Tan et al., 2020). Hyperphagia is, therefore, an important treatment target in obesity-related monogenic conditions. Treating hyperphagia is the highest priority for caregivers (Tsai et al., 2018), and a recent study conducted on young people with PWS reported that all participants were in favour of participating in future clinical trials of new medications that would curb their hunger (Dykens et al., 2021). There is an urgent need for objective, robust, reliable and reproducible treatment biomarkers to evaluate the effectiveness of new drug therapies in clinical trials.

5.1.2 Challenges in measuring hyperphagia

New drugs targeting the leptin-melanocortin signalling pathway are being developed for PWS, and their effectiveness in reducing hyperphagia is a key clinical endpoint (Baldini et al., 2019; Saeed et al., 2018). However, reliable measurement of hyperphagia is a challenge. Relying on physical measures such as Body Mass Index (BMI) fails to account for strict environmental control measures ('food security') used to manage hyperphagia. Individuals can present with a BMI within the healthy range and still be greatly affected by hyperphagia, preventing these individuals from being truly independent. Safety and ethical concerns have been raised in relation to food observation studies where participants are permitted unlimited access to food, and the food quantity consumed is measured as an indicator of hyperphagia (Heymsfield et al., 2014). These may also be influenced by social desirability bias as individuals with PWS tend to demonstrate socially desirable behaviours in a laboratory compared to community settings, reducing the effectiveness of direct behavioural observations of food consumption (Holland, Treasure, Coskeran, & Dallow, 1995). Several caregiver report questionnaires exist, e.g. the Food Related Problems Questionnaire (Russell & Oliver, 2003) and the Hyperphagia Questionnaire (HQ) (Dykens et al., 2007), which psychometrically sound measures. However, both these questionnaires are informant-report-based measures of hyperphagia that may be subject to respondent bias. For instance, a placebo was associated with a modest improvement

in hyperphagia as measured by the HQ in a recent randomised controlled pilot of intranasal oxytocin (Hollander et al., 2021).

5.1.3 Attentional bias to food stimuli

The PWS clinical trials consortium (PWS-CTC) identified new endpoint development for hyperphagia, including novel objective biomarkers using eye tracking, as an area for further development. Attention processes play a critical role in controlling eating behaviours and provide an alternative means to measure atypical satiety, which is responsible for appetitive feelings and frequency of eating (Drapeau et al., 2013). An attentional (AB) consists of the preferential allocation of attention to specific stimuli within the environment. Food cues tend to attract attention during eye tracking studies, particularly if the food is highly caloric and looks appetising (Castellanos et al., 2009; Nijs et al., 2010; Werthmann et al., 2015). This attention bias has been proposed as an adaptive mechanism during evolution that helped detect nutrient-dense foods in the environment, enabling adequate food intake and, consequently, survival (Potthoff & Schienle, 2020). A recent meta-analysis found attentional bias robustly negatively associated with proximal food intake but not BMI (Hardman et al., 2021). Another systematic review and meta-analyses found little evidence to show that food attentional bias differed in overweight/obese participants compared to healthy weight participants (Hagan et al., 2020). These results support the hypothesis that food-related attentional bias is not related to obesity or individual body weight but is instead related to satiety and the motivational value of food and is therefore influenced by factors related to satiety, such as proximal food intake, cravings, and hunger (Field et al., 2016; Hardman et al., 2021). Assessing the change in attentional bias to food from premeal to postmeal when participants experience satiety-related decreases in hunger and motivation to consume food may provide an objective and robust measure of satiety and, consequently, an absence of satiety in hyperphagia.

5.1.4 Eye tracking as a method of measuring attentional to food stimuli

AB can be measured directly and indirectly. Direct measurement approaches measure brain activity using techniques such as electroencephalography or eye movements using technology such as eye tracking in response to food cues. Indirect approaches measure reaction times to food cues using behavioural paradigms such as the Stroop or Dot Probe task (Hagan et al., 2020). In the dot-probe task, a pair of cues (food cue, neutral cue) is presented and followed by a probe that appears in place of either the food or neutral cue. Participants are asked to react to the probe quickly, typically with a key press. Faster reaction times to probes that replace food versus neutral stimuli indicate attentional bias toward food stimuli. Dot-probe tasks vary in the duration of cue presentation, which impacts interpretation (Field & Cox, 2008). In the emotional Stroop task, participants are presented with a series of food and neutral words that appear in different colours (Gotlib & McCann, 1984). Participants are instructed to name the colour of the words that appear and ignore the content of the words. For example, if the word "bread" is printed in green, the participant should say "green." Reaction time to name the colour of the word is recorded and used as an index of AB. Longer reaction times are thought to reflect greater attentional bias to the word's emotional content versus colour (Field & Cox, 2008).

Several studies have investigated the relationship between attentional bias and "satiety" induced by food intake, using indirect methods such as the Stroop and Dot Probe tasks.

However, these have poor reliability (Ataya et al., 2012; Channon & Hayward, 1990; Jonker et al., 2020; Rodebaugh et al., 2016; Stamataki et al., 2019). One study used direct and indirect approaches combining eye tracking and the Dot Probe task (Doolan et al., 2014), but this requires good language abilities to understand task instructions and the requisite cognitive capacity and motor skills to perform the task. This approach is unsuitable for the many individuals with syndromic or monogenic obesity who have co-occurring cognitive impairments. Passive viewing paradigms are non-invasive and accessible measurements that have demonstrable utility in research in autism and syndromes associated with intellectual disability to investigate ABs for social stimuli (Kong et al., 2022). They can be adapted to investigate ABs in other contexts for individuals with intellectual disabilities.

5.1.5 The Food Attention Bias task – a paradigm to measure attentional bias to food stimuli

The primary objective of this study was to develop a passive-viewing eye tracking task that could effectively assess changes in attentional bias for food stimuli before and after eating, thus serving as an indicator of satiety. Drawing from relevant literature recommendations, which emphasise the use of standardised open-access image databases (Hagan et al., 2021; Hardman et al., 2020), longer stimulus presentation times (≥3000 ms) (Van Ens et al., 2019), and capturing within-subject fluctuations in attentional bias (Hardman et al., 2021), we developed the Food Attentional Bias (FAB) paradigm. The FAB paradigm incorporated stimuli arrays of images from the Food Pics Database (Blechert et al. 2019), a carefully curated and standardised collection of food images designed for food-related research. These images represent various food categories and are standardised in size, resolution, and visual attributes, ensuring consistency and comparability across different studies. By employing this standardised resource, researchers can draw more reliable and valid conclusions from the collective body of research in this area. Each stimulus within the FAB paradigm was displayed for an extended duration of 10,000 ms to capture top-down attentional processing of the stimulus array, hypothesised to have higher test-retest reliability in attentional bias studies (Van Ens et al., 2019). The primary aim of the paradigm was to measure and compare attentional bias for food stimuli across two meal conditions: premeal and postmeal, enabling the assessment of within-subject changes in AB. Furthermore, this study sought to validate the FAB task in a sample of healthy-weight young adults, focusing on three objective eye tracking measures: duration of gaze fixation, number of gaze fixations (indicative of directed attention), and time to first fixation for food stimuli compared to non-food stimuli. By examining these eye tracking measures, the study aimed to investigate the FAB task as an effective tool for assessing attentional bias towards food stimuli in satiety. A second objective of this study was to identify potential order effects within the FAB task paradigm concerning meal condition order. Order effects refer to potential biases or influences from the sequence in which different conditions are presented in a study. In the context of the FAB task, order effects may arise if the premeal or postmeal condition systematically influences participants' responses or ABs, regardless of the effect of satiety. To address this, a second group of participants was recruited, and the study was conducted with counterbalanced meal conditions. Specifically, the post-meal condition was administered before the premeal condition to mitigate any potential influence of order effects.

5.1.6. Aims and Hypotheses

The primary aim of this chapter was to develop and test a novel eye tracking paradigm, the FAB task, specifically designed to assess variations in visual attention towards food stimuli in typically developing participants across hunger and satiety conditions. I hypothesised that, in a healthy-weight population, the total duration and number of fixations on food stimuli, compared to non-food stimuli, would decrease following a meal (hungry state) compared to before a meal (satiated state). Additionally, I hypothesised that participants would exhibit faster identification of food stimuli in an array during the premeal condition than in the postmeal condition as measured by time to first fixation within a food stimulus area of interest (AOI).

The secondary aim of the study was to investigate potential order effects within the FAB task regarding meal conditions. To address this, a second group of participants was recruited and underwent the task in reverse order. I hypothesised that the findings from the second group would demonstrate similar patterns, such as reduced total duration and number of fixations on food stimuli relative to non-food stimuli, in the postmeal (hungry) condition compared to the premeal (satiated) condition.

5.2 Methods

Seventy-seven healthy-weight participants were recruited from the student and staff community over two separate recruitment periods. Inclusion criteria were aged over 18 years and the ability to fast for 4 hours. Exclusion criteria included a history of eating disorders, special diets (e.g., vegetarian, vegan), or a BMI classification OF 30.0 or higher. Previous studies have highlighted an 'approach-avoidance' attentional pattern observed when individuals view food associated with negative connotations, such as a meat product for a vegetarian (Knight et al., 2020; Hollitt et al., 2010). This visual pattern typically involves an initial attraction toward the food (approach) followed by a subsequent inclination to avert attention from it (avoidance). Similar patterns have been documented among individuals with specific dietary restrictions for weight loss or food preferences, as reported in studies by Hollitt et al. (2010) and Werthmann et al. (2011). To mitigate the potential impact of negative associations related to food stimuli impacting on visual attention, individuals with specific dietary preferences were excluded. Studies have reported an increased attentional bias to food stimuli in obese adult (Castellanos et al., 201) and children (Folkvord et al. 2020). However, in a systematic review of attentional bias to food stimuli in overweight and obese participants, the authors concluded that further research was necessary to understand the relationship between obesity and food related attentional biases (Hagan et al., 2020). As the evidence is not conclusive on this relationship, I chose to exclude participants who were considered obese (BMI over 30) to eliminate any potential confounding factor.

The self-report questionnaires used were the satiety labelled intensity magnitude scale (SLIM) (Cardello et al., 2005), food stimuli rating scale, and Honesty Questionnaire (see Chapter 2, section 2.1.3 for full descriptions). Eye tracking set collected using a Tobii X-260. Participants were positioned 55cm from the screen and a 5-point calibration (see Chapter 2, section 2.3.3 for a full description of the set up).

5.2.1 Recruitment Period 1

Participants (n=47) were instructed to fast for 4 hours before their lab visit and completed the FAB task in the premeal condition (Table 5.1). Visits were scheduled between 12 pm and 2 pm. On arrival, participants completed the SLIM to determine their perceived level of satiety. To reduce the emphasis on hunger/satiety, the researchers designed replica scales of the SLIM in which participants were asked to rate their energy and happiness levels. Participants were also asked questions related to all five stimuli categories in the task so that equal emphasis was placed on all categories, not just food stimuli (see Appendix 6). Participants then completed the FAB task. Participants were then given a standardised meal consisting of a piece of fruit, a sandwich, and a bottle of water, where calorie content had to fall in the range of 450-550 calories (Table 5.1).

Participants completed the post-meal condition 30 minutes after consuming the standard meal, as maximum feeling of fullness, referred to as "peak fullness," occurs immediately after food consumption (Forde et al., 2021). Previous research on healthy-weight individuals who consumed a standardised breakfast revealed that peak fullness, measured by the SLIM scale, was reported around 15 minutes postmeal. To ensure an adequate timeframe for the majority of participants to reach satiety, a 30-minute postmeal period was allowed before initiating the post-meal condition. Following this, participants completed the SLIM scale, emotions rating scale, energy level rating scale, and a second administration of the FAB task. Participants completed an honesty questionnaire to assess questions related to their food consumption during the fasting period, speculations about the study's purpose and any behaviour changes made based on their understanding of the study. When finished, participants completed the food stimuli rating scale to assess if the food stimuli used in the paradigm were food that participants would usually like to eat. The study visit lasted approximately 1.5 hours.

5.2.2 Recruitment Period 2

To account for order effects in the premeal and postmeal design, a second period of recruitment was carried out where participants (n=35) completed the postmeal condition first (Table 5.1). Participants were instructed to fast for four before the study visit (i.e., not consume any food). Visits were scheduled between 8 am and 10 am. On arrival, participants were provided with a standardised meal consisting of a toast/bagel, a piece of fruit, and water so that the calorie content matched the content for the lunch meal (450-550 calories). Thirty minutes after consuming the standardised meal, participants completed the post-meal condition. The SLIM scale and emotion and energy level rating scales were administered, followed by the first administration of the FAB task. During this visit, participants were instructed to abstain from eating and to return to the lab in four hours. The FAB task was administered in the pre-meal condition after the four-hour period where they abstained from eating. The SLIM scale and emotion/energy level rating scales were repeated, followed by the second run of the FAB task. Participants completed the food stimuli appealingness scale and the honesty questionnaire when finished. The study visit lasted 1 hour, followed by a four-hour wait period and a further 30 minutes in the lab for the second condition.

Table 5.1: Counterbalanced protocols for recruitment periods 1 and 2

Procedure	Recruitment Period 1	Recruitment Period 2
Before Study Visit	4 hour fast	4 hour fast
First Meal Condition	SLIM and Distractor Questions ↓ FAB Task (premeal condition) ↓ Standardised meal	Standardised Meal ↓ 30-minute wait ↓ SLIM + Distractor Questions ↓ FAB Task (postmeal condition)
Second Meal Condition	30-minute wait ↓ SLIM and Distractor Questions ↓ FAB task (postmeal condition) ↓ Honesty Questionnaire & Food Stimuli Rating Scale	4 hour fast ↓ Slim and Distractor questions ↓ FAB task (Premeal condition) ↓ Honesty Questionnaire & Food Stimuli Rating Scale

5.2.3 Statistical Analysis

A combination of parametric and non-parametric analyses was employed to compare participant characteristics between the two recruitment periods. Independent t-tests were utilised to assess differences in age, BMI, and food stimuli rating scores between the two recruitment groups. A chi-squared test of independence was applied to examine any variations in gender distribution. Additionally, Wilcoxon signed-rank tests were conducted to evaluate potential distinctions in SLIM scores for both the premeal and postmeal conditions.

For the eye tracking analysis, the selected variables of interest were the total duration of fixations and the total number of fixations within each AOI, as both have been used in prior studies, and the latter may reflect the importance of the noticeability of a stimulus (Castellanos et al., 2009; Doolan et al., 2014; Nijs et al., 2010; Poole & Ball, 2006). Time to first fixation within each AOI was also selected as a dependent measure to see if food intake influenced how quickly participants were oriented to food stimuli. Data were collapsed into averages for each AOI category, participant, and each meal condition. We conducted three separate 5x2 repeated measures ANOVAs for each Period with AOI category (clothes, food, instruments, household items, and vehicles) and meal condition (premeal, postmeal) as the repeated factors, and total

duration of fixations, number of fixations, and time to first fixation as the three dependent variables. Initially, the data from period one of recruitment was analysed.

5.3. Results

5.3.1. Demographics and Self-Report Results

For recruitment period one, 33 out of 47 participants (70% of the initial sample) were included for analysis. Out of the remaining 14 participants, one participant did not meet the SLIM criteria for the premeal condition, indicating that they did not feel hungry before starting the task. Additionally, three participants failed to meet the criteria for the postmeal condition, as they reported still feeling hungry even after the provided meal. One participant was excluded due to not meeting the criteria on the food rating scale, revealing their dislike for the majority of food stimuli used in the task. Four participants were excluded for self-reported intentional behaviour alteration during the task. Lastly, five participants were excluded due to the poor quality of the eye tracking data.

For recruitment period two, 24 of 35 participants (73%) were included for analysis - two participants were excluded as they reported in the honesty questionnaire that they did not complete the four-hour fast. Two participants did not meet the SLIM criteria for the premeal condition. One participant was excluded due to not meeting the criteria on the food rating scale, and two participants were excluded for self-reported intentional alteration of their behaviour during the task. Lastly, two more participants were excluded due to the poor quality of the eye tracking data. Table 5.2 illustrates that no statistically significant differences were observed regarding group characteristics and self-report measures across the two recruitment periods.

Table 5.2: Demographics and self-report measures for all participants

	Period 1 of Recruitment *n =33*	Period 2 of Recruitment *n =24*	Statistic	P Value
Age	24.4 (7.7)	27.6 (6.0)	$t(56) = -1.71$	$p = .09$
BMI	23.6 (4.7)	24.0 (4.7)	$t(56) = -0.44$	$p = .66$
Sex (M: F)	10:23	13:12	$X^2(1) = 2.8$	$p = .09$
Food Stimuli Rating (Total)	77.5 (56-100)	79 (68 – 99)	$t(56) = -1.8$	$p = .29$
SLIM (premeal)	-60 (-80 - -20)	- 40 (-60 - -20)	$U = 502.5$	$p = .08$
SLIM (postmeal)	40 (20 - 80)	40 (20 - 60)	$U = 308.5$	$p = .11$

* Median and ranges are reported for Satiety Labelled Intensity Magnitude (SLIM) and food stimuli rating scales and means, and standard deviations are reported for age and BMI. No significant differences were found between groups for age, BMI, gender ratio, food stimuli rating, or SLIM ratings.

5.3.2. Eye tracking Results

Recruitment Period 1 (premeal - postmeal) Results

(i) Total Duration of Fixations

The effects of the AOI category (food, clothes, household, instrument, and vehicle) and meal condition (premeal, post-meal) on the total duration of fixations within an AOI were assessed using a 5x2 repeated measures ANOVA. Analysis of the studentised residuals showed that the normality assumption was met, per the Shapiro-Wilk test of normality. Two outliers, indicated by studentised residuals greater than ± 3 standard deviations, were included in the analyses since there was no *a priori* reason to exclude these. The assumption of sphericity for the interaction term was violated as assessed by Mauchly's test of sphericity ($p < .05$); therefore Greenhouse-Geisser correction was applied. Data are mean ± standard deviation unless otherwise stated.

There was a statistically significant two-way interaction between meal condition and AOI category, $F(2.6, 87) = 9.8$ $p < .001$, partial eta squared = 0.329. Therefore, simple main effects analyses were run to test the effect of meal conditions on each of the five AOI categories, and the effect of the AOI category within each meal condition and LSD correction was applied.

Considering the main effect of meal condition, the mean total duration for food AOIs was 649ms (95% CI, 373, 926) longer in the premeal condition compared to the post-meal condition, a statistically significant difference, $F(1, 33) = 22.8$, $p < .001$. The other AOI categories did not see this effect between hunger and satiety. The difference in mean total duration for clothing was 5.9 ms (95% CI: -139, 127, $p = .928$), for household AOIs 51.4 ms (95% CI: -74, 177, $p = .412$), for instrument AOIs -185 ms (95% CI: -234, 114, $p = .146$) and vehicle AOIs it was -60 (95% CI: -439.1, 68.1, $p = .488$) (see Figure 5a).

Considering the main effect of the AOI category, there was a main effect of category in the premeal condition $F(1.98, 65.6) = 23.9$, $p < .001$. Pairwise comparisons showed the mean total duration of fixations for food AOIs (2170, ±718) was significantly higher than all other AOI categories (Clothes: 1205, ±322; Household: 1299, ±347; Instrument: 1274, ±351; Vehicles: 1367, ±416). The AOI category had no statistically significant effect on the total duration of fixations in the post-meal condition, $F(2.3, 76) = 2.4$, $p = .06$ (see Figure 5a).

(ii) Number of Fixations

The effect of the AOI category and meal condition on the number of fixations within an AOI was assessed using a two-way repeated-measures ANOVA. Analysis of the studentised residuals showed that the assumption of normality was met, as assessed by the Shapiro-Wilk test of normality. Three outliers, as assessed by studentised residuals greater than ± 3 standard deviations, were included in the analysis. The assumption of sphericity for the interaction term was violated as assessed by Mauchly's test of sphericity ($p < .05$); therefore Greenhouse-Geisser correction was applied. Data are mean ± standard deviation unless otherwise stated.

There was a statistically significant two-way interaction between meal condition and AOI category on the number of fixations within an AOI, $F(3.0, 99.6) = 8.5$ $p < .001$, partial eta squared = 0.298. Analyses of simple main effects were conducted, and LSD correction was applied.

Considering the main effect of meal condition, the mean number of fixations for food AOIs was 1.5 (95% CI:0.8, 2.2) higher in the premeal condition compared to the post-meal condition, a statistically significant difference, $F(1, 33) = 18.8$, $p < .001$. This effect of meal condition was not significant for any of the other AOI categories. The mean total duration difference for clothing was -0.2 (95% CI: -0.6, 0.2, $p = .399$), for household AOIs .34 (95% CI: -0.1, 0.8, $p = .114$), for instrument AOIs -0.4 (95% CI: -1, 0.1, $p = .132$) and vehicle AOIs was -0.4 (95% CI: 1,1.9, $p = .178$) (see Figure 5b).

Considering the main effect of the AOI category, there was a significant effect of the category in the premeal condition ($F(1.98, 65.6) = 23.9$, $p < .001$). Pairwise comparisons showed the number of fixations for food AOIs (7.1 ±1.9) was higher than all other AOI categories (Clothes: 4.9 ±1.0; Household: 5.0 ±1.4; Instrument: 5.0 ±1.3; Vehicles: 5.6 ±1.2). The effect of the AOI category on the total duration of fixations in the post-meal condition was not significant when LSD correction was applied F (2.9, 98.9) = 2.9, $p = .037$ (see Figure 5b).

(iii) Time to First Fixation

A two-way repeated-measures ANOVA assessed the effect of AOI category and meal condition on time to first fixation within an AOI. Analysis of the studentised residuals showed that the assumption of normality was met, as assessed by the Shapiro-Wilk test of normality. Two outliers, as assessed by studentised residuals greater than ± 3 standard deviations, were included in the analysis. Mauchly's test of sphericity indicated that the assumption of sphericity for the interaction term was met for the two-way interaction.

There was no statistically significant two-way interaction $F(4, 132) = 0.5$, $p = .765$ and no main effect of meal condition ($F(1, 33) = 3.6$, $p = .065$). There was a main effect of AOI category $F(4, 132) = 20.2$ $p < .001$. Post hoc analysis with a LSD adjustment revealed that mean time to first fixation across both meal conditions was significantly faster for food AOIs compared to clothing AOIs (666 ms (95% CI: 482, 850), $p < .001$) household AOIs (644ms (95% CI: 444, 845), $p < .001$) instrument AOIs (491 (95% CI: 312, 669), $p < .001$) and vehicle AOIs (498 (95% CI, 316 to 680), $p < .001$) (see Figure 5c).

Recruitment Period 2 (postmeal - premeal) Results

Participants recruited during period 2 participated in the study with the meal conditions counterbalanced, i.e., the postmeal condition followed by the premeal condition, to address the possible effects of the meal order on the outcome variables. The same analyses that were conducted for the data collected during period one were conducted with the data collected from participants recruited in period 2.

(i) Total Duration of Fixations

The effects of the AOI category (food, clothes, household, instrument, and vehicle) and meal condition (premeal, postmeal) on the total duration of fixations within an AOI were assessed with a 5x2 repeated measures ANOVA. There was a significant two-way interaction between meal condition and AOI category, $F(2.7, 61.7) = 3.31$, $p = .031$, partial eta squared =.194 Therefore, simple main effects analyses were conducted to test the effect of meal conditions on each of the five AOI categories and the effect of the AOI category within each of the meal conditions.

Considering the main effect of the meal condition, the mean total duration for food AOIs was 393ms (95% CI: 138, 648) longer in the hungry condition compared to the satiated condition, a statistically significant difference, $F(1, 23) = 10.2622.8$, $p < .004$. This effect was not seen across hunger and satiety for any of the other AOI categories. The mean total duration difference for clothing was 107 ms (95% CI: -218, 3, $p = .056$), for household AOIs 84ms (95% CI: -244, 77, $p = .294$), for instrument AOIs -177 ms (95% CI: -386, 31, $p = .09$) and for vehicle AOIs it was 1260ms (95% CI: -53, 307, $p = .488$) (see Figure 5d).

(ii) Number of Fixations

A two-way repeated-measures ANOVA assessed the effect of the AOI category and meal condition on the number of fixations within an AOI. There was a significant two-way interaction between meal condition and AOI category on the number of fixations within an AOI $F(2.63, 60.5) = 3.31$, $p = .031$, partial eta squared = 0.126. Simple main effects were analysed, and the LSD correction was applied. Considering the main effect of the meal condition, the mean number of fixations for food AOIs was .96 (95% CI: .21, 1.71) higher in the hungry condition compared to the satiated condition, a statistically significant difference, $F(1, 23) = 7$, $p = .014$. The meal condition effect was not significant for any of the other AOI categories. The mean number of fixations for clothing was -0.12 (95% CI: -0.5, 0.2, $p = .452$), for household AOIs -.12 (95% CI: -0.6, 0.4, $p = .608$), for instrument AOIs -0.4 (95% CI: -1, 0.1, $p = .094$) and vehicle AOIs, it was -0.2 (95% CI: 1, .5, $p = .577$) (see Figure 5e).

(iii) Time to First Fixation

A two-way repeated-measures ANOVA assessed the effect of AOI category and meal condition on time to first fixation within an AOI as There was no significant two-way interaction $F(4, 92) = 1.65$, $p = .169$ and no main effect of meal condition $F(1, 23) = 2.491$, $p = .128$). There was a main effect of AOI category $F(4, 92) = 6.8$, $p < .001$. Pairwise comparisons with a LSD adjustment revealed that mean time to first fixation across both meal conditions was significantly faster for food AOIs compared to household AOIs (-507ms (95% CI, -880 to -135), $p = .003$) instrument AOIs (-424ms (95% CI, -769 to -79 to 669), $p = .009$) and vehicle AOIs (-469 (95% CI, -875 to -65), $p = .015$) but not clothes AOIs (-371 ms (95% CI, -846 to 104), (see Figure 5f).

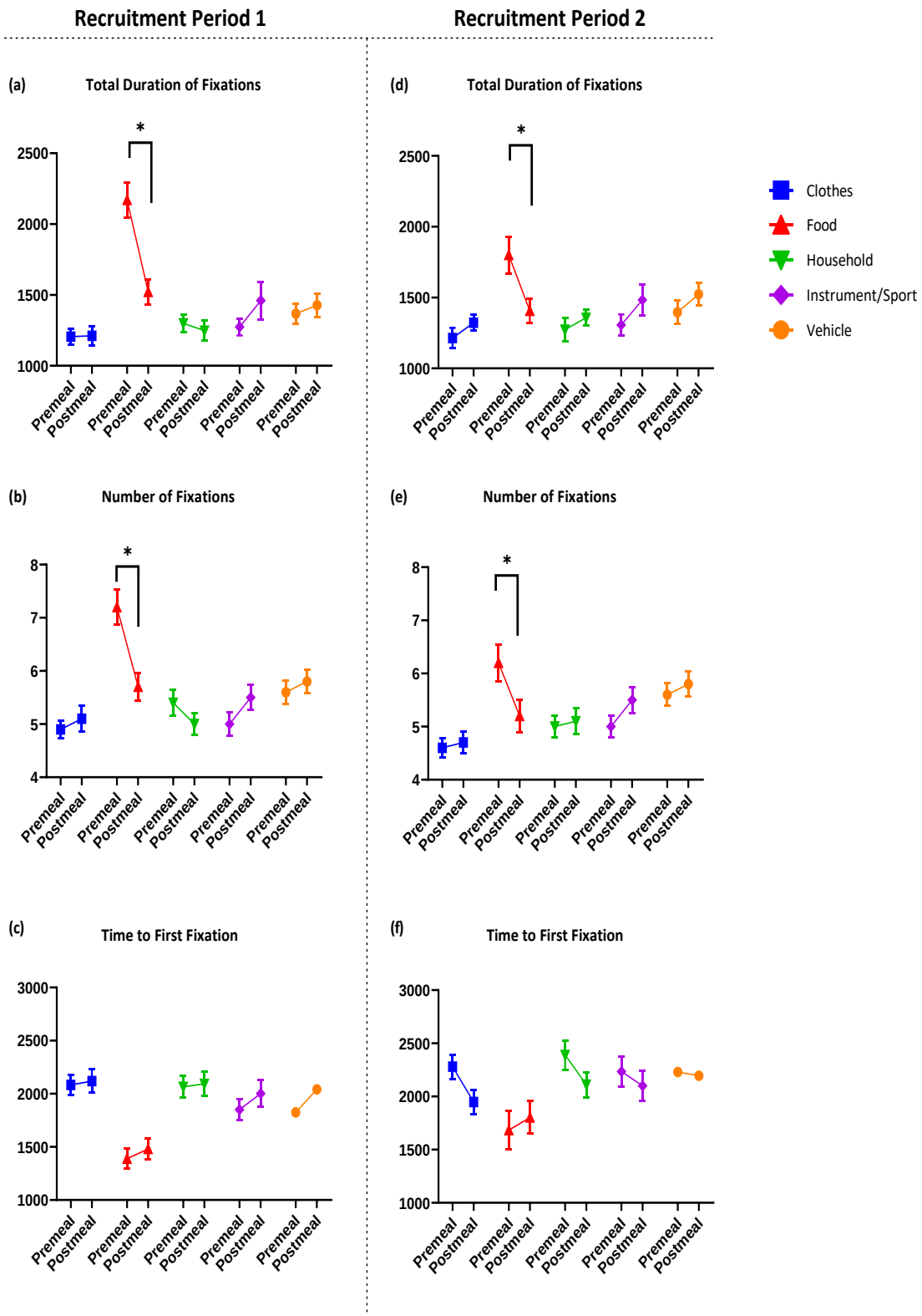

Figure 5.1: Eye tracking measures for each stimulus category for pre and postmeal conditions for (a) total duration of fixations, (b) number of fixations and (c) time to first fixation from recruitment period one and (d) total duration of fixations (e) number of fixations and (f) time to first fixation from recruitment period 2.

Table 5.3: The mean and standard deviation for each AOI category across pre and post-meal conditions for recruitment periods one and two.

Dependent Variable	AOI	Recruitment Period 1		Recruitment Period 2	
		Premeal Mean (SD)	Postmeal Mean (SD)	Premeal Mean (SD)	Postmeal Mean (SD)
Total Duration of Fixations (ms)	Clothes	1205 (322)	1211 (384)	1214 (351)	1322 (274)
	Food	2170 (719)	1521 (515)	1800 (631)	1406 (412)
	Household	1299 (347)	1248 (402)	1273 (400)	1357 (277)
	Instrument	1274 (351)	1460 (777)	1306 (367)	1483 (525)
	Vehicle	1367 (416)	1427 (476)	1396 (405)	1523 (397)
Number of Fixations	Clothes	4.9 (1.0)	5.1 (1.4)	4.6 (0.9)	4.7 (1.0)
	Food	7.2 (1.9)	5.7 (1.5)	6.2 (1/7)	5.2 (1.5)
	Household	5.4 (1.4)	5.0 (1.2)	5.0 (1.0)	5.1 (1.1)
	Instrument	5.0 (1.3)	5.5 (1.4)	5.0 (1.0)	5.5 (1.2)
	Vehicle	5.6(1.2)	6.0 (1.7)	5.6 (1.3)	5.8 (1.3)
Time to First Fixation (ms)	Clothes	2083(547)	2119 (641)	2280 (558)	1948 (542)
	Food	1389(549)	1480 (584)	1683 (889)	1802 (752)
	Household	2065 (598)	2093 (668)	2390 (677)	2111 (581)
	Instrument	1850 (568)	2002 (732)	2234 (684)	2100 (694)
	Vehicle	1825(531)	2041 (679)	2230 (638)	2194 (640)

5.4. Discussion

This chapter aimed to develop and test the FAB task, an innovative eye tracking paradigm, to explore variations in visual attention towards food stimuli in typically developing individuals under different hunger and satiety conditions. The hypothesis predicted reduced attention to food stimuli in the postmeal condition, as measured by the duration and frequency of participants' gaze. Additionally, the order effects of meal conditions were investigated by recruiting two groups of participants who underwent the FAB task protocol in counterbalanced conditions.

The development of the FAB task was guided by recommendations aimed at enhancing the reliability of food attentional bias measurements (Hagan et al., 2020; Hardman et al., 2021; van Ens et al., 2019). Several key aspects were incorporated into the study protocol to address these recommendations. Firstly, direct measurements of AB, eye movements, were used to assess participants' attentional focus towards food stimuli more precisely and objectively. Secondly, the task employed stimuli from the standardised Food Pics Database (Blechert et al., 2019), ensuring consistency in stimulus properties and enhancing comparability across studies. Thirdly, the FAB task extended the duration of the stimulus presentation to 10,000 ms, aligning with the recommendation of longer presentation times. This extended duration provided sufficient time for participants to process and engage in the top-down visual processing of the food stimuli, facilitating more robust measurements of ABs. Lastly, a repeated measures design was employed to capture within-subject fluctuations in AB, comparing performance across two distinct conditions: premeal and postmeal. This design enabled the examination of changes in ABs within individual participants, reducing the influence of between-subject variability. By incorporating these methodological considerations, the FAB task offers an improved approach to measuring food AB. This is the first study to use a free viewing paradigm to investigate whether attentional bias for food fluctuates pre and post-meal intake in healthy-weight adults.

In line with my first hypothesis, a significant difference in the total duration of fixations on food stimuli between the premeal and postmeal conditions suggested that participants looked longer at food stimuli when hungry than when satiated. The significant difference in the total duration of fixations on food stimuli between the premeal and postmeal conditions was consistently observed across both counterbalancing conditions. This suggests that the observed variations in attentional bias towards food stimuli are more likely to be attributed to manipulating the meal condition (premeal vs postmeal) rather than the sequence in which the conditions were presented. In other words, it is more likely that the satiety level induced by the meal condition influenced participants' attentional focus on food stimuli rather than any order effects introduced by the experimental design.

The findings of this study align with a prior investigation that demonstrated a reduced duration bias for food stimuli in satiated compared to hungry conditions using a dot-probe task in healthy-weight females (Castellanos et al., 2009). In contrast, other studies employing the Dot Probe paradigm found no differences in duration bias between pre- and post-meal conditions (Doolan et al., 2014; Nijs et al., 2010). Dot Probe tasks typically present stimuli for a short duration, such as 2000 ms, to detect "bottom-up" attentional bias. In contrast, the FAB task utilised an extended stimulus presentation time of 10,000 ms, aligning with recommendations to improve the reliability of food attentional bias measurements (Hagan et al., 2020; Hardman

et al., 2021; van Ens et al., 2019). This longer duration allows for the assessment of "top-down" attentional processes involved in the sustained attention towards food stimuli (Akcay et al., 2022), potentially providing a more robust measure of attentional bias compared to Dot Probe tasks (Waechter et al., 2014).

However, it is essential to interpret the total duration of fixations cautiously since it represents a combination of fixation duration and count, each having distinct psychological interpretations (Orquin & Holmqvist, 2018). Differences in total duration can arise from three independent conditions: (1) when AOI A receives more fixations or dwell time than AOI B, (2) when fixations to AOI A have a longer duration than fixations to AOI B, and (3) when AOI A is fixated with a higher likelihood than AOI B. These conditions have diverse psychological implications (Orquin & Holmqvist, 2018). A higher number of fixations on AOI A suggests a greater likelihood of refixations, potentially indicating top-down control or stimulus complexity. Longer fixations on AOI A may suggest complexity or higher relevance, while a higher likelihood of fixating on AOI A can be attributed to both top-down and bottom-up processes, such as salience or relevance (Orquin & Holmqvist, 2018). Therefore, relying solely on total fixation duration makes it challenging to draw definitive conclusions about the specific top-down components of attention involved in the task.

In line with my second hypothesis, a significant difference was observed in the number of fixations to food stimuli between the two conditions. It appears that differences in the total duration of fixations are arising from condition one - the food AOIs received more fixations than non-food AOIs indicating top-down control. It has been argued that the importance of the noticeability of an object increases the number of fixations in the AOI allocated to that object (Poole & Ball, 2006). These findings suggest that participants found food stimuli more "important" or "noticeable" when hungry than satiated. An increased number of fixations likely represents top-down control (Orquin & Holmqvist, 2018). Therefore, the number of fixations to food stimuli may also be a good measure of how hungry or satiated a person is as it may show how interesting or rewarding food is at that time. This is the first time this variable has been reported to measure hunger-related fluctuations in attentional bias for food stimuli.

The data did not support my third hypothesis that the time taken to locate a food stimulus would be lower in the premeal condition. Participants located food stimuli consistently more quickly than non-food stimuli in both pre and postmeal conditions. Other studies using the dot-probe task similarly showed that individuals were more likely to orient to food stimuli before non-food stimuli (Doolan et al., 2014; Nijs et al., 2010), while another study found that healthy-weight individuals were more likely to look initially at food when hungry compared to when satiated (Castellanos et al., 2009). These studies measured initial orientation likelihood, i.e., the percentage of first fixations towards food versus non-food, whereas this study looked explicitly at the time taken in ms to the first fixation within a food AOI compared to non-food AOIs. The FAB task had a comparatively more complex display with four additional distractor categories compared to these previous studies, but despite this, food was still located faster than non-food. It is generally considered that a short entry time to a target AOI reflects high efficiency in locating that stimulus (Holmqvist et al., 2011), suggesting participants were more efficient at locating food stimuli than other categories. The consistent orientation towards food stimuli, observed both when participants were hungry and satiated, may be attributed to the inherent

salience of food throughout human evolution. Food has always possessed a salient quality, serving as an adaptive mechanism that facilitated the detection of nutrient-dense sources in the environment. This enhanced sensitivity towards food stimuli likely played a crucial role in ensuring adequate food intake for survival (Potthoff & Schienle, 2020).

Interestingly, a location effect was found within the array, in which participants initially orientated to the top-left of the screen. This tendency to initially direct their gaze to the left of a display has been previously reported and may be explained as the "reading effect," i.e., wherein the English language, words are read top to bottom and left to right (Durgin et al., 2008; Guo et al., 2012). This was accounted for by counterbalancing the location of stimulus categories across arrays so that all stimulus categories were presented an equal number of times in each possible location, and therefore, location effects did not influence this finding.

The results of this study support the theory that the motivational value of food influences attentional bias and that the motivational value of food fluctuates in response to food intake (Field et al., 2016). Moreover, different components of attentional processing may be impacted by motivational states of hunger and satiation. The later component (top-down processing), as measured by duration bias and fixation count, is influenced by hunger, whereas the earlier component (bottom-up processing), measured by time to first fixation, may be less influenced by hunger. Early attention processing of food stimuli could therefore be a trait, while later processing of food attention may be subject to the fluctuating motivational value of food. The later motivational value of food may be an important marker of satiety with utility for trials of therapeutic interventions for hyperphagia in individuals across age ranges and developmental abilities. Due to its passive nature, the paradigm could be beneficial as a behavioural biomarker of impaired satiety in cohorts of patients with syndromic obesity. There is an urgent need for objective, robust, reliable, and reproducible treatment biomarkers to evaluate the effectiveness of new drug therapies in the context of clinical trials, as existing measures rely on subjective questionnaires which do not account for environmental control measures and are vulnerable to placebo effects (Hollander et al., 2021).

A limitation of the current study is that participants were not randomly assigned to counterbalance conditions; data was instead collected during two separate recruitment periods that used different orders of presentation of the FAB task. However, group comparisons revealed no demographic or self-report differences across the two recruitment periods. Although effect sizes were smaller in the second group, which used the counterbalanced task order (satiety condition first), the statistical interaction between meal condition and stimulus category was reproduced, as were all other statistical effects. This demonstrates that the FAB task robustly captures the effect of satiety on AB, supporting its potential value as an objective behavioural biomarker of impaired satiety in clinical cohorts.

Another limitation is that participants may have realised the purpose of the paradigm was to assess their patterns of viewing food before and after eating. Six (7.8%) participants were excluded for reporting that they had purposely altered their behaviour while completing the task, suggesting social desirability factors may have impacted results. Participants might have altered their behaviour if they thought it was not socially acceptable to still feel hungry after eating and were conscious of looking at food stimuli. To try to mitigate against this bias and to reduce the emphasis on food, a questionnaire asking about all the category items included in

the FAB task was given before participants viewed the FAB task. Also, all participants that reported purposefully altering their behaviour at any stage were excluded, but some participants may still have altered behaviour. While the FAB task has been primarily designed for PWS, adapting it for studies involving typically developing populations requires modifications to minimise desirability bias. Gamifying the FAB task has the potential for adapting it to typically developing populations, leveraging immersive VR environments where participants authentically interact with gamified tasks. This approach reduces social desirability bias and discourages intentional behaviour modification.

Furthermore, recent findings show that food selection in VR environments generalises to real-world settings without precise matching (Cheah et al., 2020). Significant correlations between real-world and VR food selections persist even with a one-week time lag, supporting VR's utility for studying psychological and behavioural food-related processes. Consequently, gamified VR tasks hold promise for enhancing attentional biases towards food stimuli across diverse populations.

This study demonstrates the robustness of the FAB task in measuring attentional bias towards food stimuli, as indicated by the total duration and number of fixations. The findings consistently show a reduction in attentional bias under conditions of satiety following a meal compared to before a meal. The FAB task, designed as a passive eye tracking paradigm, holds the potential for measuring attentional bias to food stimuli across different age groups and cognitive abilities. Its objective nature suggests that the FAB task could serve as an objective biomarker for hyperphagia PWS. Further replication of these findings in diverse populations is needed to assess the sensitivity of the FAB task to attentional bias in such cohorts. In the subsequent chapter, the adaptation of the original FAB task protocol for use in a PWS cohort will be discussed, aiming to explore the task's potential usefulness in studying therapeutic interventions for hyperphagia.

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Chapter 6: A participatory approach to the adaptation of the Food Attentional Bias Task, a protocol for measuring hyperphagia in participants Prader-Willi Syndrome

6.1. Introduction

6.1.1 Impact of hyperphagia

As outlined in Chapter 1, individuals with PWS experience hyperphagia, characterised by persistent hunger, abnormal satiety, preoccupation with food, and a range of problematic behaviours related to food (Schwartz et al., 2021). Hyperphagia significantly impacts the lives of people with PWS and has been linked to reduced quality of life and increased social isolation (FPWR, 2014). Embarrassment or shame related to their eating behaviours has been reported by individuals with PWS, and they feel they often must avoid social situations where food is present to avoid food-related anxiety (FPWR, 2014). Hyperphagia is a major contributor to the caregiver burden in PWS (Kayadjanian et al., 2018) and treating hyperphagia is the highest priority for caregivers (Tsai et al., 2018). A recent study conducted with young people with PWS reported that all participants favoured participating in future clinical trials of new medications that would curb their hunger (Dykens et al., 2021). However, as discussed in Chapter 5, a lack of objective measures of hyperphagia is a significant barrier to evaluating the efficacy of new drug therapies. The food attentional bias (FAB) task tested in Chapter 5 demonstrated a reduction in attentional bias after eating, suggesting potential as a cognitive marker of "satiety." Lack of satiety is a hallmark feature of hyperphagia, and therefore the FAB task may offer an objective approach for measuring hyperphagia by assessing the lack of reduction of attentional bias to food stimuli post-food intake.

6.1.2 Challenges of implementing the FAB task protocol in a PWS cohort

Using the FAB task protocol, as previously described in Chapter 5, presents several challenges when working with individuals with PWS. Providing a standardised meal or asking participants to fast can be particularly challenging, given the insatiable hunger associated with PWS. This challenge is further complicated by other behaviours observed in PWS, such as becoming agitated or anxious in the presence of food or becoming upset or exhibiting temper outbursts when food is denied (Schwartz et al., 2021b). Additionally, the required disruption to routine associated with the FAB task protocol, e.g. consuming a meal in a novel environment, may add to distress, as individuals with PWS tend to rely on structure and routine to regulate their eating behaviours and manage their anxiety (Angulo et al., 2015). Most individuals with PWS have intellectual disabilities or communication difficulties, which can affect their ability to understand the purpose of the task or communicate their discomfort (Angulo et al., 2015). It is crucial to consider not only the potential challenges faced by individuals with PWS when asked to participate in this type of research but also the impact of these challenges on their caregivers. The fasting and meal provision in the protocol and change in routine associated with these procedures may cause additional distress or burden for caregivers if not carefully considered. For these reasons, adaptations to the FAB task, informed by caregivers and professionals expert in PWS, are required to ensure that the study is feasible for individuals with PWS.

6.1.3 Public and Patient Involvement

The importance of public and patient involvement (PPI) in research is increasingly recognised, and funding bodies like the National Institutes of Health and the European Commission have encouraged its implementation (NIHR, 2019; Coulter et al., 2014). PPI can lead to better recruitment and retention of participants, improved research relevance, and increased dissemination and implementation of study findings. Involving patients and the public in various stages of research can also result in more meaningful and effective research outcomes (NIHR, 2019; Coulter et al., 2014). PPI principles and values, such as those promoted by PPI Ignite in Ireland, have been developed to standardise and promote good practice (https://ppinetwork.ie/). However, challenges can arise in using PPI in study protocol design, particularly in balancing the perspectives of patients/public and researchers with scientific expertise. Conflicts may arise between what patients/the public want in a research protocol and what researchers view as important for scientific rigour. Ongoing communication and stakeholder collaboration is essential to balance these competing perspectives (NIHR, 2019; Staniszewska et al., 2018; Coulter et al., 2014).

6.1.4 Participatory Research

Participatory research involves stakeholders' meaningful and active involvement in the research process (Cargo & Mercer, 2008). In this context, stakeholders refer to individuals with lived experience of PWS, such as parents, caregivers, service providers, advocates, health professionals and most importantly, the individuals. Using a participatory approach for adapting the FAB task protocol for the PWS population has important value, given the specific needs and challenges this cohort faces, including insatiable hunger, intellectual disabilities, and challenges with social communication. In general, participatory research is valued for its potential to enhance research's relevance, validity, and impact by engaging stakeholders in the research process and promoting co-learning and empowerment. Participatory approaches have improved the quality and relevance of research and interventions in various health-related fields, including mental health and disability (Cargo & Mercer, 2008; Israel et al., 2008; Viswanathan et al., 2004). In addition, participatory approaches can help to build trust and relationships between researchers and participants, facilitate the identification of relevant outcomes and strategies, and enhance the dissemination and uptake of findings (Cargo & Mercer, 2008; Israel et al., 2008; Viswanathan et al., 2004). Qualitative research methods, such as focus groups, can be beneficial in this context, as they provide opportunities for in-depth exploration of individuals' perspectives, experiences, and concerns. This is especially important given the limited existing research on collecting eye tracking data in a PWS cohort. Overall, the use of participatory and qualitative methods can help ensure that adaptations to the FAB task are tailored to the specific needs and experiences of individuals with PWS and their caregivers and have the potential to improve the task's overall feasibility and validity in this population.

6.1.5 Aims and Hypotheses

This study aimed to use a participatory approach with parents and caregivers of individuals with PWS and with PWS professional experts to inform the adaptation of the FAB task from Chapter 5 for use with individuals with PWS. A second aim of the study was to consult with the scientific advisory panel to seek their views on the impact of proposed adaptations from the focus groups

on the scientific rigour of the study and to determine which focus group recommendations would be implemented and which would not. Ultimately, the goal was to create an adapted version of the FAB for use in children and adults with PWS that was informed by parents/caregivers and professional experts that was also scientifically rigorous.

6.2. Methods

6.2.1 Study Design

A qualitative research design was used to gather data from participants with lived or professional experience with PWS. Focus groups were chosen as the preferred research method due to their interactive and collaborative nature, which enables the collection of rich and nuanced data that may be difficult to obtain through quantitative approaches (Krueger & Casey, 2015). To ensure high-quality data collection, a well-constructed discussion guide was developed. A discussion guide serves as a structured outline to guide the facilitator in leading the group discussion and typically includes open-ended questions designed to elicit specific information from participants (Fridberg et al., 2021; Moretti et al., 2011). The process of developing a discussion guide for the focus groups involved several steps. Initially, a comprehensive review of prior studies involving fasting and meal provision within PWS was conducted to identify gaps in knowledge and define key objectives for the study (Haqq et al., 2007; Holsen et al., 2006; Key et al., 2020; Purtell et al., 2015). The study objectives were identified as (1) obtaining feedback on the study design for the hunger and satiety conditions, (2) gathering feedback on the meal provided for the satiety condition, (3) collecting feedback on the questionnaires to be used in the study, and (4) inviting participants to provide any other feedback or considerations regarding the study protocol (see figure 6.1).

The research team conducted two focus groups to ensure maximum input from caregivers and professionals. This decision was based on recognising that these two groups may have distinct perspectives and insights regarding adapting an eye tracking paradigm for individuals with PWS. By conducting separate groups, the research team could provide more time and depth of discussion for each group. To tailor the focus groups to the specific needs of each group, separate objectives were developed (see Table 6.1). It was also necessary to tailor aspects of the discussion guide to each group to ensure the questions were relevant and appropriate. This approach allowed for targeted questions and discussion topics to maximise the insights and perspectives provided by each group (Krueger & Casey, 2015; Moretti et al., 2011). For example, there was scope in the parents/caregivers' discussion guide for suggestions and guidance on making research visits more comfortable for the person with PWS and their caregivers on the day of the research visits. This approach allowed for targeted questions and discussion topics to maximise the insights and perspectives provided by each group (Krueger & Casey, 2015; Moretti et al., 2011).

6.2.2 Participants

Parents, caregivers, advocates, health care professionals and service providers with either lived experience or professional experience of PWS were invited to participate in the focus groups. Potential participants were identified from a database of PWS families who had previously participated in the research survey from Chapter 3 and had agreed to be contacted about future PWS research conducted by the Trinity Neurodevelopment Research Group. These participants

had previously been recruited through the national advocacy group, the Prader-Willi Syndrome Association of Ireland (PWSAI; https://pwsai.ie/) association and the national paediatric centre for PWS clinical care at Children's Hospital Ireland https://www.orpha.net/PWS/ireland Fourteen parents/caregivers and eight professionals were invited by email to participate in the study.

6.2.3 Procedure

The focus groups were delivered via video conferencing and lasted 90 minutes. An overall introduction to the study and the study aims were presented to the participants. The participants were separated into two breakout groups (participants with lived experience and participants with professional experience), and a group facilitator from our research team chaired each. The facilitator used a discussion guide developed for each group to ensure that all relevant topics were covered. The discussion guide included open-ended questions and prompts that encouraged participants to share their experiences, opinions, and perspectives on the study design of the hungry and satiated meal conditions and the study protocol (Fridberg et al., 2021; Moretti et al., 2011). All participants and facilitators joined in debriefing each group's main learnings after the breakout groups. Participants were thanked for their time, and the research team shared the future directions and timeline of the research project. The audio from each focus group was recorded and saved.

6.2.4 Focus Group Data Analysis

The focus group data analysis was conducted using inductive content analysis. This method involves analysing data in a systematic process to identify themes and patterns in the data (Elo & Kyngäs, 2008; Hsieh & Shannon, 2005; Lindgren et al., 2020). By using multiple coders and conducting independent data reviews, this study ensured the validity and reliability of the findings. It also allows researchers to explore and identify themes and patterns within the data without preconceived assumptions or theoretical frameworks, which is particularly useful when there is limited previous literature on the topic (Bengtsson, 2016). The steps taken to analyse the focus group data were as follows:

1. Transcription: Both focus groups were transcribed by a professional medical transcription service (TranScribe. i.e.), ensuring data accuracy and consistency.

2. Coding: The transcripts of the focus groups were independently reviewed by two research team members, me and Ms Áine McNicholas to identify the questions asked (open vs closed) and map each question to the study objectives. This process helped to ensure that the data were categorised according to the research questions.

3. Initial analysis: The transcript was reviewed again to highlight participant answers to the questions asked. This step helped to identify initial themes and patterns in the data.

4. Theme identification: A third review of the transcripts was completed to identify themes relating to each question. This process involved identifying words, phrases, or ideas that were repeated or emphasised by participants.

5. Overlapping themes: A joint review of transcripts was completed to identify overlapping themes or ideas. This step helped to refine the themes identified in Step 4 and ensure consistency in the coding process.

6. Emergent overarching themes: Finally, emergent overarching themes were assigned to each study objective. This process involved synthesising the themes identified in steps 4 and 5 into higher-order concepts or categories representing the study's main findings.

In examining the credibility, dependability, confirmability, and transferability of the developed themes, akin to the reliability and validity considerations in quantitative research, Lincoln and Guba's (1985) criteria provide a framework for assessing the trustworthiness of qualitative studies. Credibility, aligning with internal validity in quantitative research, focuses on the trustworthiness of findings and their faithful representation of participants' experiences. In this study, credibility was bolstered through an iterative analytical process involving stages of discussion, coding, categorization, and theme development, ensuring the themes identified were trustworthy and accurate. Transferability evaluates the potential applicability of findings to other contexts. To address for transferability, the current study provided a comprehensive description of settings, participants, data collection procedures, and analysis methods, enabling other researchers to assess the relevance of the findings in similar contexts. Dependability, akin to reliability, focuses on the stability and consistency of findings over time and conditions. This study enhanced dependability through referential adequacy, by storing raw data and providing a clear description of the analysis process for step-by-step replication and validation. Confirmability, the influence of participants and context over the researcher's biases, was ensured through two measures: a comprehensive report shared with focus group participants for review and feedback, and a feedback session with stakeholders to validate the researcher's understanding of participants' perspectives, aligning intentions with interpretations. These strategies collectively contribute to the robustness and trustworthiness of the qualitative research conducted

Key objectives for the study
1. To get feedback on my study design for the hunger and satiety conditions 2. To obtain feedback about the meal provided for the satiety condition 3. Gather feedback on the questionnaires to be used in the study 4. Invite participants to highlight any other considerations or feedback on the protocol

Specific objectives for parent/caregiver focus group	Specific objectives for professional focus group
1. To gather feedback on the study design for the hunger and satiety conditions 2. To gain insight into whether caregivers think having a meal in the lab environment (outside of routine) would be disruptive for people with PWS and how to prepare someone with PWS for this disruption to their routine. 3. To gain feedback on the study design for the standardised meal for the satiety condition 4. To ask parents/caregivers for their feedback on the suitability of self-report measures in PWS individuals 5. Seek suggestions and guidance on how to make research visits more comfortable for the person with PWS	1. To gather feedback on the study design regarding the fast length of the hunger and satiety condition. 2. To gain feedback on the study design for the standardised meal for the satiety condition 3. To obtain experts' feedback on capturing the states of hunger and satiety 4. To ask experts for their feedback on the suitability of self-report measures in PWS individuals 5. Invite experts to highlight any other considerations to account for in the study protocol.

Figure 6.1: Objectives for focus groups

6.3. Results

6.3.1 Themes

Nine parents/caregivers consented to participate, of whom six were ultimately able to attend the focus groups. Parents/caregivers of children, adolescents and adults were all represented in the final group of participants. Five PWS professionals consented to participate in the study, and all attended. The professional participants comprised advocates (PWSAI, IPWSO), residential service providers and healthcare professionals from the national paediatric centre for PWS clinical care. In this section, the themes that emerged from the focus groups about each of the study objectives are reported. Quotations have been selected as typical examples of the main themes to explore the differences between participants or to highlight issues of particular interest. All names used in the results section have been changed to ensure anonymity. A summary of the key themes identified through the inductive content analysis is outlined below (see Figure 6.2).

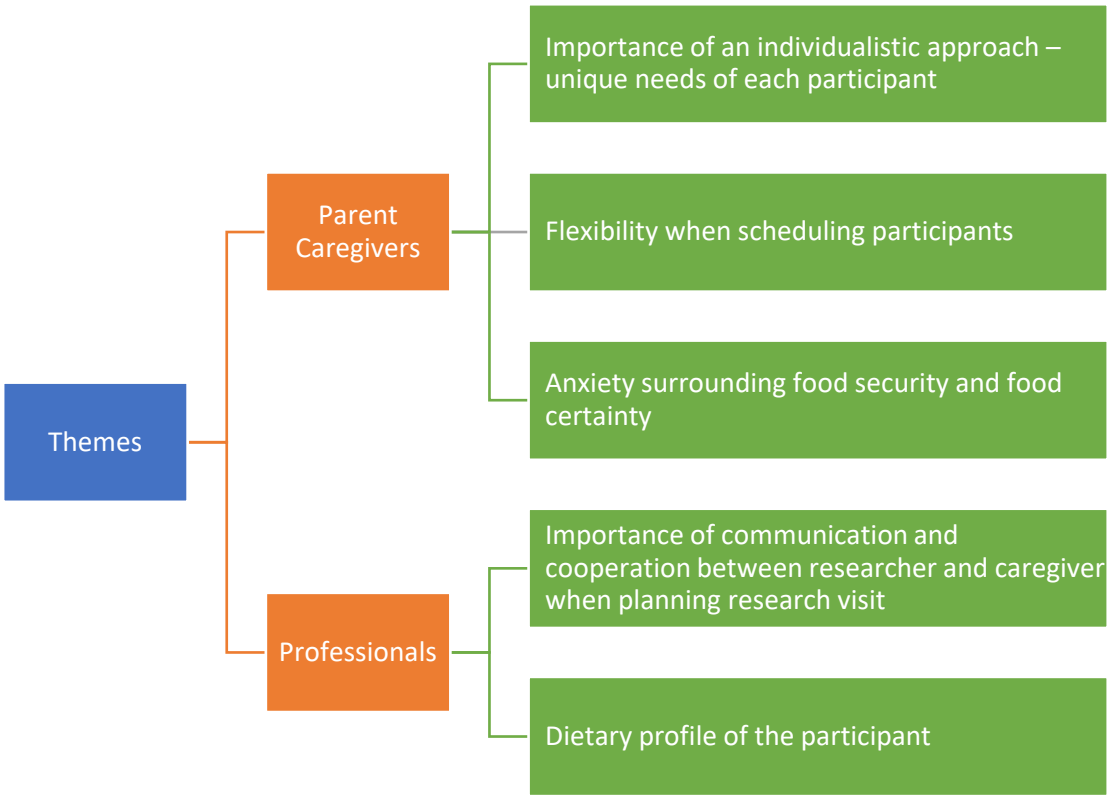

Figure 6.2: Schematic map of themes that emerged from thematic analysis of focus groups

Objective 1: Study design for the hunger and satiety conditions

In the existing FAB task design, the "hunger" condition was defined as a 3-hour fast for participants aged 4-11 years and a 4-hour fast for all participants aged 11 years and older (see Table 4.1 for original protocol). "Food certainty" was a central theme that emerged from the parent/ caregiver focus group in relation to PWS individuals and fasting. In this context, food certainty refers to establishing a structured and predictable mealtime routine to reduce anxiety. This involves setting consistent mealtimes, limiting access to food outside of scheduled meals, and providing clear guidelines and expectations around food choices and portion size (Miller et al., 2011). This was the most critical factor for parents and caregivers rather than any challenges associated with providing a meal in an unfamiliar context.

> *"He has no problem having a change of environment, as long as he is guaranteed lunch or dinner or coffee or whatever he doesn't mind where it is."*

The PWS professional focus group feedback identified anxiety arising from a possible delay or postponement of a meal as a key theme. The importance of flexibility in scheduling participants was a second theme that emerged. The professionals thought that integrating the study to fit with the participant's meal routines based on their typical individual meal schedule would increase the likelihood of the participant completing the fast for the hunger condition.

> *"I don't think you are going to get an adult [with PWS] too fast for 4 hours. In our place [residential setting], breakfast is at 11, lunch at 1, snack at 4, dinner at 6, supper at 9. I*

think if they [the PWS resident] was to skip one of those it would cause them huge anxiety."

Objective 2: The content of the meal provided in the study protocol

The original study design used a standardised meal approach, i.e., all study participants were provided with a standardised menu before the study visit, from which they had few choices. This allowed all meals to be analysed for calorie and macronutrient content so that meal content was consistent across participants (see Chapter 5, section 5.2). The parent/caregiver group feedback identified two potential challenges with this standardised meal approach: anxiety related to providing a standardised meal and the importance of accounting for individual differences in planning for the FAB task study visit. Differences in portion size, the provision of sweet treats and deviations from typical meals were identified as potential sources of anxiety for the PWS participants. Parents/caregivers emphasised the importance of liaising with them first on the menu in advance.

"It [the standardised meal] would be a disaster waiting to happen, he would be very stressed out by that. It wouldn't suit him, I think, because we have his packed lunch for school every day. That is what he would be expecting to eat at lunchtime, it's what he knows is his packed lunch."

The central theme that emerged from the professional group was the importance of communication and cooperation between the parent /caregiver/residential setting and the researcher when planning the standardised meal. The group expressed that it would be imperative for the researcher to engage with the participant's parent/caregiver in advance to view the menu and identify any items that may increase stress or anxiety for both the person with the PWS.

Objective 3: Behavioural questionnaires included in the study protocol.

In the FAB task study discussed in Chapter 5, participants were asked to complete a hunger rating scale before completing eye tracking in both conditions (premeal and postmeal). Those who did not report a subjective sense of hunger or satiety consistent with the condition were excluded. Two main themes emerged from the parent/caregiver group concerning using a hunger rating scale. The first was related to the concept of hunger. Specifically, the parent/caregiver group identified that their family member with PWS did not have insight into their feelings of hunger and would have difficulty reporting hunger subjectively. They believed that completing a hunger questionnaire would be a source of stress. A further theme that emerged was parent/caregiver anxiety. Some parents/ caregivers reported that conversations surrounding "hunger" were avoided at home; there was also parental anxiety in discussing hunger with their family member with PWS.

"We don't really talk about being hungry in our house, food just happens. I don't know how my son would know to answer this because we don't talk about hunger".

The professional's group's feedback was consistent with the parent/caregiver group concerning measuring hunger. They reported from experience that questions relating to hunger were avoided within some households or residential settings. Concerning the hunger scale, the

professionals highlighted that social desirability effects might influence participant reporting, i.e., they might feel obliged to say they are full after receiving a meal even if they are not.

> *"You need to take into account, people with PWS [can be] people pleasers and may give you the answers that you want to hear, and I don't know how you would avoid that because if they are eating in there and especially if you are supplying the meal they may just want to [say they feel full] as thanks".*

In the study described in Chapter 5, participants completed a food stimuli rating scale after the protocol to identify if they regarded the food stimuli used in the paradigm as palatable. Each food stimulus was rated on a scale of 1 to 5: 1 ('I would hate to eat this'), 2 ('I would not eat this'), 3 ('neutral'), 4 ('I would eat this'), 5('I would love to eat this'). Participants were excluded if they scored lower than a three on more than 50% of the food stimuli. The focus groups were asked if the rating scale was appropriate for the study and whether a self-report or parent/caregiver version should be implemented. Most parents/caregivers thought asking people with PWS whether they like, or dislike different food types depicted in the FAB task stimulus arrays was appropriate. However, they thought the rating scale administration timing was important. They suggested it should be completed after lunch to avoid increasing food cravings.

> *"Mary would be happy to answer that [stimuli rating scale] when she thought she was full instead of coming up to snack time. If she saw that, she would want her snack, lunch, or whatever. You would have to time it."*

The theme that emerged from the professional group was the potential impact of specific food stimuli on attentional bias/looking behaviours. For some participants with PWS, certain foods may be considered "forbidden food," i.e., the food they know they are not supposed to eat, such as chocolate, which they thought could potentially influence their attentional bias towards specific food stimuli. The group emphasised knowing what foods are allowed and what is liked. A second theme emphasised using simplified questionnaires wherever possible, e.g., reducing the number of responses from five to three points.

Objective 4: Additional considerations relating to the study protocol

Finally, we invited both groups to contribute additional perspectives on improving the research design and optimising the accessibility for individuals with PWS. The primary theme evident in parent/caregiver participants' narratives was the importance of a person-centred approach to ensure a successful and enjoyable research visit. They indicated that researchers should communicate with the parent/caregiver to accommodate each participant's unique needs as much as possible within the research design. Specific recommendations included 1/ creating a good rapport with the participant before the study, 2/ accommodating anxiety and repetitive questioning by providing FAQs to the participant and allowing the opportunity to meet and discuss any questions the participant may have before the research visit, 3/reassuring the participant by structuring the research visit and providing a schedule ahead of the visit, and 4/ valuing the participant by identifying a personalised thank-you gift that was agreed upon in advance with the caregiver.

Proposed protocol changes and consultation with the scientific advisory panel

After the inductive content analysis of the focus groups was complete, the themes that emerged from the FAB task and proposed adaptations to the study protocol (Table 6.1) were discussed in a meeting with the scientific advisory panel of experts in hyperphagia research in PWS. These themes were discussed with the scientific advisors in the context of feedback provided and the potential implications on the scientific objectives.

Objective 1 related to the study design for hunger and satiety conditions. All parents/caregivers agreed that their loved ones with PWS could complete a 3-4 hour fast and expressed the importance of structured and predictable mealtimes to reduce anxiety. The professional group suggested scheduling participants based on their typical mealtime routine to increase the likelihood of completing the fast. To accommodate individual lunch times, flexibility surrounding the hunger condition time was proposed, with a fast occurring between breakfast and lunch. The scientific advisory panel agreed that there could be flexibility around the timing of the meal but that it would be important to ensure that every participant received the same type of meal (i.e. lunch). Despite variations in timing across participants, this amendment aimed to minimise disruption to meal schedules and increase the chances of participants completing the protocol (see Table 6.1).

The study's second objective was to standardise the meal content provided in the study protocol. The parent/caregiver group emphasised the importance of prior communication regarding the menu to avoid potential sources of anxiety. The professional group highlighted the importance of communication and cooperation among the researcher, parent/caregiver, and residential setting to identify any aspects that may increase stress or anxiety. While acknowledging the difficulties of standardising the meal for individuals with PWS, the scientific advisory panel emphasised that not doing so would be a significant study weakness. Variances in meal composition can impact satiety levels across participants, leading to potential confounding factors in assessing attentional bias for food cues (Hobden et al., 2017). Standardising the meal would allow the research team to control these variables and enhance the study's internal validity, ensuring that it accurately measures what it is intended to measure. On this basis, the meal was standardised by providing all participants with a basic lunch consisting of a piece of fruit, a sandwich, a yoghurt and a bottle of water. The team also discussed the meal options with the parent/caregiver before the visit. If the standardised meal option was not feasible for a participant, a second option was available, where the parent/caregiver could bring their version of the standardised meal from home that would consist of the same items, a sandwich, yoghurt and a piece of fruit. All details of the home meal would be provided to the researcher in advance, who would calculate the caloric and nutrition content to ensure it was within the parameters of the standardised meal. The cost of the meal was reimbursed to the parent/caregiver.

The third objective addressed the adaptation of the behavioural questionnaires in the study protocol, specifically the SLIM scale and food stimuli rating scales. The parent/caregiver group identified that their family member with PWS did not have insight into their feelings of hunger and would have difficulty reporting hunger subjectively. In contrast, the professional group highlighted the social desirability effects that may influence participant reporting. The scientific

advisory panel agreed it would be best to omit the SLIM measure, and there was a lack of valid or accurate questionnaire-type measures to capture states of hunger and satiety. The panel also agreed that a simplified three-point version of the food stimuli rating scale would be useful for collecting from PWS participants. The team proposed adding a parent/caregiver version of the food stimuli rating scale to the protocol. The parent/caregiver would be asked to complete the same scale as the PWS participants about how much their loved one with PWS liked each food and how often their loved one with PWS would be allowed to eat the food shown from "often," "sometimes" and "never." The panel agreed that collecting the food stimuli rating scale in parents/caregivers would be interesting as it would indicate if certain food stimuli were "forbidden foods", and then this would be accounted for in the analysis.

Objective four was to gather additional perspectives on improving the research design and accessibility for individuals with PWS. Parents/caregivers emphasised the importance of a person-centred approach. They suggested recommendations such as creating a good rapport with the participant before the study, accommodating anxiety and repetitive questioning, structuring the research visit, and providing a personalised thank-you gift. Additional preparatory steps to the study protocol included a remote consultation with participants to introduce the research team and answer questions about the study visit (Appendix 7). A visual schedule was developed for participants before the visit to provide greater certainty (figure 6.3). Visual schedules have been recommended to reduce anxiety surrounding research visits and improve communication with people with autism (Knight et al., 2015).

Table 6.1: Overview of adaptations made to the FAB task protocol

	Original FAB Task Protocol	Adapted FAB Task Protocol for PWS
Design of Hunger and Satiety Conditions	Hunger Condition: 3-4* hour fast Satiated Condition: 30 min postmeal	Hunger Condition: 3-4 hour fast Satiated Condition: 30 minutes postmeal In addition, testing in the hunger condition will be scheduled for each participant immediately before the typical lunchtime.
Meal for Satiety Condition	Standardised meal provided by the research team	More flexibility was provided with the standardised meal. Food choices were discussed with the parent/ caregiver before the visit. Option included for parent/ caregiver to bring a home version of the standardised meal. A detailed description

		of the home meal will be collected, and the calorie and macronutrient content will be accounted for.
Hunger/ Satiety Rating Scales	Satiety Labelled Magnitude Scale (Adults) Teddy the Bear Scale (Children)	Hunger/ satiety rating scales were removed from the protocol
Food Stimuli Rating Scale	Food stimuli rating scale (5-point Likert scale)	Food stimuli rating scale (3-point Likert Scale) for participant An additional rating scale (3-point Likert Scale) was included to be completed by the participant and parent/caregivers. This scale would ask if each food stimulus was food the participant had "often," "sometimes", or "never."
Debrief	Honesty Questionnaire	The Honesty Questionnaire was removed from the protocol

* Participants under 12 years were asked to fast for 3 hours, and participants over 12 years were asked to fast for 4 hours.

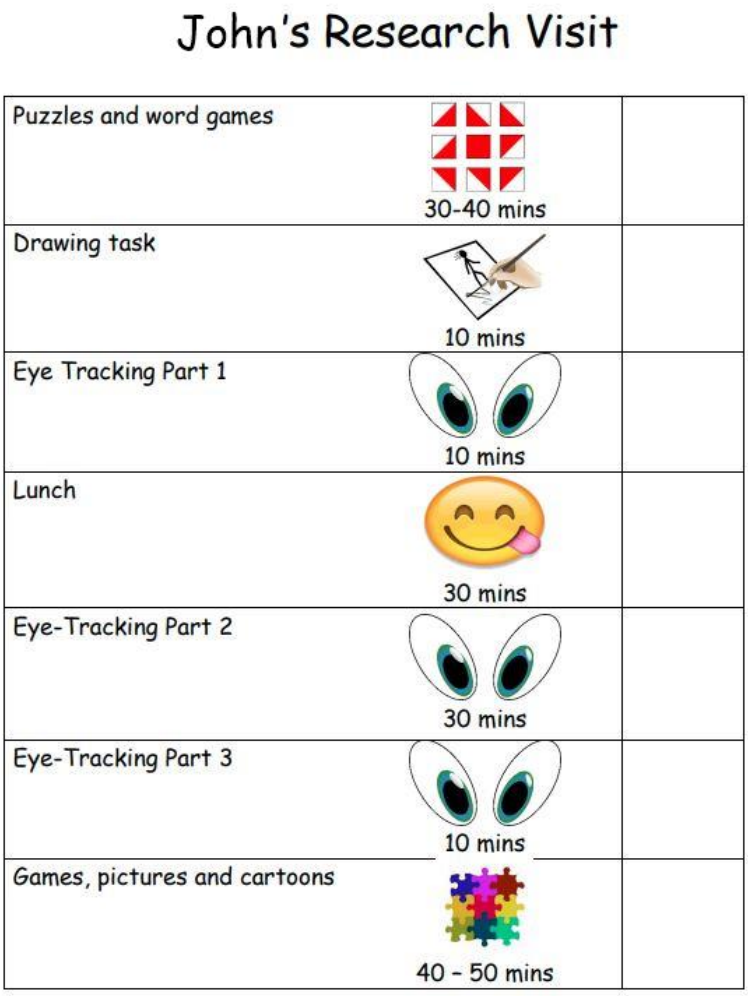

Figure 6.3: Sample visual schedule for the participant (pseudo name used).

6.4. Discussion

6.4.1. Overview of the study

In this chapter, I describe a study that aimed to develop an adapted version of the FAB task protocol for use in a PWS cohort using a participatory approach that involved parents, caregivers, and professionals. Focus groups were conducted to anticipate potential challenges and issues that may arise during the study, particularly regarding the hunger condition, standardised meal approach, and use of behavioural questionnaires. To ensure the scientific rigour of the study was maintained, a scientific advisory panel was consulted during the adaptation process. The ultimate goal was to adapt the FAB task protocol for children and adults with PWS. It was hypothesised that involving parents, caregivers, and professionals in the adaptation process would improve the relevance and suitability of the FAB task protocol for individuals with PWS, thereby increasing the likelihood of their participation and completion.

6.4.2. Key adaptations

The study identified key adaptations based on themes from the focus groups with parents/caregivers and professionals. The original FAB task defined the "hunger" condition as a 3-hour fast for participants aged 4-11 years and a 4-hour fast for all participants aged 12 years and older. Findings from parents/caregivers and professionals indicated that the fasting length would be feasible but delays to a meal could cause significant anxiety for participants with PWS. The importance of providing a structured and predictable mealtime routine was emphasised, with parents/caregivers and professionals stressing the need for clear communication and cooperation when planning the standardised meal. The protocol was adapted to allow flexibility in scheduling the research visit so that the satiety condition would align with the participant's typical meal schedule.

Regarding the meal content provided in the study protocol, the original design used a standardised meal approach, with all participants provided a standardised menu from which they had a small number of choices. However, feedback from the parent/caregiver group identified two potential challenges with this approach: anxiety related to providing a standardised meal and the importance of accounting for individual differences in planning for the FAB task study visit. To address this, parents/caregivers emphasised the importance of liaising with them to view the menu and identify any items that may increase stress or anxiety for the participant with PWS. The professional group also emphasised the importance of communication and cooperation between the researcher and the participant's parent/caregiver or residential setting. The protocol was adapted so that there was a second option for participants regarding meal options - parent/caregiver could bring their version of the standardised meal, and the researcher would calculate the caloric and nutrition content to ensure it was within the parameters of the standardised meal.

Finally, the hunger rating scale was removed from the study protocol due to concerns expressed by parents/ caregivers and professionals regarding using a hunger rating scale with participants with PWS. The parent/caregiver group identified that their family member with PWS may not have insight into their feelings of hunger and would have difficulty reporting hunger subjectively. The professional group reported that questions about hunger were avoided within some households or residential settings and that social desirability effects may influence participant reporting. The food stimuli rating scale was also simplified from a five point-scale to a three-point scale to make it easier to understand and complete as fewer response options reduce the cognitive demand of making a choice. An additional preparatory step in the form of a remote consultation was added to the protocol to accommodate any potential anxiety and repetitive questioning from the participant.

One potential challenge encountered in this study was the potential for contradictory views between the perceived necessary adaptations by stakeholders and the scientific rigour required by researchers. To address this issue, ongoing communication and collaboration were essential in balancing these competing perspectives. During the focus groups, stakeholders were invited to express their preferences on communicating the study's findings and decisions. It was agreed upon that the research team would generate a report (Appendix 7) and a concise one-page summary of all the findings and adaptations (Appendix 7), which would be disseminated before an additional online conference meeting. The stakeholders were presented with the focus

group's findings and feedback from the scientific advisory panel. The adapted FAB task protocol was presented, and the majority of the session was devoted to a question-and-answer period between the research team and the stakeholders.

This study holds significant importance in codesign and engagement research for PWS, considering the number of ongoing clinical trials for hyperphagia (Mahmoud et al., 2023). Codesign and engagement approaches prioritise the active involvement of stakeholders. By employing a participatory approach that involves parents, caregivers, and professionals, the current study exemplifies these principles and contributes to the broader field of codesign and engagement research in PWS. This study also shows the importance of promoting a more inclusive and collaborative approach to research, recognising the expertise and perspectives of individuals with developmental disabilities. Notably, this study represents the first of its kind in the context of PWS, where a participatory approach was employed to adapt a study protocol. The absence of similar studies highlights the novelty and need for this type of research in PWS. By involving PWS stakeholders in research design and implementation, the study sets an example for future endeavours in engaging individuals with PWS and other rare genetic syndromes and their support networks, ultimately leading to more impactful and person-centred outcomes. Additionally, Crocker and colleagues' (2018) findings support the case for involving PWS stakeholders in future clinical trial planning, as patient enrolment significantly increases with the inclusion of participatory approaches.

6.4.3. Strengths of the study

The study's strength lies in its participatory approach, which involved parents/caregivers and professionals with lived experience in the research process. By incorporating the perspectives of stakeholders and researchers with scientific expertise, the study enabled the development of a protocol adapted to the PWS community's needs through engagement and co-development. Participatory research has led to more relevant research questions, improved study design, and greater participant satisfaction and retention (Wallerstein & Duran, 2010). Codesign, a type of participatory research, has been shown to improve the relevance and acceptability of interventions and increase participant engagement and satisfaction (Greenhalgh et al., 2016). Therefore, using a participatory approach, focus groups, and codesign in adapting the protocol will likely lead to a more rigorous and meaningful study that better meets the needs of people with PWS and their caregivers. The adaptation process was guided by expert input and scientific rigour. The panel's insights and recommendations contributed to the credibility and validity of the adapted FAB task protocol.

Additionally, the communication plan implemented in the study was crucial for maintaining transparency and collaboration with stakeholders. Generating a comprehensive report and a concise summary of findings and adaptations facilitated effective communication between the research team and stakeholders. The online conference meeting allowed stakeholders to ask questions, express their views, and contribute to decision-making. This collaborative approach enhanced the study findings' relevance, acceptability, and applicability. This approach aligns with the literature on participatory research and codesigns, which suggests that involving stakeholders in the research process can lead to more meaningful and effective research outcomes (Wallerstein & Duran, 2010).

6.4.4. Limitations of the study

A limitation of the current study was the omission of engagement with participants with PWS concerning the protocol adaptation. While involving parents/caregivers in the focus group is valuable, they may not have the same perspectives and experience as individuals with PWS. This may result in a protocol that does not fully capture the experiences of individuals with PWS or meet their needs. The challenge for the present study was to ensure that individuals with PWS remained blinded to the objectives of the FAB task, as this could influence their behaviour during the administration. As PWS is a rare condition, I wished to maximise the number of participants available to the study and therefore did not engage them in this work. An important future research direction of this research is to invite people with PWS to share their experiences participating in the study and involve them in the codesign process for any additional adaptation to the FAB task protocol.

A potential limitation of the current study is the reliance on focus groups rather than one-on-one interviews. It is important to recognize that a group setting can amplify social desirability bias—a tendency to present oneself and one's social context in a manner perceived as socially acceptable but not entirely reflective of reality (Bergen & Labonte, 2019). Opting for one-on-one interviews might have established a more confidential setting, potentially mitigating social desirability bias. The degree of social desirability is influenced by the sensitivity of the topic and prevailing societal attitudes (Grimm et al., 2010). It's worth noting that the present study focused on gathering information from participants related to a study protocol rather than delving into personal disclosures. Given the comparatively lower sensitivity of the topics discussed in these focus groups, it is plausible that social desirability bias may not have been as pronounced.

Another potential limitation of the focus group setting is the possibility of participants succumbing to groupthink, where consensus overshadows critical evaluation, a risk mitigated by one-on-one interviews (Fusch et al., 2022). To comprehensively understand the impact of social desirability and groupthink on our results, future research should triangulate focus group data with other qualitative measures. This might involve a mixed-methods approach, integrating surveys, social desirability scales, or follow-up individual interviews to assess and mitigate social desirability bias.

6.4.5. Future Directions

This study successfully adapted the FAB task protocol for use in a PWS cohort through a participatory approach involving parents, caregivers, and professionals. Key adaptations were made to the hunger condition, standardised meal approach, and behavioural questionnaires, emphasising communication and cooperation between researchers and participants' parents/caregivers. Future directions include testing the adapted FAB task in PWS and collecting data on acceptability. The study's findings have implications beyond the PWS population, as the findings can be applied in other research contexts, such as collecting eye tracking data in neurodevelopmental conditions or in other cohorts who experience hyperphagia. This chapter highlights the importance of considering the needs of individuals with PWS in research protocols and the benefits of involving stakeholders in the research process.

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Chapter 7: The food attention bias (FAB) task – Eye tracking as a marker of hyperphagia in PWS

7.1 Introduction

In chapters 5 and 6, I described hyperphagia and its significant implications for individuals diagnosed with PWS and their families. Addressing hyperphagia holds utmost importance for individuals with PWS, as evidenced by a recent qualitative study where all participants strongly expressed a desire to participate in future clinical trials involving new medications to regulate hunger (Dykens et al., 2021) Hyperphagia contributes significantly to the burden experienced by caregivers (Kayadjanian Id et al., 2021), and it also emerges as their primary treatment priority (Tsai et al., 2018). These findings collectively emphasise the critical role of effectively managing hyperphagia in promoting the overall well-being of individuals with PWS. However, despite the recognised significance of addressing hyperphagia, the lack of objective measures for assessing its severity poses a substantial barrier to evaluating the effectiveness of emerging interventions. The Food Attention Bias (FAB) task was developed to address this gap in measures of hyperphagia. In Chapter 5, the task was tested in a cohort of typically developing participants to understand how attentional bias interacts with states of hunger and satiety. In Chapter 6, through stakeholder consultation and co-design, the FAB task protocol was thoughtfully adapted to ensure its appropriateness in the PWS population. The overall aim of this final chapter was to use the FAB task in a cohort of individuals with PWS to determine if there are observed differences in their attentional bias to food in states of hunger and satiety compared with an age and gender matched comparison group.

7.1.1 Therapies for managing hyperphagia in PWS

Hyperphagia in PWS manifests as an intense, persistent sensation of hunger accompanied by food preoccupations, an extreme drive to consume food, food-related behaviour problems, and a lack of normal satiety (Schwartz et al., 2021b). Currently, there is a lack of effective treatments for appetite suppression and weight control in individuals with PWS (Tan et al., 2020), and the standard approaches mainly involve food-related interventions and environmental controls to prevent unsupervised access to food (Tan et al., 2020). Dietary restriction and modifications can reduce weight gain but increase caregiver burden, while regular physical activity is hindered by low activity levels, fatigue, and exercise-induced pain (Morales et al., 2019; Muscogiuri et al., 2021). Growth hormone replacement therapy can improve body composition in PWS but has limited efficacy in reducing hyperphagia and may even exacerbate appetite in some cases (Grugni et al., 2016).Bariatric surgery, although effective for inducing weight loss in morbid obesity, showed unfavourable outcomes in long-term use for PWS patients (Tan et al., 2020). These approaches offer limited long-term efficacy in improving hyperphagia in individuals with PWS.

The pharmacotherapeutic options for PWS are expanding, with several drugs in development showing promise for treating PWS-associated hyperphagia and obesity. These potential treatments include beloranib, setmelanotide, a diazoxide choline controlled-release tablet (DCCR), an unacylated ghrelin analogue, oxytocin and related compounds and glucagon-like

peptide 1 receptor agonists (Mahmoud et al., 2023; Miller et al., 2023; Roof et al., 2023). These drugs target various pathways, such as oxytocin receptors, leptin, and gut peptides. Ongoing evaluation aims to determine their efficacy and safety in individuals with PWS. While some have completed Phase 2 or 3 trials, others are still in preclinical or early trial stages (Mahmoud et al., 2023). The emergence of these promising pharmacological interventions holds great potential for treating obesity and potentially hyperphagia.

Nevertheless, as highlighted above, an urgent and pressing demand exists for implementing objective, robust, reliable, and reproducible treatment biomarkers of hyperphagia. Such biomarkers are essential in ascertaining the true efficacy of these novel therapeutic approaches. The absence of well-defined and quantifiable indicators poses significant challenges in accurately assessing and validating the effects of these interventions. Thus, it is crucial to emphasise the immediate necessity for developing new measures of hyperphagia to address this critical gap in current research.

7.1.2 Current methods of measuring hyperphagia

Existing measures of hyperphagia, including the Hyperphagia Questionnaire (HQ) and Food Related Problems Questionnaire (FRPQ), are limited as these do not account for environmental control measures that may be in place for individuals with PWS. They are vulnerable to placebo effects in clinical trials (Hollander et al., 2021). A strategic goal of the PWS Clinical Trials Consortium (CTC) is to develop outcome measures to assess the efficacy of novel drug therapies in treating hyperphagia. The US Federal Drug Administration (FDA) emphasised the need for reliable measures at a Critical Path to Innovation meeting with the PWS Clinical Trials Consortium and underlined novel hyperphagia biomarkers as an area for development (FPWR-CTC, 2019). Several studies have highlighted the importance of investigating biomarker outcome measures to mitigate the placebo response observed in caregiver and clinician-reported outcome measures (Duis et al., 2019; Hollander et al., 2021; Key et al., 2020).

The incomplete understanding of the underlying neurobiology of hyperphagia in PWS has impeded the progress of biomarker development in this domain. Behaviour investigations of hyperphagia have shown that people with PWS experience a delayed onset of satiety compared to healthy weight controls when provided with unrestricted access to food, and a higher caloric load is required to reach a perceived state of satiety as measured by self-report hunger rating scales (Holland et al., 1995). Functional magnetic resonance imaging (fMRI) studies have revealed disturbances in cortical and subcortical structures associated with satiety and reward processes in individuals with PWS, suggesting hyperphagia is a result of both heightened reward sensitivity to food and diminished satiety response (Huang & Cai, 2023). Following glucose ingestion, a notable delay in activation was observed at the hypothalamus and other satiety-associated brain regions, such as the insula, ventromedial prefrontal cortex (VMPFC), and nucleus accumbens, in individuals with PWS compared to a healthy weight control group (Shapira et al., 2005). Heightened activation within the VMPFC was observed when exposed to food-related stimuli, which supports the involvement of neural pathways governing reward-related behavioural regulation of food responses in individuals with PWS (Miller et al., 2007). More pronounced altered brain functioning was found in individuals with PWS compared to control groups under a satiety condition compared with a fasting condition (Holsen et al., 2012), which supports investigating hyperphagia across states of hunger and satiety.

7.1.3 Eye tracking as a methodology for measuring hyperphagia

As discussed in Chapter 1 and Chapter 4, eye tracking approaches represent an alternative approach to measuring cognitive processes (Eckstein et al., 2017) and, therefore, may provide an opportunity to objectively measure the cognitive processes that underlie hyperphagia in PWS. As previously introduced in Chapter 5, eye tracking methodologies can be used to measure attentional bias (AB), where an individual's perception is affected by selective factors in their attention and may be affected by altered reward sensitivity in the brain. An attentional bias to food occurs when food cues capture and hold visual attention (Field et al., 2016). As shown in Chapter 5, attentional bias to food stimuli was reduced in healthy participants after consumption of a standardised meal. These findings from Chapter 5 indicate that after eating, there is a decline in attentional bias towards food stimuli, which may indicate a decrease in the motivational significance of food. Therefore, evaluating attentional bias towards food in individuals with PWS may offer an objective and reliable measure to assess the absence of satiety.

In Chapter 5, I discussed how many existing attentional bias eye tracking paradigms often demand language proficiency, cognitive abilities, and motor skills, which restrict their applicability in individuals with PWS (Hagan et al., 2020; Hardman et al., 2021). Free-viewing paradigms have emerged as a promising approach for assessing attentional bias in individuals with limited language or cognitive abilities (Kong et al., 2022). Key and colleagues (2020) demonstrated the feasibility of employing a free-viewing paradigm to measure visual attention to food in individuals with PWS. Their study used stimulus arrays consisting of 20-40 stimuli, with three categories: food stimuli, animal stimuli (considered high interest), and household items (deemed low-interest stimuli). The stimulus arrays were presented with food and animals (competing high-interest stimuli) or food and household items (competing low-interest stimuli). The results indicated that individuals with PWS explored and revisited a significantly higher number of food stimuli only when food was presented in combination with low-interest stimuli and not when food stimuli were presented alongside low-interest stimuli.

This study provides valuable insights into the accessibility of using eye tracking in PWS. However, it is important to acknowledge certain limitations that may have influenced the findings. Building upon the insights from Chapter 4, it is known that individuals with PWS exhibit automatic orientation to faces and prolonged gaze towards faces. Considering the inclusion of animal faces and the existing literature demonstrating the cross-species face pop effect, these factors may have influenced the measurement of attentional bias towards food. Another limitation pertains to the measurement of participants in a single condition. MRI studies have suggested that the contrast between controls and individuals with PWS is most pronounced in the satiated condition. In Key et al.'s study, all tracking sessions were completed between 9–11:15 am, implying variation in time elapsed since food intake.

Additionally, attentional bias was measured only once, whereas recent eye tracking studies have highlighted the significance of assessing fluctuations in attentional bias to food (intra-participant variability). The FAB task protocol was specifically developed to capture attentional biases towards food stimuli in individuals with PWS during pre and postmeal conditions to further expand on the valuable findings and address the limitations. This approach allows for a

more comprehensive assessment of attentional processes related to food, taking into account the impact of hunger and satiety states on attentional bias.

7.1.4 Aims and Hypotheses

The primary objective of this chapter was to investigate and compare the performance of individuals with PWS and a matched comparison (COM) group on the FAB task following a period of fasting (premeal) and 30 minutes after eating (postmeal). I hypothesised that attentional bias to food stimuli would decrease in the postmeal condition ("satiated state") in the COM group, as reported in a TD group in Chapter 5 but not in the PWS group. I further hypothesised that attentional bias to food stimuli in the postmeal condition would inversely correlate with hyperphagia symptoms as measured by caregiver-report questionnaires, the HQ and FRPQ.

7.2 Methods

7.2.1 Methods and Clinical Assessments

Fifty-four participants (27 PWS and 27 COM) were recruited to the study (see Chapter 2, section 2.1.3 for inclusion and exclusion criteria). Members of PWS and COM groups were assessed using several questionnaires to measure demographics, eating behaviours and cognition, which are outlined fully in Chapter 2, section 2.2.1. For cognitive assessment, participants under six underwent evaluation using the Mullen Scales of Early Learning (MSEL), while older participants had their IQ assessed using the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II). Caregivers were asked to complete the Hyperphagia and Food Related Problems questionnaire to assess hyperphagia and food-related behaviours. The FAB task from Chapter 5 was used to measure attention to food stimuli across two conditions; premeal and postmeal. Detailed information about the task, set-up, and data collection procedure can be found in Chapter 2. sections 2.3.2 and 2.3.3.

7.2.2 Adapted FAB Task Protocol for PWS

The study protocol was co-designed based on the recommendations of PWS caregivers and professionals, as described in Chapter 6. The key recommendations from the study were that the protocol was adapted to allow flexibility in scheduling the research visit so that the premeal and postmeal conditions would align with the participant's typical meal schedule. The protocol was adapted so that there was a second option for participants regarding meal options – the parent/caregiver could bring their version of the standardised meal, and the researcher would calculate the caloric and nutrition content to ensure it was within the parameters of the standardised meal (450-550 calories). Finally, the Satiety Labelled Intensity Magnitude (SLIM) scale was removed from the study protocol due to concerns expressed by parents about causing participants distress by asking them about their hunger. To incorporate these recommendations into the protocol, a number of preparatory steps were added to the study visit.

A pre-visit phone call with parents or caregivers of PWS participants and COM participants (Appendix 7). The purpose of this pre-visit phone call was to action the recommendation in Chapter 6 regarding the personalisation of the food choices for individuals with PWS and to determine the optimal timing of the fast for the PWS individual. The fast length was set at 3h

for participants aged 12 years and younger and at 4h fast for participants 12 years and above. The fast was timed to 3 or 4h following the participant's typical breakfast time. The composition of the standardised lunch was also discussed with the caregiver. This consisted of a sandwich, a piece of fruit, a yoghurt and a bottle of water If caregivers thought that the provision of a standardised lunch by the research team would induce anxiety in the participant, flexibility was offered to allow the caregiver to prepare a lunch consisting of the same components at home and take it to the study visit and ensure it fell within the calorie range of 450-550 calories. The nutrient content of this meal was provided to the researcher on the day. Three of the 54 participants (all PWS) opted to bring their standardised lunch (n=3 participants). A visual schedule was also prepared for each participant before the research visit so they would be fully informed of what tasks they would be doing on the day (Appendix 7). Participants were also offered an opportunity to meet the research team via Zoom before the visit to ask any questions about the visual schedule so that participants were more familiar with the research team.

On the day of the study visit, participants complete the IQ assessment. The FAB task was presented to participants as described in Chapter 2, the premeal condition. Participants were then given a standardised meal consisting of a piece of fruit, a sandwich, a yoghurt and a bottle of water. Thirty minutes after consuming the standard meal, the FAB task paradigm was presented again, the postmeal condition.

7.2.3 Data Analyses

Participants were required to have a minimum of three valid trials from each meal condition (premeal and postmeal) to be included in the analysis. A trial was considered valid if two conditions were met: firstly, the participant directed their gaze towards the stimulus array for more than 5000ms out of the 10000ms duration, and secondly, the proportion of valid samples exceeded 50%. In other words, the eye tracker successfully captured and recorded the participant's eye location more than 50% of the time the eyes were sampled—the Tobi X2-60 eye tracker used in the study samples at a rate of 60 times per second. Out of the initial 27 participants with PWS, 23 were included in the data analysis, along with 23 matched COM participants. The exclusion criteria were applied to three PWS participants: one did not complete the postmeal condition of the task, and the other two were excluded due to having less than three valid trials per meal condition. Only participants over the age of 12 years were included in the eye tracking analysis (n = 15). Younger children were removed as they may not have entered hyperphagia yet. According to Miller et al., 2011, Nutritional Phase 3 in PWS is characterised by hyperphagia and the median age of onset of phase three is eight years and the quartiles are 5-12 years. Only participants over age 12 years were included to ensure that the PWS participants included in the analysis were hyperphagic.

The proportion of valid trials in which the participants' orientation was directed towards the food AOI first was calculated for each group (COM, PWS) and meal condition (Premeal and Postmeal). To examine if the participants' orientation towards the food exceeded the chance level of 0.2, given the presence of five stimuli within each array, four one-way t-tests were conducted. The LSD correction was applied to account for multiple comparisons. Group differences between individuals with PWS and the COM group regarding their orientation towards food stimuli were assessed using two-way repeated measures ANOVA.

Proportional dwell time to the food areas of interest (AOI) and proportional fixation count to food AOIs were selected as dependent variables to measure attentional bias to food stimuli. To ensure that all participants with PWS were in nutritional phase three, commonly referred to as "hyperphagic," the analysis included only participants over the age of 12 years (15 PWS and 15 COM; Miller et al., 2007). The effects of group (PWS and COM) and meal conditions (premeal and postmeal) on the dependent variables were assessed using linear mixed models, as PWS participants were more likely to have more missing trials than the COM group.

The model-building process followed a maximal-that-improves-fit approach, with Akaike's information criterion (AIC) used to assess model fit (see Chapter 2, section 2.5 for a full description of linear mixed models). Random effects were initially established for intercepts and slopes of the two variables (participant and stimulus array), and redundant or detrimental random effect estimates were subsequently removed. Fixed effects were introduced, including the within-subject variable of meal condition, the between-subject variable of group, and their interaction (meal condition*group). Age and IQ were considered as covariates in the model but were removed if redundant or worsened model fit. Spearman's rank correlations were conducted to explore associations between the primary variables of interest, phenotypic characteristics, and controlling for IQ and age.

7.3 Results

7.3.1 Participant Demographics and Behavioural Data

Forty-six participants aged 5 to 42 participated in the study. Twenty-three participants with PWS (M age = 16.8, SD = 7.2; 15 female) and 23 typically developing participants (M age = 17.9, SD = 8,2; 15 female) (see table 7.1). The groups did not differ statically in terms of age or BMI, but as expected, statistically significant differences were found in mean total IQ scores and hyperphagia measures (see table 7.1)

Table 7.1: Participant characteristics and behavioural data

	COM (n=15, 15F/8M)	PWS (n=15, 15F/8M)		
	M (SD)	*M (SD)*	*t statistic*	*P value*
Age	20.7 (5.0)	21.6 (6.3)	-0.47	.64
IQ	103.7 (12.9)	64.2 (13.3)	4.74	<.001
BMI	21.5 (8.1)	20.5 (5.3)	0.40	.69
Hyperphagia Questionnaire				
Behaviour	5.0 (0.0)	9.8 (5.1)	-1.28	.22
Drive	4.0 (0.0)	10.9 (3.7)	-2.59	.02
Severity	2.0 (0.0)	4.0 (1.5)	-1.54	.10

Total	12.0 (1.7)	24.7 (9.1)	-2.35	.03
Food Related Problems Questionnaire				
Preoccupation with food	5.7 (3.1)	10.8 (3.4)	-2.39	.03
Impairment of satiety	9.3 (3.1)	20.1 (3.8)	-4.48	<.001
Stores food	2.0 (2.0)	7.7 (5.8)	-1.65	.12
Eat inedibles	0.0 (0.0)	2.3 (2.6)	-1.46	.17
Negative responses	1.3 (1.5)	8.7 (4.5)	-2.74	.17
Total	17.5 (3.3)	49.6 (15.5)	-4.01	<.001

7.3.2 The proportion of first looks to Food AOIs.

The proportion of first looks to the food AOI for the COM group in the premeal condition was significantly above the chance level (p = .05) but did not survive LSD correction. The proportion of first looks to the food AOI for the PWS group in the premeal condition was not significantly above chance (p = .18) (Table 7.2, Figure 7.1). The proportion of first looks to the food AOI was not statistically above chance level for the COM group in the postmeal condition (p = .33) or postmeal (p = .08) conditions (Table 7.2, Figure 7.1). Group differences in the proportion of first looks to food AOIs across the two meals condition were evaluated using repeated measures ANOVA. No significant main effects were found (group, p = .59; meal condition, p = .68; group*meal condition, p = .50).

Table 7.2: The number of valid trials and proportion of trials with the first look at the food AOI

Meal Condition	AOI	Measure	COM (n=15) Mean (sd)	PWS (n=15) Mean (sd)
Premeal	Food	number of valid trials	9.07 (1.16)	7.13 (2.64)
		Proportion of trials with the first look at food	.28 (.14)	.28 (.21)
Premeal	Non-Food	number of valid trials	9.07(1.16)	7.13 (2.64)
		Proportion of trials with the first look at food	.18 (.04)	.18 (.05)
Postmeal	Food	number of valid trials	8.87 (1.6)	6.53 (2.2)
		Proportion of trials with the first look at food	.24 (.14)	.9 (.18)
Postmeal	Non-Food	number of valid trials	8.87 (1.6)	6.53 (2.2)
		Proportion of trials with the first look at food	.19 (.03)	.18 (.05)

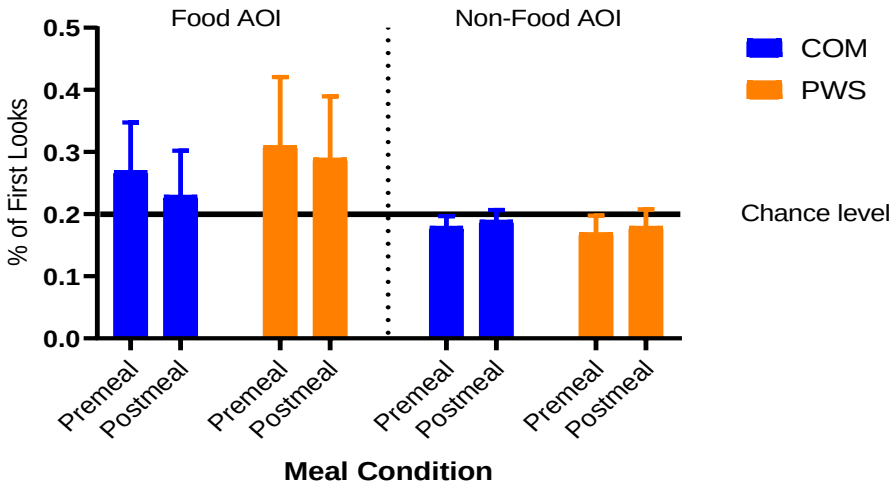

Figure 7.1: Proportion of first looks to food and non-food AOIs for PWS and COM. The proportion of first looks to food AOIs was not statistically above chance level for either group or meal condition, and no statistically significant group differences were observed.

7.3.3 Proportion dwell time

A linear mixed effects analysis was conducted with proportional dwell time to food AOIs as the dependent variable. Fixed factors included group (PWS, COM), meal condition (Premeal, Postmeal) and their interaction (group*meal condition). The model included a random factor for participant slope (p = .005). IQ and age were included as covariates but did not improve the fit of the model and were removed (see Table 7.3). The analysis revealed no significant main effect of Group; F(1, 180.128) = 2.0, p = .159. A significant effect of meal condition was observed F(1, 463) = 7.09, p =.008. There was a significant two-way interaction between the group and meal condition, F(1, 469.073) = 5.71, p = .017 (see Table 7.3). The effect observed of group*meal interaction suggests that groups differed across meal conditions (Figure 7.2). The descriptive statistics in Table 7.4 show that the proportional dwell time for food AOIs in the COM group decreased from pre to postmeal conditions, while the proportional dwell time for food AOIs in the PWS group increased from pre-to-postmeal conditions.

Table 7.3: Fixed and random effect estimates for proportion dwell time for food AOIs

Fixed effects			
	Estimate (Std. error)	*95 % CI*	*p*
Intercept	.43 (.01)	[.23, .62]	<.001
Group	-.09 (.06)	[-.21, .04]	.159
Meal Condition	-.14 (.05)	[-.25, -.04]	.008
Group*Meal Condition	.08 (.03)	[.01, .15]	.017
Random effects			
	Variance (Std. error)	*p*	*95 % CI*

Residual	.04 (.002)	< .001	[.02, .03]
Participants (Intercept)	.01 (.002)	.005	[-.00, .01]

Degrees of freedom estimation: Satterwaitte.

Table 7.4: Proportional Dwell time for Food and Non-Food AOIs

		PWS (n=15) Mean (SD)	COM (n=15) Mean (SD)
Premeal	Food	.27 (.22)	.28 (18)
	Non-Food	.18 (.05)	.18 (.05)
Postmeal	Food	.30 (.26)	.22 (.15)
	Non-Food	.18 (.06)	.20 (.04)

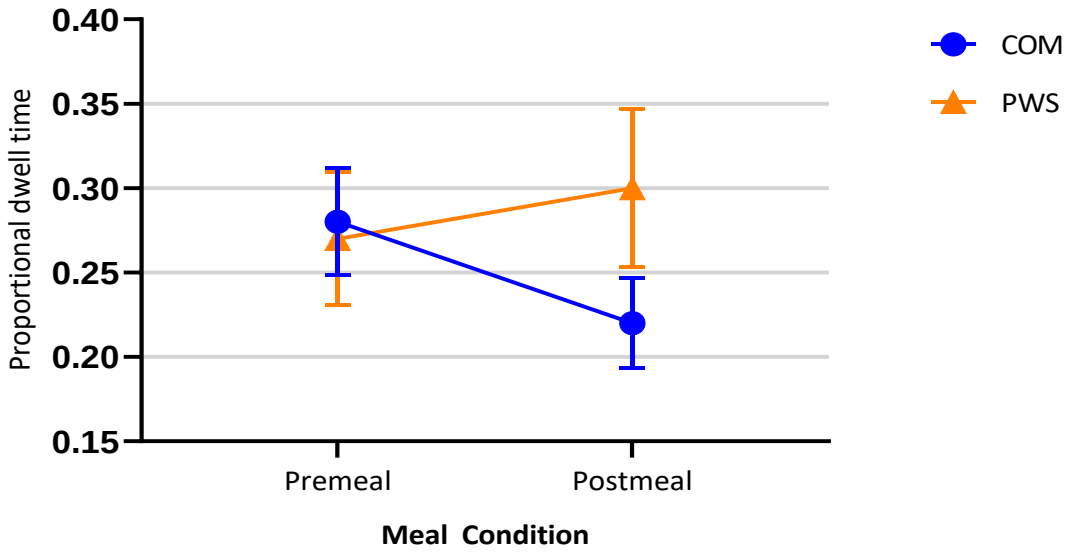

Figure 7.2: Proportional dwell time to food stimuli between groups and across meal conditions. Error bars correspond to 95% CIs.

7.3.4 Proportional Fixation Count

A linear mixed effects analysis was conducted with proportional fixation count to food AOIs as the dependent variable. Fixed factors included group (PWS, COM), meal condition (Premeal, Postmeal) and their interaction (group*meal condition). The model included a random factor for participant slope (p = .005). IQ and age were included as covariates but did not improve the fit of the model and were removed (see Table 7.5). The analysis revealed no significant main effect of Group; F(1, 180.128) = 1.6 p <.221. A significant effect of meal condition was observed F(1, 463) = 5.57, p =.019. There was a significant two-way interaction between the group and meal condition, F(1, 470) = 4.72, p =.030 (see Table 7.5 and Figure 7.3). The effect observed of

group*meal interaction suggests groups differed across meal conditions. The descriptive statistics in Table 7.6 show that the proportional fixation count for food AOIs for the COM group decreased from pre to postmeal conditions, while the proportional fixation count for food AOIs in the PWS group increased from pre-to-postmeal conditions.

Table 7.5: Fixed and random effect estimates for proportional fixation count for food AOIs

Fixed effects			
	Estimate (Std. error)	*95 % CI*	*p*
Intercept	.36 (.08)	[.19, .53]	<.001
Group	-.06 (.05)	[-.17, .04]	.211
Meal Condition	-.11 (.04)	[-.19, -.02]	.019
Group*Meal Condition	.06 (.03)	[.01, .12]	.03
Random effects			
	Variance (Std. error)	*p*	*95 % CI*
Residual	.04 (.002)	< .001	[.02, .03]
Participants (Intercept)	.01 (.002)	.005	[.00, .01]

Degrees of freedom estimation: Satterwaitte.

Table 7.6: Proportional fixation count for Food and Non-Food AOIs

		PWS	COM
		Mean (SD) (n=15)	Mean (SD) (n=15)
Premeal	Food	.28 (.20)	.31 (.19)

	Non-Food	.18 (.05)	.17 (.05)
Postmeal	Food	.30 (.25)	.26 (.19)
	Non-Food	.18 (.06)	.18 (.05)

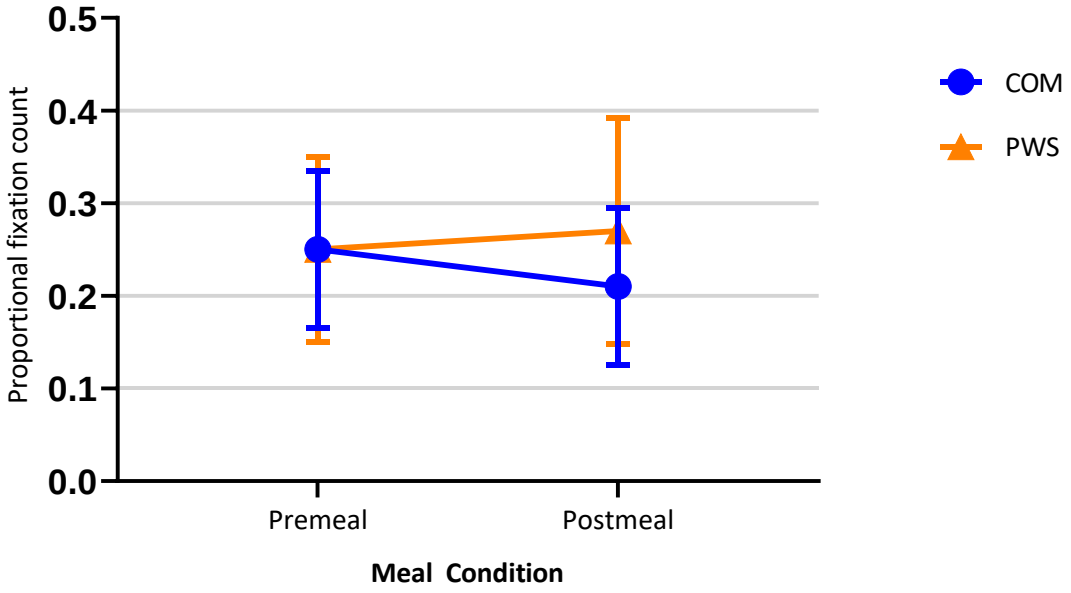

Figure 7.3: Proportional fixation count to food stimuli between groups and across meal conditions. Error bars correspond to 95% CIs.

7.3.5 Association between eye tracking measures and behavioural measures of hyperphagia

As highlighted above, it was hypothesised that proportional dwell time and proportional fixation count would be correlated with food and eating behaviours measured by HQ and FRPQ. Additionally, the relationship between eye tracking variables and the "Impairment of Satiety" subscale of the FRPQ was explored, considering its relevance to the FAB task, which aimed to capture a lack of satiety. The results indicated that the total score on the HQ did not show a significant association with increased proportional dwell time to food AOIs in either the premeal condition (r_s = .19, p = .49) or the postmeal condition (r_s = .47, p = .07). The total score on the FRPQ did not exhibit a significant correlation with increased proportional dwell time in the premeal condition (r_s = .25, p = .36). However, a significant positive correlation was observed in the postmeal condition (r_s = .68, p = .004), as illustrated in Figure 7.4. Scores on the "Impairment of Satiety" subscale were not found to be correlated with proportional dwell time in the

premeal condition (r_s = .02, p = .93). However, a significant correlation was observed in the postmeal condition (r_s = .59, p = .02). As proportional dwell time and proportional fixation count were highly correlated, (*r* =.91, p <. 001), only the relationships for proportional dwell time are reported.

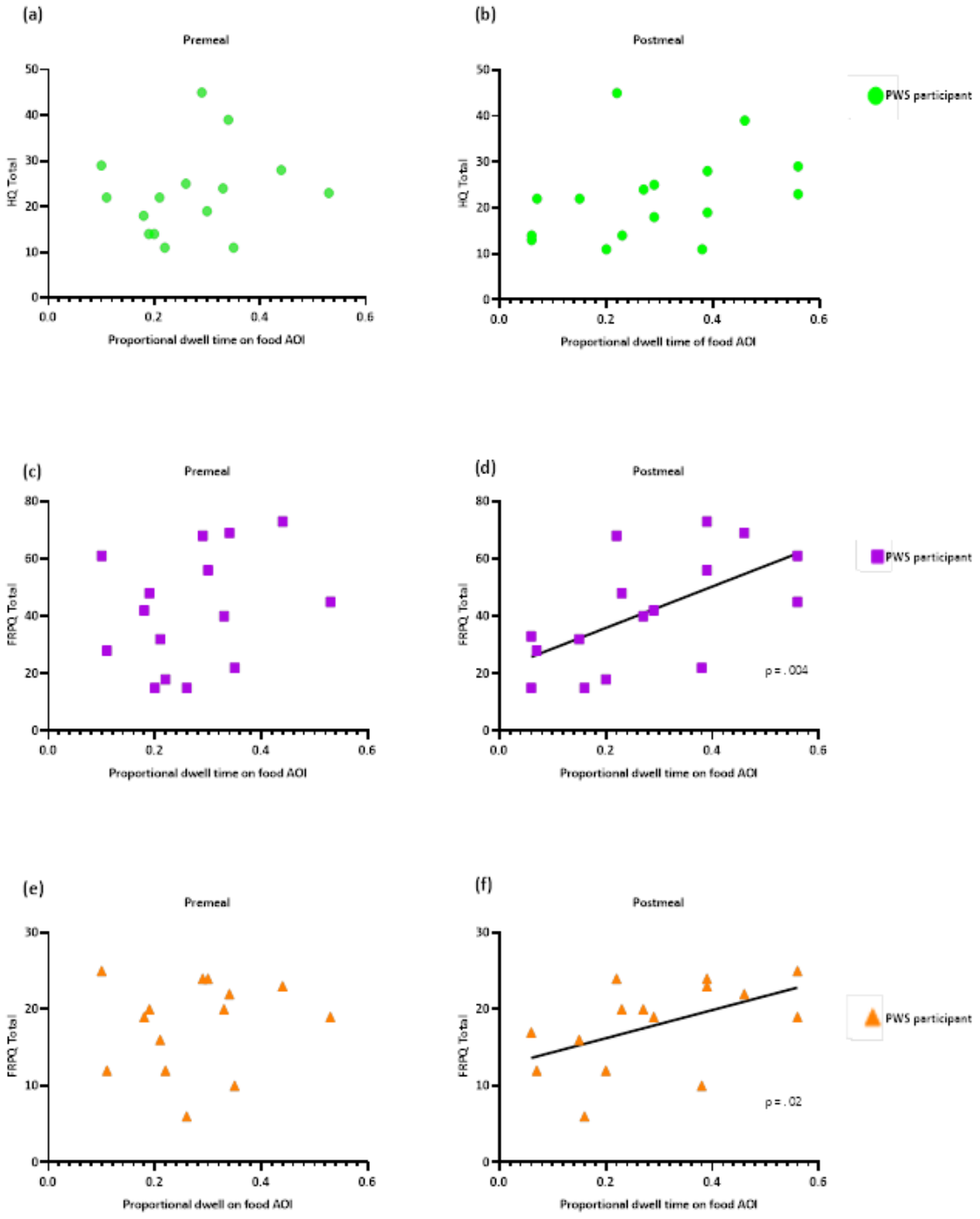

Figure 7.4: Scatterplots of proportional dwell time to food AOIs in the premeal and postmeal condition for (a + b) Hyperphagia Questionnaire Total Score, (c + d) Food Related Problems Questionnaire Total Score and (e + f) Impaired satiety subscale from the Food Related Problem Questionnaire.

7.4 Discussion

The primary objective of this chapter was to examine and compare the performance of individuals with PWS and a matched COM group using the adapted version of the FAB task protocol, which was developed through co-design with PWS stakeholders in Chapter 6. I hypothesised that attentional bias towards food stimuli would diminish from the premeal to postmeal condition in the COM group, while no significant change would be observed in the PWS group. A second hypothesis was that that the degree of attentional bias towards food stimuli would correlate with the severity of hyperphagia symptoms as measured by the caregiver-reported questionnaires, the HQ and the FRPQ.

The analysis of the proportion of first looks to the food AOI revealed that neither the COM nor PWS groups exhibited statistically significant deviations from chance levels. These findings indicate that participants in both groups did not demonstrate a significant automatic attentional bias towards food stimuli compared to non-food stimuli in either the premeal or postmeal conditions. These results are particularly interesting when considering the outcomes from Chapter 4, which explored the face pop task and consistently found that faces captured attention above chance level in both the PWS and COM groups. Although not directly comparable, these findings suggest that food stimuli may not possess the same level of salience as faces for individuals in both groups.

Furthermore, no notable differences in first looks to food were observed between the premeal and postmeal conditions for both the PWS and COM groups. In Chapter 5, the variable of time to first fixation (measured in milliseconds) was used to assess the speed of attentional orientation towards food stimuli. However, due to reduced processing speed in PWS, this variable was replaced in this chapter with the proportion of first looks to food used to determine if food stimuli captured attention first, rather than the specific processing time for locating food. The pilot study conducted with typically developing controls showed no differences in how quickly participants located the food stimuli across the premeal and postmeal conditions. This suggests that hunger or satiety did not impact the speed of attentional orientation towards food stimuli. Interestingly, the measurement of the proportion of first looks to food stimuli in the current study aligned with this finding for both the PWS and COM groups. This finding aligns with Nijs and colleagues (2010) where they also observed no difference in orientation bias (first look to food stimuli) across hunger and satiety conditions in both obese and healthy weight participants. On the other hand, Castellanos and colleagues (2009) did find an orientation bias in obese females compared to normal-weight females during a state of satiety. It is important to note that both studies utilized dot probe tasks with the presentation of two competing stimuli, while the FAB task design involves the presentation of five competition stimuli. Therefore, direct comparisons between these studies may be challenging due to the differences in task design and stimulus presentation. The results from this study indicate that although food stimuli may not be as salient as faces, they still exhibit greater salience than non-food stimuli and are not influenced by food intake. These findings provide valuable insights into the relative salience of food stimuli and highlight the importance of their attentional capture, irrespective of satiety state or meal conditions.

A significant effect of meal condition (Premeal, Postmeal) was observed on proportional dwell time towards food AOIs, meaning the amount of time participants spent looking at food AOIs

differed between the premeal and postmeal conditions. Furthermore, a significant interaction between group and meal condition was observed, indicating distinct patterns of change in the relative duration of gaze towards food AOIs from premeal to postmeal conditions for the PWS and COM groups. Specifically, the COM group decreased their gaze duration towards food AOIs in the postmeal condition compared to the premeal condition, while the PWS group displayed an increase. These results indicate differences in visual attention to food stimuli post intake between the two groups, with the COM group demonstrating reduced attentional focus on food after eating. In contrast, the PWS group exhibited an augmented attentional bias towards food stimuli in the postmeal condition. When analysing the proportional fixation count, a similar pattern was observed. Specifically, the PWS group exhibited an increase in the number of fixations towards food AOIs in the postmeal condition, indicating a heightened level of attentional engagement with food stimuli after eating. In contrast, the COM group reduced fixations toward food AOIs postmeal. The findings from the COM group exhibit a similar trend compared to the original FAB task results in chapter 5, where the healthy-weight group also demonstrated reduced interest in food AOIs postmeal.

The present study revealed significant correlations between participants' gaze towards food stimuli and the FRPQ, specifically the "impaired satiety" subscale, but only in the postmeal condition. In contrast, no such correlations were found in the premeal condition. These findings align with previous research suggesting that a high reward value for food and atypical satiety responses drives hyperphagia in PWS. Interestingly, my study did not find differences in reward value between individuals with PWS and typically developing individuals before a meal, as evidenced by the lack of relationships or group differences in the mixed models. However, the main differences emerged in the postmeal condition, where sustained attention towards food stimuli was observed, indicating that the reward value of food remains unchanged after eating. These findings align with fMRI studies in PWS that have reported more pronounced altered brain functioning under the condition of satiety (post-calorie intake) compared to control groups (Holsen et al., 2012; Miller et al., 2007; Shapira et al., 2005) specifically in the VMPFC supports the involvement of neural pathways governing reward-related behavioural regulation of food responses (Miller et al., 2007). The lack of change in attentional bias towards food AOIs following food intake in this study may reflect a delay or absence of satiety in individuals with PWS. These findings underscore the importance of considering the postmeal condition when assessing differences between individuals with PWS and TD and investigating the associations between gaze patterns and eating behaviour problems.

Interestingly, no correlation was observed between scores on the HQ and the duration of gaze towards food stimuli in the postmeal condition. This finding reveals that individuals scored differently on the HQ than on the FRPQ. The FRPQ encompasses a wider range of food-related problems beyond hyperphagia. These problems include sensory issues, dietary restrictions, and challenges during mealtime (Russell & Oliver, 2003). Food security measures may directly influence these aspects. Parents or caregivers may have implemented food security measures to counter hyperphagic behaviours, such as locking the fridge or kitchen (Schwartz et al., 2021b). For example, food security measures may reduce or eliminate behaviours specific to the HQ, such as "bin foraging". Overall, the broader scope of the FRPQ in capturing a range of food-related challenges beyond hyperphagia, combined with potential caregiver-implemented strategies specifically targeting hyperphagic behaviours, may explain why participants' gaze

duration to food stimuli correlated with the FRPQ but the HQ. Although no studies have directly compared the FRPQ and HQ in PWS, participants are more likely to score higher on the FRPQ than the HQ in Smith-Magenis syndrome, another rare genetic condition associated with hyperphagia (Gandhi et al., 2022).

The findings from this chapter shed light on the salience of food stimuli for individuals with PWS and healthy weight participants. These results contrast with the findings of Key and colleagues (2020) who examined the visual processing of food stimuli in children with PWS and found that food items were not exceptionally salient compared to animal stimuli. One possible explanation for this discrepancy could be facial features within the animal stimuli used. As demonstrated in Chapter 4, individuals with PWS displayed heightened attention towards faces compared to the COM group, indicating the salience of facial stimuli. Previous fMRI studies have provided robust evidence for specialised neural systems involved in visual face processing (Pitcher & Ungerleider, 2020). These specialised neural systems, which encompass both cortical and sub-cortical structures, may contribute to the dominance of facial stimuli in terms of salience.

An alternative explanation for the divergent findings could be related to the differences in meal timing and content between the studies. In the FAB task protocol used in my study, the postmeal condition was set at 30 minutes based on the anticipated peak satiety as the maximum feeling of fullness, referred to as "peak fullness," occurs immediately after food consumption (Forde, 2018). In contrast, Key and colleagues (2020) examined participants with PWS within 90 minutes of their last meal or snack. The meal content and timing variations may have contributed to the differences in attentional bias observed between the COM and PWS groups. Additionally, the longer delay time in the study conducted by Key and colleagues (2020) may have captured delayed satiety, specifically in individuals with PWS. Future iterations of the FAB task protocol could incorporate an additional postmeal condition to provide further insights into the presence or absence of satiety in individuals with PWS. This additional condition could involve participants completing the FAB task 30 minutes after and possibly 90 minutes after a meal. Fifteen or 30-minute intervals have been commonly used in studies measuring satiety using rating scales to capture time frames of satiety response (Forde, 2018). Implementing additional time points to assess attentional bias to food in the post-meal condition could be informative regarding the potential absence or delayed onset of satiety in individuals with PWS.

The study demonstrates several notable strengths, including incorporating a co-design protocol and utilising eye tracking data across two meal conditions (hunger and satiety) to assess hyperphagia. However, it is important to acknowledge certain limitations within the study. One such limitation is the absence of strict standardisation in the composition of the meal consumed prior to the experimental tasks. In line with the recommendations stakeholders provided during the FAB task protocol development in Chapter 6, flexibility was allowed to accommodate individual preferences and dietary requirements. Although efforts were made to maintain consistency in the meal content, variations in portion size and micronutrient composition could have occurred, resulting in a range of tracked calorie intake between 450-550 calories. Despite this, it is noteworthy that no participants were excluded based on meal-related factors, such as tantrums over the meal content, incomplete consumption of the meal, or refusal to fast or consume the entire meal. This approach prioritised inclusivity and ensured that a broader range of participants could be involved in the study.

A potential limitation of the study is the absence of a counterbalanced design for meal conditions. The order in which the premeal and postmeal conditions were administered was not systematically alternated between participants, which raises the possibility of order effects. As demonstrated in Chapter 5, implementing a counterbalanced design would have required participants who underwent the reverse order (starting with the postmeal condition) to remain on-site for a longer duration of 5 hours, significantly burdening individuals with PWS and their caregivers and potentially affecting study recruitment. However, the findings from Chapter 5, which replicated the reduced attention towards food stimuli in the postmeal conditions regardless of order, supported the decision to forego counterbalancing. Given these results and in consideration of reducing the study visit burden for families of participants with PWS, it was deemed appropriate to proceed without a counterbalanced design. Implementing a counterbalanced design could be considered in future studies where participants can stay overnight or undergo additional testing across multiple days.

The findings of this study provide valuable insights into attentional processes that underlie hyperphagia in PWS. Further developing the FAB task protocol as a clinical endpoint in drug trials for hyperphagia treatment will require considerable future research. First, establishing the reliability and validity of the FAB task protocol is crucial. This entails conducting rigorous psychometric analyses, including assessments of test-retest reliability and measures of convergent and discriminant validity, to ensure the robustness and accuracy of the protocol (Kraus, 2018). Additionally, continued collection of normative data from individuals without PWS and other genetic conditions associated with obesity will be important to establish appropriate baseline values and facilitate comparisons with the PWS population. Normative studies will aid in interpreting results and determining the clinical significance of attentional biases in postmeal conditions as measured by the adapted FAB task in individuals with PWS (Kraus, 2018). Longitudinal studies are needed to evaluate sensitivity to change in the FAB task, which is relevant to capturing treatment-related improvements in attentional processes associated with hyperphagia (Kraus, 2018). This step will require careful design and integration of the adapted FAB task within clinical trials assessing the efficacy of drugs targeting hyperphagia.

Additionally, a major challenge will be to validate the FAB task against clinical outcomes of hyperphagia, as such a measure is currently lacking in the field of PWS. However, this will be required to provide evidence for the protocol's relevance and utility as a clinical endpoint in drug trials. By undertaking these future steps, the adapted FAB task protocol can be further refined, validated, and established as a meaningful and reliable clinical endpoint in clinical trials for drugs targeting hyperphagia in individuals with PWS.

This chapter has provided valuable insights into the attentional processes underlying hyperphagia in individuals with PWS and the promising potential of the adapted FAB task protocol as a robust assessment tool. Future research endeavours should refine and validate the protocol, gather normative data to establish baseline values, conduct longitudinal studies to assess sensitivity to change over time and investigate the protocol's relationship with clinical outcomes to advance our understanding. These future approaches have the potential to unravel the intricacies of attentional processes associated with hyperphagia and enhance our ability to assess and measure this challenging aspect of PWS.

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Chapter 8: Discussion

8.1 Introduction

Mental health and behaviour are major challenges for people with PWS. Hyperphagia and autism are key features of this complex behavioural phenotype that are significantly impairing and reduce adaptive functioning. Interventions that target these core features are needed to improve the well-being and quality of life of individuals with PWS. However, a deeper knowledge and understanding of the neurocognitive underpinnings of these behaviours is required to develop targeted treatments. The aim of this thesis was to investigate the behavioural phenotype of PWS and provide an enhanced understanding of the underlying neurocognitive processes, specifically in relation to autism behaviours and hyperphagia. In this section, I will review the key findings and contributions of the five experimental chapters, discuss the implications of these findings, address the thesis' limitations, and suggest future directions for research.

8.2 An Overview of Thesis Findings and Implications

8.2.1 A Profile of Mental Health and Behaviour in Prader-Willi Syndrome in Ireland

The main aim of Chapter 3 was to profile the mental health and behavioural needs of people with PWS in Ireland. The survey results revealed a high prevalence of psychiatric disorders in this population, with anxiety being the most diagnosed condition. Psychotropic medications were prevalent among older participants, particularly antipsychotics and prescribed more frequently in individuals with the mUPD subtype of PWS. The study also found that behaviours associated with hyperphagia, and repetitive questioning were prevalent across all age groups. Caregivers reported a significant negative impact on their employment, family relationships, and emotional well-being, highlighting the challenges individuals with caregivers and families face. The findings from Chapter 3 illuminated the complex mental health and behavioural needs of individuals with PWS and emphasised the challenges these individuals face. Importantly, this research has provided a profile of these needs within an Irish context for the first time, providing insight into gaps in service provision and resource allocation and highlighting the pressing demand for skilled professionals and specialised behaviour support services.

Data-driven advocacy efforts carry weight and can strengthen an organisation's ability to influence decision-makers, leading to positive changes in the mental health landscape (Halley et al., 2022). The availability of this survey data and the publication of these findings were important for the Irish PWS community when advocating for access to respite and improved mental health support and policies specifically tailored to the unique need of individuals with PWS. These data formed the basis of a report that I co-wrote that was submitted to the Ministry for Health in Ireland by the Prader-Willi Syndrome Association of Ireland (PWSAI) to make a business case to fund specialist residential care settings for individuals with PWS that is available to read here https://pwsai.ie/surveyreport. The Ministry provided €500,000 in funding for a pilot program to provide specialist residential services in the 2018 government budget,

which has now been regularised into recurrent funding. This house provides accommodation to four young adults with PWS.

8.2.2 The role of social cognition in autism behaviours within PWS

The overarching aim of Chapter 4 was to investigate social cognition in individuals with PWS using the face pop task, a well-established paradigm used to measure visual attention to faces in autism (Gliga et al., 2009). I initially hypothesised that individuals with PWS would display a reduced preference for faces, similar to that described in autism (Chita-Tegmark, 2016; Frazier et al., 2017), considering the high prevalence of autism behaviours and reduced social functioning reported in PWS (Bennett et al., 2015; Dykens et al., 2017; Fernández-Lafitte et al., 2022). However, I unexpectedly found that the PWS group exhibited an increased preference for viewing faces compared to the comparison group, challenging the hypothesis that reduced social interest may explain reduced social functioning in PWS and contrasting findings in autism cohorts. A recent meta-analysis of studies measuring social interest in autism reported a reduced preference for social stimuli in the context of non-social stimuli in autistic people (Chita-Tegmark, 2016; Frazier et al., 2017). These meta-analyses were conducted in young children and adults, respectively. A recent study that investigated preference for social stimuli in autistic participants (N = 650) and neurotypical participants across a wide age range (6–30 years) also found a pattern of less looking to social stimuli (faces) in the autism group as a whole (Del Bianco et al., 2018). This study is the first time preference for social stimuli has been investigated in PWS. The findings from this chapter suggest that there appear to be differences in neural processes between autism and PWS, as indicated by eye tracking based measures of preference for social versus Nonsocial stimuli.

I also investigated if there were differences in face preference between the two genetic subtypes of PWS: DEL and mUPD. Based on the higher prevalence of autism diagnosis within the mUPD subtype, I hypothesised that a reduced preference for social stimuli would be more evident in this group. However, no significant differences in face preference were found between groups defined by genetic subtype, contrasting with previous research that reported distinct differences in other aspects of social cognition (Debladis et al., 2019; Key et al., 2013). For example, mUPD individuals have been reported to exhibit similar patterns of face processing to those seen in autistic populations, such as spending more time looking at mouths than eyes (Debladis et al., 2019). The lack of observed genetic subtype differences in the present study may be attributed to the reduced statistical power resulting from the smaller number of individuals in each group, as discussed in Chapter 4.

Although no genetic subtype differences were observed, higher levels of autism traits, as measured by average look duration in the PWS group, were associated with reduced sustained attention to social stimuli in line with the third hypothesis. These findings align with Del Bianco and colleagues (2021), who also identified significant differences in temporal dynamics between the autism and comparison groups. The latter showed a prolonged engagement with faces, potentially indicating further successive components of social attention after the initial attention-grabbing effect had diminished. In contrast, the autistic group exhibited a substantial reduction in attention after the initial orientation to faces, suggesting the involvement of different attentional components influenced by factors like motivation, relevance, and experience (Orquin & Holmqvist, 2018). While social stimuli initially captured the attention of

participants with autism, they failed to sustain their attention. These observations align with the results seen in PWS participants with high autism severity scores in this study, as reduced sustained attention to faces correlated with autism severity. Individuals with higher autism traits co-occurring with PWS may experience a diminished endogenous deployment of attention to social stimuli, similar to what is observed in autism alone (Del Bianco et al., 2018). This finding suggests that individuals with PWS and higher autism traits exhibit attention patterns similar to those observed in autism. The face pop task is currently considered a potential stratification biomarker in autism (Loth et al., 2017; Webb et al., 2020). If validated, it could have implications for future clinical trials testing interventions targeting social interest and social attention in autism. Additionally, these interventions may also prove beneficial for individuals with PWS and increased autism behaviours, and the face pop task could also have value as a stratification biomarker in the PWS group.

8.2.3 Development and adaptation of the Food Attentional Bias (FAB) task

As highlighted throughout this thesis, hyperphagia is a highly prevalent and impairing symptom in PWS. However, there are limitations to the objective measurement of hyperphagia, a barrier to progress in clinical research. One of the key factors contributing to hyperphagia in the context of PWS is an absence of satiety (Huang & Cai, 2023). Next, I focused on developing an objective measure of satiety using an eye tracking task modified from the 'face pop' task described in Chapter 4. Using eye tracking methodology, the protocol was intentionally designed to address existing limitations in measuring attentional bias to food stimuli. Previous measures often focused on measuring attention to food as a standalone trait instead of capturing fluctuations in attention to food depending on hunger and satiety states. To overcome this limitation, I used a repeated measures study design to assess whether there was a change in attentional to food stimuli from premeal to postmeal when participants were expected to experience a satiety-related decrease in hunger and motivation to consume food.

In Chapter 5, the task was initially tested in a group of healthy weight, typically developing adults. I hypothesised that this group would show reduced attention to food stimuli after a meal compared to before. In this study, participants showed a clear reduction in attention to food stimuli in the postmeal condition, as evidenced by shorter durations and fewer fixations on food stimuli in the postmeal condition. This finding supports the theory that attention to food stimuli reduces after food intake. This outcome confirmed the study's primary objective and highlighted the importance of measuring attention to food stimuli both before and after the meal to capture individual variations in attention to food.

To the best of my knowledge, this study represents the first free-viewing paradigm to measure visual attention to food stimuli in a typically developing cohort across conditions of "hunger" (premeal) ad satiety "postmeal" using a stimulus display length of more than 3 seconds. The only other eye tracking studies investigating the impact of satiety on visual attention to food stimuli have used the dot-probe task, which features a significantly shorter stimulus display duration, making direct comparisons challenging. Van Ens and colleagues (2019) argued that the reliability of eye tracking attentional biases increases with longer stimulus durations as this allows for capturing top-down attention driven by motivation – a crucial aspect when studying satiety. Castellano and colleagues (2009) reported a reduction in time spent looking at food stimuli after food intake in their study, while two others (Doolan et al., 2014; Nijs et al., 2010)

found no reduction. Notably, all these tasks had much shorter stimulus presentation durations (<3000ms). This difference in stimulus duration may contribute to discrepancies in findings among dot-probe studies. Longer stimulus durations, as presented in the FAB task paradigm, may better capture attentional processes influenced by motivation, offering more insight into the impact of satiety on visual attention to food stimuli.

An important finding from Chapter 5 is that the observation of reduced attention to food in the post-meal state was consistent when the order of pre- and post-meal tasks was counterbalanced. This supports the hypothesis that attention to food stimuli is related more directly to the meal condition and not influenced by order effects. The findings from this chapter offer valuable insights into the attentional bias towards food and the impact of hunger on visual attention in typically developing populations. The results of this chapter informed the study in Chapter 7, which aimed to investigate attentional bias to food stimuli in pre- and post-meal states and the potential to identify a marker of atypical satiety in PWS.

In order to extend the application of the FAB task to measure attentional bias to food in PWS, it was necessary to determine if adaptations would be required to successfully implement the same protocol in individuals with PWS who typically present with developmental delay, speech and language impairments and intellectual disability. A participatory design approach was used to address the FAB task adaptation for PWS. The participatory design facilitates stakeholders' active involvement in the research process to enhance the research process's relevance, validity, and impact (Cargo & Mercer, 2008). The study conducted here involved focus groups with caregivers and professionals to identify barriers and facilitators to implementing the protocol in PWS individuals and to obtain recommendations for adaptations.

Several key adaptations emerged from the focus groups and were integrated into the protocol for use in individuals with PWS (Chapter 7). Key adaptations included 1/ Allowing flexibility in scheduling the research visit to align with the participant's typical meal schedule 2/ Designing a second meal option, allowing parents/caregivers to provide their version of the standardised meal and 3/ Adding preparatory steps involving a remote consultation and provision of a visual schedule to address potential anxiety and repetitive questioning from the participant. These key adaptations were incorporated into the protocol and then implemented in a cohort of individuals with PWS in Chapter 7. This was a novel approach as PWS was the first time stakeholders were actively involved in designing a neurocognitive study for PWS. The participatory design allowed for a collaborative and inclusive process, valuing the insights and perspectives of caregivers and professionals. By incorporating the key adaptations suggested by the stakeholders, the study became more attuned to the specific challenges faced by individuals with PWS. It made the study more manageable and achievable, as evidenced in Chapter 7 by the number of PWS participants who completed the protocol.

The final chapter (Chapter 7) aimed to investigate attentional bias to food in PWS based on the hypothesis that there would be no difference in attentional bias to food between the pre-and post-meal states. This hypothesis was consistent with the theory that impaired satiety contributes to hyperphagia in PWS. To test this, the adapted FAB task protocol was conducted in individuals with PWS compared with an age and gender-matched comparison group using the same pre- and post-meal design described in Chapter 5. The study results supported these hypotheses, revealing that participants with PWS did not display a significant decrease in the

number and duration of fixations on food stimuli in the post-meal condition. In contrast, the comparison group showed reduced visual attention to food stimuli in the post-meal condition, similar to the healthy-weight group in Chapter 5. This outcome validated the primary objective of the FAB task protocol, showing that participants with PWS maintained their interest in food stimuli even after eating, suggesting atypical satiety. These findings align with previous fMRI studies on PWS, which have reported more pronounced altered brain functioning under conditions of satiety when compared to control groups. Specifically, the VMPFC (ventromedial prefrontal cortex) has been implicated in neural pathways governing reward-related behavioural regulation of food responses (Holsen et al., 2012; Miller et al., 2007; Shapira et al., 2005). Consequently, it supports further exploration of visual attention to food stimuli as a potential marker of hyperphagia. Identifying atypical satiety patterns as a potential marker has important implications for interventions and monitoring hyperphagia in individuals with PWS.

8.3 Implications

8.3.1 Implications for Autism Biomarker in PWS

This thesis explored the preference for social stimuli within PWS, a biomarker currently being investigated in autism. The finding that reduced attention to social stimuli was related to autism behaviours warrants further investigation as it may have broad implications for exploring autism biomarkers in the context of PWS. Currently, in the autism field, the search for biomarkers has garnered significant attention due to their potential clinical relevance (Molloy & Gallagher, 2021). The lack of a valid diagnostic biomarker for autism and the inconsistencies observed in studies comparing autism to typically developing control groups has been attributed to the extensive heterogeneity observed in autism cohorts (Loth, 2023). In autism research, there is a growing emphasis on unravelling clinical and biological heterogeneity and identifying stratification biomarkers that can define subgroups based on shared biology (Loth, 2023). It is plausible that the distinct autism profile observed in individuals with PWS may align with subgroups within autism that may be identified with autism-related biomarkers. Given the correlational nature of the findings, further in-depth research is necessary to understand the relationship between autism behaviours in PW and markers of social cognition. This cross-pollination of knowledge and approaches between PWS and autism research has the potential to provide valuable insights and potentially innovative strategies for addressing social communication in individuals with PWS. However, it is imperative to acknowledge the preliminary nature of the findings and the need for more robust experimental designs and extensive investigations to draw firm conclusions and guide future diagnostic and intervention approaches within the broader field of autism research.

8.3.2 The Importance of Stakeholder Inclusion in Designing and Conducting PWS Research: Implications for Clinical Trial

The use of a participatory approach in developing a potential marker of hyperphagia in this thesis holds several significant implications. By involving key PWS stakeholders, this study demonstrated the feasibility and value of integrating stakeholder perspectives into the co-design process of the adapted FAB task protocol. The participatory approach created a more relevant and accessible paradigm for measuring hyperphagia in PWS. This study's successful implementation of patient and public involvement (PPI) highlights its importance for future PWS research endeavours, particularly in clinical trials. With numerous ongoing clinical trials in

PWS (25 active trials), engaging stakeholders in the study design can enhance recruitment and retention rates (Bagley et al., 2016). The study's outcome of adapting the FAB task protocol in Chapter 6 shows how involving caregivers and individuals with PWS in the research process can address barriers specific to this population, which may also affect other success and impact of clinical trials. PPI ensures that research efforts align with the real-world experiences of individuals with PWS, promoting evidence-based care and improving outcomes for this complex condition. The feasibility and benefits of the participatory approach demonstrated in this study extend beyond PWS and have broader implications for other neurodevelopmental and neurogenetic conditions with complex behavioural phenotypes. Involving stakeholders in the research process can provide valuable insights and expertise, leading to the development of more comprehensive and tailored interventions for diverse populations. By embracing PPI, research efforts in other conditions can be more inclusive and patient-centred, ultimately driving progress in understanding and managing these complex neurodevelopmental disorders.

8.3.3 The FAB Task as a Biomarker for Hyperphagia in PWS

This thesis's findings highlight the FAB task's potential utility as a marker of hyperphagia. To date, studies have shown ambiguous results because of a lack of a meaningful measure of hyperphagia. This means that some potentially beneficial therapies may not meet regulatory requirements to be considered an effective treatment. An eye tracking biomarker of hyperphagia has implications beyond PWS. Hyperphagia is common in various monogenetic syndromes associated with obesity, including Bardet-Biedl Syndrome, Alstrom syndrome, Cohen syndrome, Kleine-Levin, and Rubinstein-Taybi (Chandler, 2021; Yu et al., 2019; Zorn et al., 2022). Therefore, an eye tracking biomarker that identifies aberrant visual attention patterns related to hyperphagia in the context of PWS could also be relevant for individuals with these syndromal conditions that are also associated with cognitive and speech and language delays. Potentially life-changing clinical trials of pharmaceutical interventions for syndromic and monogenic forms of obesity targeting hyperphagia are increasing (Müller et al., 2021). Reliable, objective measures of hyperphagia could be used to stratify individuals with NDD-associated obesity and allow for more objective measures to evaluate hyperphagia in response to therapies.

In recent times, drug treatment options for obesity have been limited, but the FDA's approval of glucagon-like peptide 1 (GLP-1) receptor agonists dulaglutide and semaglutide for weight management has sparked excitement in the field of obesity (Lafferty et al., 2023). GLP-1 analogues are reported to induce weight loss through various mechanisms, including insulin stimulation, glucagon secretion inhibition, delayed gastric emptying, appetite and food reward regulation, and enhanced satiety (Aldawsari et al., 2023). Only 11 studies have investigated the effect of (GLP-1) receptor agonists, all of which used a Visual Analog Scale (VAS) to evaluate hunger and satiety. Concerns have been raised about the validity and reliability of this tool for assessing hunger and satiety (Aldawsari et al., 2023). Relying solely on the group average responses from the VAS might overlook critical information related to appetite control and specific responders to treatment (Forde, 2018). It is recognised that individual variability in appetite expression must be considered (Forde, 2018). Accurate quantification of satiety using eye tracking methodology could advance our understanding of obesity treatment and management with novel therapies such as glucagon-like peptide 1 (GLP-1) receptor agonists.

For example, applying the FAB task protocol in this context could allow for a reliable, objective measure of satiety that could be used to stratify individuals in response to dulaglutide and semaglutide in clinical trials. However, further steps are required to validate the FAB task protocol as a biomarker of typical satiety before implementing it in a clinical trial.

There is also the question of how feasible it will be to implement an eye tracking protocol as part of a clinical trial. Despite the numerous benefits of eye tracking, non-invasive, accessible) research and applications have been limited by the high cost of eye trackers and their inability to scale due to specialised hardware (e.g., infrared light source, multiple high spatiotemporal resolution infrared cameras). However, using smartphones and tablets, machine learning (ML) approaches have shown promise for eye tracking. In a study by Valliappan and colleagues (2020), machine learning was utilised with a smartphone's front-facing ("selfie") camera feed as input, along with 30 seconds of calibration. This level of accuracy would be within the calibration error set as an inclusion criterion for data quality in this thesis. Consequently, combining technological advancements and the FAB design presents the potential for a scalable digital phenotype. Such a system could screen or monitor satiety changes in response to interventions.

8.4. Limitations of the Thesis

8.4.1. The sample size for genetic subtype analysis

One of the primary limitations of this study was the PWS sample size in the experimental chapters. Despite being the third largest neurocognitive investigation of social cognition in individuals with PWS conducted to date, the sample size of 27 participants in this study was not sufficiently large to thoroughly examine genetic subtype differences within the PWS group or compare individuals with PWS and co-occurring autism to those without autism. Specifically, the investigation of genetic subtype differences between the DEL and mUPD subgroups did not yield significant findings, likely due to the limited statistical power to detect such differences. LMMs necessitate a large amount of data to attain sufficient power. Meteyard and Davies (2020) recommend at least 30-50 participants per condition, and 30-50 items per participant, totalling 900-2500 data points per condition. Therefore, at least 60 participants (30 mUPD and 30 Del) with at least 10 stimulus arrays would be needed to analyse genetic subtype differences in PWS. Similarly, the study lacked adequate participants to explore differences in autism diagnosis within the PWS population adequately. Consequently, drawing conclusive results from the genetic subtype analysis in this study is limited, and further recruitment efforts through collaboration could enhance the likelihood of effectively investigating these differences.

8.4.2. The age range of participants and cross-sectional design

As PWS is a rare condition, including individuals across a wide age range was necessary. This introduced variability in the developmental stage of hyperphagia and the emergence of autism behaviours. As discussed previously, hyperphagia has distinct nutritional phases. Therefore the wide age range and small size did not allow for analysis by stage of hyperphagia as participants were spread out across the different nutritional phases. The study's cross-sectional design also presents challenges in capturing how autism characteristics manifest and the dynamic nature of autism characteristics across different developmental stages in PWS. The complex nature of

PWS, including its distinct nutritional phases and potential influences on autism behaviour, necessitates a longitudinal approach to comprehend the interplay between these factors and developmental trajectories fully. The age and study design limitations underscore the need for larger sample sizes, focused subgroup analyses, and longitudinal designs in future research to effectively unravel the intricate relationships between PWS phases, autism behaviour, and developmental trajectories. By addressing these limitations, future studies can provide a more comprehensive understanding of the dynamic nature of autism in PWS and its association with different nutritional phases.

8.4.3. Measurement of psychosis symptoms

One major limitation of this study is the absence of measurement or screening for psychosis in individuals with PWS. Individuals with PWS have an elevated risk of psychosis, particularly in the mUPD genetic subtype (Yang et al., 2013). Psychosis is linked to differences in social cognition in the areas of emotion processing, theory of mind and social perception (Healey et al., 2016). Therefore undetected or subclinical psychosis symptoms may have influenced performance on social cognition tasks. Psychosis has also been linked to eye movement abnormalities more broadly during social/non-social free-viewing tasks, including fewer eye fixations, longer mean fixation duration, and shorter mean scanning length (Tom et al., 2023; Wolf et al., 2021). By not incorporating measures or screening for psychosis, the study missed an opportunity to explore the potential influence of psychosis on eye movements and social cognitive performance and its subsequent impact on the results and interpretations. Future research should include appropriate measures or screenings for psychosis to enhance the comprehensive understanding of the relationship between eye movements and psychosis in individuals with PWS.

8.4.4. Ecological validity of eye tracking paradigms

A limitation of eye tracking methodology in PWS lies in translating findings from controlled laboratory settings to real-life contexts. While the eye tracking studies provided valuable insights into a preference for social stimuli and the interaction between satiety and attentional biases to food stimuli gaze patterns, it is necessary to consider the ecological validity of the findings. Real-life situations involve complex stimuli, dynamic environments, and interactions that may differ significantly from the controlled stimuli used in laboratory experiments (Boraston & Blakemore, 2007). Our ability to identify eye tracking markers of hyperphagia or social cognition might be hindered by poor ecological validity of stimuli presented through eye tracking devices, as visual displays might fail to enable bodily engagement with the participant, possibly failing to fully capture the core social engagement impairments that characterise atypical social cognition or hyperphagia in PWS (Boraston & Blakemore, 2007). This limitation has been targeted by research studies that measure viewing patterns in live interactions with caregivers (Thorup et al., 2018) and studies using eye-wear technology (e.g., glasses) that records participants' viewing pattern as they go about their day or worn by an examiner to detect gaze patterns during social interactions (Edmunds et al., 2017).

Additionally, combining eye tracking measurements with other assessment tools, such as behavioural observations and self-report measures, can offer a more holistic understanding of the relationship between eye movements and real-life experiences. However, this is currently

limited for hyperphagia due to the limited sensitivity of existing clinical measures (Key et al., 2020). While eye tracking research provides valuable insights into neurodevelopmental disorders, careful consideration of the limitations and ecological validity of the findings is necessary to ensure their relevance and applicability.

8.4.5. Thesis studies were not pre- registered.

An acknowledged limitation of this thesis is the absence of pre-registration for the various methodologies and analyses employed across the studies within each chapter. Pre-registration, aligned with contemporary scientific reforms such as open science, underscores the principles of transparency, replicability, and reproducibility in research results (Logg et al., 2021). Pre-registration involves the public documentation of key components in a proposed research study, such as study design, hypotheses, and planned statistical analyses (Logg et al., 2021). Notably, the use of eye tracking methodology yields a diverse array of outcome variables. Embracing pre-registration would be particularly advantageous for eye tracking studies as it would encourage researchers to select hypothesis-driven outcome variables before undertaking analyses. Future studies using eye tracking to explore social cognition and hyperphagia would benefit significantly from engaging with pre-registration practices, as it would enhance the research integrity of a study.

8.5. Future Directions for Research

8.5.1. Perspectives from individuals with PWS

While this thesis involved a participatory element with PWS stakeholders, the perspective of individuals with PWS was not explored. Given the rarity of PWS, maintaining blinding regarding the exact purpose of the FAB task protocol was necessary, therefore participants with PWS were unfortunately not involved in the adaptation process. A crucial next step for the FAB task protocol is to explore the experiences of individuals with PWS who were part of the study. Individuals with PWS have previously been canvassed for their perspectives on how they perceiver their syndrome and how they are feeling about participating in clinical trials (Dykens et al., 2021), however no studies have yet investigated how individuals with PWS feel about participating in research. Understanding what the participants found challenging, enjoyable, beneficial, and any recommendations for adaptations to the FAB task protocol would be invaluable for improving the accessibility of the protocol. Equally important is gathering feedback from individuals with PWS who did not take part in the study. This feedback would offer insights into the motivations or barriers influencing participation. Utilizing both focus groups and structured one-to-one interviews for data collection provides flexibility, empowering participants to contribute in a manner they find comfortable. Comparing insights from these diverse data collection approaches will inform the design of future research studies. Exploring if the themes regarding the FAB task protocol that emerged from stakeholder focus groups align with the perspectives of individuals with PWS is intriguing. Such an exploration could yield valuable insights into whether the priorities of individuals with PWS are genuinely understood by other stakeholders.

8.5.2. Collaboration

International collaboration and developing registries that create opportunities for data pooling and collaborative research studies are crucial next steps in PWS research. The Global PWS Registry, launched in 2015 and hosted on the National Organization for Rare Disorders "IAMRARE" Registry Platform, exemplifies such collaboration. The registry facilitates large-scale

data collection by documenting detailed information about individuals with PWS, including clinical features, treatments, and outcomes. Pooling data from multiple sources increases statistical power, enabling researchers to identify patterns, trends, and rare manifestations that may not be apparent in smaller individual studies. Moreover, international collaboration fosters the standardisation of data collection and research protocols, promoting comparability and consistency across studies. By working together to progress large-scale research with strong research integrity, the PWS research community can accelerate progress, inform clinical care, and advance potential therapies for this complex genetic disorder. An example of the progress that can be made through international collaboration is the AIMS-2 Trials project, a multi-centre European initiative that aims to identify and validate stratification biomarkers for autism and develop new therapeutic interventions through collaborative research (Loth et al., 2017; Webb et al., 2020). This initiative has made progress in validating autism candidate biomarkers, such as the latency of the EEG N170 event-related component known to be sensitive to face processing and in characterising the neuroanatomical basis of changes in clinical features of autism. These advancements are strengthened by multi-stakeholder collaboration, ensuring that research outputs align with community priorities and have a real-world impact (Loth et al., 2022). Lessons from such approaches in autism can guide future efforts in biomarker discovery and precision medicine across the PWS spectrum (Sahin et al., 2018; Tillmann et al., 2019). By leveraging collaborative efforts and applying lessons from successful initiatives in autism and other rare genetic syndromes, the field can advance biomarker discovery and precision medicine for individuals with PWS, ultimately improving their outcomes and quality of life.

8.5.3. Longitudinal Research

Longitudinal research is essential to overcome the limitations of wide age ranges and cross-sectional designs in PWS research. The inconsistency of results in social cognitive studies conducted in PWS so far likely stems from the developmental heterogeneity in research cohorts. The PWS behavioural phenotype changes across development - the most striking example being the switch from feeding difficulties in infancy to the development of hyperphagic behaviours in late childhood (Goldstone et al., 2008). As previously discussed in Chapter 7, we now know this transition happens across seven distinct nutritional phases (Miller et al., 2007). However, research has not investigated the changes in cognitive processes accompanying these nutritional phases. Adequately powered longitudinal cohort studies would provide a valuable opportunity to examine developmental trajectories and the dynamic nature of hyperphagia, autism behaviours, and social cognition across the different nutritional phases of PWS, leading to a more comprehensive understanding of their interplay. Longitudinal studies offer the advantage of tracking the progression of the behavioural phenotype in PWS at the group and individual levels. They can identify critical periods of change, allowing for the investigation of complex interactions between genetic, neurobiological, and environmental factors that contribute to the diverse manifestations of PWS. This longitudinal perspective provides insights into the factors contributing to the increase in the severity of autism-related features during adolescence and adulthood in individuals with PWS. It will allow researchers to investigate potential interventions or therapies to address these specific challenges and improve outcomes for individuals with PWS.

8.5.4. Validation of the Food Attentional Bias Paradigm as a Biomarker

An important next step in validating the FAB task is to conduct comprehensive validation testing. Validation involves assessing the biomarker's reproducibility by collecting repeated measurements from a subset of participants or across multiple testing sessions to establish its stability and reliability (Califf, 2018). Replicating and validating the findings in independent cohorts through collaboration with other research groups or multi-centre studies are required to determine the robustness and generalisability of the task (Califf et al., 2018). However, a current challenge that needs to be solved is how to compare the performance of a biomarker such as the FAB task where there is a lack of gold standard measurements for hyperphagia (Schwartz et al., 2021). Validation of the FAB task is necessary prior to registration for either the FDA or European Medicine's Agency (EMA) or other regulators' Biomarker Qualification Program (*FDA*, 2018; *EMA*, 2020). Qualification streamlines regulatory processes, facilitating the adoption of the biomarker in drug development and clinical practice, ultimately leading to more effective patient therapies

8.6. Final Conclusions

This thesis has contributed to understanding autism, social cognition, and hyperphagia in individuals with PWS. The findings have shed light on the complex needs of individuals with PWS, highlighting the prevalence of psychiatric disorders and the negative impacts on caregivers. The thesis has also provided novel insights into social cognition within the PWS population, revealing a distinct attentional pattern towards faces and the relationship between attention to faces with autism severity and socialisation abilities. Additionally, the development and adaptation of the FAB task offered valuable insights into the attentional bias towards food stimuli and its potential as a biomarker for hyperphagia in PWS. This thesis emphasises the need for comprehensive support and interventions to address the psychiatric and behavioural aspects of PWS and improve the overall well-being of affected individuals and their caregivers. Identifying attentional patterns related to social cognition and hyperphagia provides opportunities for developing targeted treatments and interventions addressing this challenging aspect of PWS. Moreover, the participatory approach employed in this thesis highlights the importance of stakeholder involvement in study design, emphasising the need for patient and public involvement in all future PWS research endeavours.

Future research should focus on expanding the sample size through collaborative multi-site studies with longitudinal designs. Larger samples will allow for subgroup analyses and a better understanding of developmental trajectories in PWS. Additionally, integrating alternative methodologies and measures that capture real-life contexts and experiences can enhance the ecological validity of research findings. By addressing these future directions and building upon the contributions of this thesis, further research can advance the knowledge and understanding of PWS, leading to more effective interventions, improved clinical outcomes, and better support for individuals with PWS and their families. The ultimate goal is to continue research that will enhance the quality of life for individuals with PWS and promote their overall well-being.

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Appendices

Appendix 1: Consent materials and script for contacting potential participants.

PARTICIPANT INFORMATION LEAFLET

Study Title: The impact of social cognition and reward processing on mental health and behaviour in Prader-Willi Syndrome

Research Team:

- Prof. Louise Gallagher, Consultant and Chair in Child and Adolescent Psychiatry, Trinity College Dublin
- Dr. Ciara Molloy, Postdoctoral Research Fellow, Trinity College Dublin
- Ms. Sarah Feighan, PhD Candidate, Trinity College Dublin
- Ms. Áine McNicholas, Research Assistant, Trinity College Dublin
- Ms Linda Lisanti, Research Assistant, Trinity College Dunlin

Thank you for your interest in our research study. Please read this information sheet carefully and discuss it with your family, and your family doctor if you wish. Take your time to read it and don't feel under any pressure to make a decision right away. It is important for you to understand the study fully before you decide to participate or not. If anything is unclear to you, or if you have any questions at all about the study, please don't hesitate to get in touch with us by phone or email.

PART 1 – THE STUDY

Why is this study being done?

The purpose of this study is to gain a better understanding of behaviour and mental health in PWS. Autism like behaviours and atypical social behaviour are common in PWS and can be challenging for individuals with PWS and their families. Social cognition, which refers to the ability to perceive and understand the thoughts and feelings of other people, is impaired in autism and is thought to underpin many of the social challenges in autism. Studies suggest that social cognition is impaired in PWS but this has not been comprehensively evaluated. This project will determine if atypical social cognition underlies social challenges in PWS and if it contributes to behaviours of concern.

Next, this study will consider whether there is an effect of altered social reward processing in PWS that drives poor social understanding. Reward circuitry, which responds and places values on things in our environment, is fundamentally impaired in PWS and contributes to

hyperphagia. It is notable that autism behaviours become more prominent in PWS in later childhood as hyperphagic symptoms are increasing. We think that this may be due to a shift away from social reward in brain circuitry. We will study if social reward has less value for people with PWS and if this is related to increased value of food

This project will address our gap in understanding the neurocognitive nature of social behaviour in PWS and how these contribute to behaviours of concern. It will provide knowledge as to whether core PWS deficits in reward underlie poor social functioning which may indicate that treatments targeted at hyperphagia will be effective for social behaviour. Finally the study will provide measures that can be further validated as biomarkers of autism traits in PWS in treatment studies.

Why am I being asked to take part?

You are being asked to take part in this study as you are either:

- A person with a confirmed genetic diagnosis of Prader-Willi Syndrome
- A parent/legal guardian of a person with a confirmed genetic diagnosis of PraderWilli Syndrome
- A person with no know medical condition

Do I have to take part? What happens if I say no? Can I withdraw?

It is important to know that you and/or your child do not have to take part in this study if you do not wish. You and/or your child can change your mind about participating in this study at any time, even after the study has begun. If you and/or your child decide that you do not want to participate, this will have no effect on your or their current or future medical care.

You and/or your child do not have to provide a reason for deciding not to participate or changing your mind. If you or your child wish to opt out, please contact Prof Louise Gallagher (Email: lgallagh@tcd.ie, Phone: (01) 896 2144), the principal investigator of this study and she will be able to organise this for you.

How will the study be carried out?

The study will take place at the Trinity Centre for Health Sciences based on the St James's Hospital Campus in Dublin. Participants will be asked to come for two visits and an optional third visit. On the first visit we will ask the participant and their caregiver to take park in a clinical assessment. On the second visit, we will ask individuals to complete tasks using eye tracking technology. We will also ask participants if they are willing to give us a small sample of their blood for genetic testing. If they would prefer not to give a blood sample we will ask if they are willing to provide us with a saliva sample for genetic testing. On the third (optional) visit, we will ask participants to perform tasks while we record brain activity using electroencephalography (EEG). EEG is a safe research technique that can be used to investigate how specific regions of the brain are connected to one another.

What will happen to me if I agree to take part?

After you have read this information sheet, and if you decide that the study is something you (or your child) would still be interested in, we will have a short conversation over the phone to ensure that you are/your child is eligible to participate. We will then organise the first visit.

Visit 1: St James's Hospital, approximately 2 hours (including breaks as needed) The first visit will take place in either St James's Hospital or the participant's home, depending on which location the participant prefers. First the researcher will go through the PILS and answer any questions the participant or caregiver may have about the study. The participant and caregiver will then be asked to sign forms to indicate that they consent to take part in the study.

We will ask the participant to complete two assessments, a cognitive assessment which will give us an indicator of their cognitive ability. We will also ask the participant to take part in a behaviour assessment called an ADOS. This will give us an idea of their social communication strengths and difficulties. We will ask the caregiver to take part in an interview which will ask all about the developmental history of the participant. We will also leave questionnaires with the caregiver to complete which will ask about the participant's mental health and behaviour.

Visit 2: St. James's Hospital, approximately 2 hours (including breaks as needed) For the second visit, we will ask the participant and their caregiver to come to our research centre at St James's Hospital where our cognition lab is based. In order to look at social cognition and reward processing we use an eye tracker machine. This will involve the participant sitting in front of a computer and watching different videos on the screen. The eye tracker sits on top of the computer and it records the eye movements of the participant during the videos. The researchers can then look at where the participant's eye were looking throughout the task which will give us information about how the participant's brain processes faces, emotions and social interactions. We will also ask participants if they are willing to give us a small sample of their blood for genetic testing. If they would prefer not to give a blood sample we will ask if they are willing to provide us with a saliva sample for genetic testing. This genetic testing will be for research purposes only so will not routinely feedback the results of the DNA or provide you with your genetic sequence.

Visit 3 (optional): St. James's Hospital approximately (90 minutes)

If the participant is happy to return for a third day, we will ask them to come back to the Trinity Centre to undergo an EEG. EEG is a technique that uses electrodes placed on the skin to record brain activity in regions of the brain beneath the electrodes. It is important to note that this EEG will be carried out for research purposes only. Participating individuals will have an EEG cap fitted on their head. The cap contains the electrodes, but there will be a blunt syringe used to apply electrode gel to the skin. It will not penetrate the skin. Participants will be asked to perform short computerised tasks while wearing the cap. The researcher will fully describe the tasks before the experiment and there will be lots of time to practice.

Are there any benefits to me or others if I take part in the study?

There is no direct benefit to taking part in this study. It is important to remember that this is a research study and so is not designed to replace any clinical assessment or treatment. While individual participants will not receive direct benefit from participating in this research, taking part will help to provide information to understand more about the neurocognitive nature of autism behaviours, hyperphagia and how these contribute to behaviours of concern. This project can inform treatment approaches for mental health in PWS.

Are there any risks to me or others if I take part in the study?

The risks involved in participating in this study are minimal, and our procedures are widely used by groups at many different research laboratories across the world. Before consenting to take part though, it is important to be aware of the potential risks.

EEG

There are minimal risks associated with EEG. Occasionally, subjects experience mild skin irritation due to application of the electrode gel. The electrode cap may leave a faint trace on your skin, but this will resolve in a few minutes. If you feel uncomfortable at any point, and wish to discontinue the experiment, you need only say this to the investigator and the experiment will be ended immediately.

Eye tracking

There are minimal risks associated with eye tracking. Sometimes, subjects report their eyes getting tired from looking at a screen. If this happens we can stop and take as many breaks as needed.

Blood-drawing

There are minimal risks associated with drawing blood with a needle, which will be performed by trained phlebotomists. Potential risks includes feeling faint, localised bruising and in very rare cases, infection. We understand that drawing blood can make some people uncomfortable so any individual who does not wish to give a blood sample need only let us know.

PART 2 – DATA PROTECTION

What information about me (personal data) will be used as part of this study? Will my medical records be accessed?

With your consent, the personal data we will collect from you (and your child) will be your name, address, contact details. We will use your contact details to contact you about the research study. Although we won't be accessing your hospital records, in some cases it may be very useful to us if we could access some relevant health records (i.e. GP or psychologist's report, genetics report). If you have access to these records yourself, we may ask you to bring copies of them when you come for your assessments with us. If you don't have access to these records, we may ask for your consent to have them shared with us by your clinician.

We will also ask for your consent to inform your family doctor (GP) of your participation. With your consent, we will then ask your GP to share information about your medical history with us. This information, along with all of the other personal information we gather from you, will be kept strictly confidential and only accessible by authorised members of the research team at Trinity College.

What will happen my personal data?

As soon as you/your child has enrolled in the study, you will be assigned a unique study ID code - this is called pseudonymisation. Any information we gather from that point will be associated with the ID code and not with your name. Only the authorised research team at TCD will have access to the 'key' that links your name to your ID code, and this information will be stored on an encrypted file on a password protected computer at TCD. Neither your name nor ID code will be used when reporting the results of this research for publication or presentation; your contribution will be entirely anonymous.

The blood/saliva samples we collect from you will also be shared and analysed by the genetics lab however your name will not be linked to these samples. We will ask if you consent for us to video record some of the interviews we conduct with you/your son or daughter, as well as the EEG and eye tracking sessions. This is for quality control and training purposes. The video files will be encrypted in the Trinity database, and will not be shared with any third party without explicit permission from you.

As Prader-Willi Syndrome is a very rare syndrome we may share data with other research collaborators in the future however we will re-seek consent to do this from every participant before sharing.

Who will access and use my personal data as part of this study?

Only the authorised researchers named above working on the research team at Trinity College will have access to your/your child's personal information. All other data will have an ID code associated with it.

Will my personal data be kept confidential?

Any information we gather from you/your child during this study will be kept entirely confidential, and we will exercise our duties in handling your personal data as per the General Data Protection Regulation (GDPR)

What is the lawful basis to use my personal data?

As part of the study you/your child's personal data will only be used under the lawful basis for processing of data defined in Article 6 and Article 9 of General Data Protection Regulation (GDPR). Under GDPR and the Freedom of Information Act, you can have access to any of the data we collect from you if you request it.

What are my rights?

As a research participant you have the following rights to your data:

- The right to erasure
- The right to access
- The right to rectification
- The right to restrict processing
- The right to object to processing

PART 3 – COSTS, FUNDING & APPROVAL

Will it cost me anything if I agree to take part?

We will refund you for any travel expenses incurred by participating in our study, so please remember to retain any receipts you get on your journey. We will also reimburse for any other expenses that may be involved including parking fares. We understand that participating in our study involves a lot of time and energy, so we will also offer a gift voucher for taking part. This study is also covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

Who is funding this study? Will the results study be used for commercial purposes?

This study was funded by the Foundation for Prader-Willi Syndrome Research.

Has this study been approved by a research ethics committee.

This study has been approved by the Tallaght University Hospital Ethics Committee.

PART 4 – FUTURE RESEARCH

Will my personal data and/or biological material be used in future studies?

We will ask for your consent to retain the biological samples that we collect from you/your child so that they may be used for future research. We will also ask if we can retain the other data that we collect from you so that it may be used in future research studies (e.g. eye tracking, EEG and questionnaire data). Future research may be conducted by our group or collaborators if you consent to this. You can always opt-out of having your data retained at a later point.

Any future research studies that make use of your data will likely be very similar to this study. They will be using the same data and information to ask slightly different questions. We hope that the results of this study will lead to new and interesting questions about PWS. By allowing your data to be shared with other researchers, they will be able to explore these new questions, and speed up the discovery of new ways of helping people with PWS and their families. By combining the data collected in this study with the data collected in many other studies, scientists can collaborate to create large-scale studies that are more likely to have impactful results.

We will retain the data that we gather from our participants at our Trinity College site for 10 years. With your consent we will contact you after 10 years to continue to store your (and your child's) data. With your consent we may also irrevocably anonymise all of your data and keep it indefinitely. This means that your data will be given a unique study ID code, and any document linking your code to you will be destroyed. Once we do this, there will be no way to link you to any of the data you have provided. The irrevocably anonymised data will be stored with our research group at Trinity College Dublin indefinitely. We will ask if you consent for us to share your fully anonymised data with other research groups or databases following completion of our study, which helps to speed up the rate of scientific progress enormously. However you are entirely within your rights state that you do not consent to data sharing.

PART 5 – FURTHER INFORMATION

Where can I get further information?

You can get further information from our research team at Trinity College or at www.trinityautismresearch.com

Principle Investigator		Research Team
Prof. Louise Gallagher	Sarah-Marie Feighan	Aine McNicholas Linda Lisanti
Dept. of Psychiatry Trinity Centre for Health Sciences	Dept. of Psychiatry Trinity Centre for Health Sciences	Dept. of Psychiatry Trinity Centre for Health Sciences
St James's Hospital Dublin 8	St James's Hospital Dublin 8	St James's Hospital Dublin 8
lgallagh@tcd.ie	feighans@tcd.ie	recruitmentNRG@tcd.ie

Data Controller

The data controllers for this study are: Trinity College Dublin

Data Processor

The data processors for this study are: the Autism Research Group, Trinity College Dublin.

Data Protection Officer

For more information about data processors and data controllers, and the data protection arrangements in Trinity College Dublin, please contact: Data Protection Officer, Data Protection Officer Secretary's Office, Trinity College Dublin, Dublin 2, Ireland. dataprotection@tcd.ie.

What happens if I wish to make a complaint?

If you have concerns or questions about any aspect of the study or if you wish to make a complaint, please speak to the researcher you are working with who will do their best to assist you. If they are unable to answer your question, please contact the study's principal investigator

Will I be contacted again?

If you consent, you may be contacted again to be informed of future PWS research in Trinity College Dublin. You may be contacted in 10 years' time to request to continue to store your data at Trinity College Dublin for further research purposes.

Participant Information Sheet

This leaflet will tell you all about a research study for people with Prader-Willi Syndrome (PWS). This study is taken place in Trinity College Dublin. We would like to invite you to take part in this study. You can read this first before and talk about to your family, friends or any member of the research team before you decide anything.

Meet the Research Team	
Louise Gallagher	
Sarah Feighan	
Linda Lisanti	

Áine McNicholas	

a) What is the Project?

We want to learn more about what it is like to have PWS. We want to know what things you find easy and what things you find difficult and what you enjoy and what you don't enjoy. We're really interested in how you see things, how you think about things and also how you feel things.

b) Why is this project important?

Some people with PWS can find it challenging to cope with their emotions. If we can learn more about people with PWS, we will be more able to help people with PWS.

c) What happens in the project?

First of all, we will tell you exactly what we would like you to do in the study. You can ask us as many questions as you want and then you can take some time to decide if you want to take part.

This study would involve meeting with us researchers twice. There is also an option to do a third day if you like. The first day, we would meet in our research building in Dublin, or in your own house or clinic. Whichever you prefer more! That day, your parents or caregivers will be asked to complete a list of questions about you, describing your behaviour on Zoom video call. We will also meet with you and spend some time looking at pictures and books and talking. This session will be recorded, and this recording will be used later to help us with our research. Only the scientists in Trinity College and St. James Hospital will see the recording.

The second day would take place in our research building or in a nearby location depending on where you live. You will be asked to play some simple computer games while we use a camera to capture your eye movements. This should take 60 minutes – 90 minutes. We will stop for a lunch break halfway through and we can take as many breaks as you like at any other time.

The third day is optional. This day will involve coming to Trinity. We will also ask you to complete a computer game while wearing an EEG cap. The EEG cap records your brain activity while playing these games. We will put some gel on your hair under the cap to help us to get the best signals from your brain. This day will take 60 – 90 minutes too.

We will also collect a blood or saliva sample from you on one of the days. A blood or saliva sample will allow us to look at your DNA. We will only do this if you decide you want to. For a blood sample, you will need to get a needle in your arm. Sometimes this can hurt a bit and you might feel a bit uncomfortable. You might have a bruise on the arm where the blood was taken. A small number of people feel dizzy or weak after a blood test. If you can't give a blood sample the researcher will ask you to give a saliva sample. There are no risks with this, you will just need to spit into a dish. As we are doing this for research purposes, we will not be able to give you the results from our DNA analysis. You only have to do this part of the project if you want to. At any time you can decide to not give a DNA sample and the researchers will not mind at all.

d) Who will benefit from this project?

Although you will not be directly helped by this project, people who have PWS in the future and their families may gain better help if we can understand PWS more.

e) Are there any risks in taking part in this project?

There are hardly any risks associated with taking part. You may get bored during some of the computer games, but you can stop and take a break at any time. Sometimes when we put the EEG hat on, the gel used can make the skin itchy, but this rarely happens. If you don't like it and want to stop at any time, you can say that to the helper, and they will stop it right away.

f) Confidentiality

We will not share any information you or your parent or caregiver give us with anyone outside of our research group. This means that the only people who know your name and information are the research team at Trinity College Dublin and your own doctor. Also, if the researcher thinks you are not safe in any way, they will tell your parents and/or a person whose job it is to keep children safe.

g) You do not need to take part if you do not want to

The really important thing about this project is that you get to decide if you want to take part or not. If you don't want to, that is fine. You can just say no to the researcher, or your parent and you will not have to do anything.

h) What do I do now?

You can ask the researcher as many questions as you want before you decide to take part. If you wish to take part in this study, you will be asked to sign a consent form. It is usual to ask parents to give their consent for young people, so we will ask one of your parents to sign the form as well.

Where can I get further information?

You can visit our website to learn more about our team and our research www.trinityautismresearch.com

You can also contact Sarah, Linda or Áine on the phone or send us an email and we would be very happy to talk to you and answer your questions.

Research Team

Sarah Feighan	Áine McNicholas	Linda Lisanti
recruitmentNRG@tcd.ie	recruitmentNRG@tcd.ie	recruitmentNRG@tcd.ie
0860742999	089 942 7621	089 940 9239

PARENT/CAREGIVER CONSENT FORM

Study Title: The impact of social cognition and reward processing on mental health and behaviour in PWS

Instructions: To consent to take part in this project would you please read these statements, tick the appropriate box and sign your names in the space below.

Consent to take part in research study	**Yes**	**No**
I have read (or been read to) the information about the study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction		
I understand the information and what taking part in this study involves.		
I am willing to participate in the project, but I feel under no obligation to do so.		
I understand that the information collected in the study will be kept strictly confidential and will only be made available to qualified scientists.		
I understand that I can withdraw from the study at any stage without giving an explanation, and withdrawal will not affect my/my family's medical care.		
I freely and voluntarily agree to be a part of this research study, though without prejudice to my ethical and legal rights.		

Consent to video/ audiotaping of interviews or assessment	**Yes**	**No**
I consent to the video or audio-recording of my child's interview with the research clinician if required.		
I agree to these audio or video recordings being shared with other authorised researchers for research reliability.		
I agree to these audio or video recordings being used to train clinician's in autism assessment.		

Consent to collection of biological sample	Yes	No
I consent to my child providing either a blood or salivary sample for the Prader-Willi Syndrome Research Study so that researchers can look at genes and DNA for the purpose of analysing genetic subtype, gene expression and methylation		
I understand this genetic testing is for research purposes only so the researchers will not feedback the results of the test		
I know that if the researchers find out something that affects my health, they may need to tell my caregiver and I might need to have some tests done with the doctor.		

Consent to data retention/sharing	**Yes**	**No**
I consent to my child's anonymised cognitive and behavioural data being retained following completion of this study for the purposes of further ethically approved research.		
I consent to my child's anonymised cognitive and behavioural data being shared with research collaborators for ethically approved research.		
I consent to my child's anonymised biological samples being retained following completion of this study for the purposes of further ethically approved research.		
I consent to my child's anonymised biological samples being shared with research collaborators for ethically approved research.		

I consent to my child's anonymised cognitive and behavioural data being shared with publicly available databases for the scientific community for the purposes of ethically approved research. I understand that if data will be shared in this way that the research team will contact the ethics committee to request permission for data sharing.		
I consent to my child's anonymised biological samples being shared with publicly available databases for the scientific community for the purposes of ethically approved research. I understand that if data will be shared in this way that the research team will contact the ethics committee to request permission for data sharing.		
I consent to my child's data being fully (irrevocably) anonymised after project completion should the Principal Investigator of the study wish to do so.		

Future Contact	**Yes**	**No**
I consent to my being contacted by a member of the research team after 10 years to ask for consent to continue to retain my data for future research.		

Participant
Name:
_____

Parent/Guardian
Name: _____
Signature: _____
Date: _____

To be completed by the researcher	**Yes**	**No**
I have fully explained the purposes and nature of this research to the participant's parent/legal guardian in a way that s/he can comprehend, and I have invited him/her to ask questions about the study.		
I have explained the potential for risks and benefits to the participant's parent/legal guardian in a way that s/he can comprehend.		

I confirm that I have provided the participant' with copies of the information leaflets and consent forms.		

Researcher

Name _____

Signature: _____

Date: _____

ADULT CONSENT FORM

Study Title: The impact of social cognition and reward processing on mental health and behaviour in PWS

Instructions: To consent to take part in this project would you please read these statements, tick the appropriate box and sign your names in the space below.

Consent to take part in study	**Yes**	**No**
I have read (or been read to) the information about the study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction		
I understand the information and what taking part in this study involves.		
I am willing to participate in the project, but I feel under no obligation to do so.		
I understand that the information collected in the study will be kept strictly confidential and will only be made available to qualified scientists.		
I understand that I can withdraw from the study at any stage without giving an explanation, and withdrawal will not affect my/my family's medical care.		
I freely and voluntarily agree to be a part of this research study, though without prejudice to my ethical and legal rights.		

Consent to video/ audiotaping of interviews or assessment	**Yes**	**No**
I consent to the video or audio-recording of my interview with the research clinician if required.		
I agree to these audio or video recordings being shared with other authorised researchers for research reliability.		

I agree to these audio or video recordings being used to train clinician's in autism assessment		
Consent to collect a biological Sample	**Yes**	**No**
I consent to providing either a blood or salivary sample for the Prader-Willi Syndrome Research Study so that researchers can look at genes and DNA for the purpose of analysing genetic subtype, gene expression and methylation		
I understand this genetic testing is for research purposes only so the researchers will not feedback the results of the test		
I know that if the researchers find out something that affects my health, they may need to tell my caregiver and I might need to have some tests done with the doctor.		

Consent to data retention/sharing	**Yes**	**No**
I consent to my anonymised cognitive and behavioural data being retained following completion of this study for the purposes of further ethically approved research.		
I consent to my anonymised cognitive and behavioural data being shared with research collaborators for ethically approved research.		
I consent to my anonymised biological samples being retained following completion of this study for the purposes of further ethically approved research.		
I consent to my anonymised biological samples being shared with research collaborators for ethically approved research.		
I consent to my anonymised cognitive and behavioural data being shared with publicly available databases for the scientific community for the purposes of ethically approved research. I understand that if data will be shared in this way that the research team will contact the ethics committee to request permission for data sharing.		
I consent to my anonymised biological samples being shared with publicly available databases for the scientific community for the purposes of ethically approved research. I understand that if data will be shared in this way that the research team will contact the ethics committee to request permission for data sharing.		

I consent to my data being fully (irrevocably) anonymised after project completion should the Principal Investigator of the study wish to do so.		

Future Contact	**Yes**	**No**
I consent to being contacted by a member of the research team after 10 years to ask for consent to continue to retain my data for future research.		

Participant

Name _____

Signature: _____

Date:

_____

Parent/Guardian

Name _____

Signature: _____

Date: _____

To be completed by the researcher	**Yes**	**No**
I have fully explained the purposes and nature of this research to the participant's parent/legal guardian in a way that s/he can comprehend, and I have invited him/her to ask questions about the study.		
I have explained the potential for risks and benefits to the participant's parent/legal guardian in a way that s/he can comprehend.		

I confirm that I have provided the participant' with copies of the information leaflets and consent forms.		

Researcher

Name _____

Signature: _____

Date: _____

U18 PARTICIPANT CONSENT FORM

Study Title: The impact of social cognition and reward processing on mental health and behaviour in PWS

Instructions: To consent to take part in this project would you please read these statements, tick the appropriate box and sign your names in the space below.

Consent to take part in Study	**Yes**	**No**
I have read the information about the study, or one of the researchers has read it to me. I understand all of this information.		
I have had the opportunity to ask questions and all my questions have been answered to my satisfaction.		
I am willing to take part in the project, but I do not feel like I have to.		
I agree to let the researchers take a small sample of my blood or saliva for testing.		
I agree to let the researchers record some parts of this study.		
I agree to the video recordings being shared to help other clinicians practice their scoring.		
I agree to the video recordings being used to help train other clinicians.		

I agree to allow the researchers at Trinity to use some personal information about me for this study, and I know that they will not share this with anyone.		
I know that if the researchers find out something that affects my health, they may need to tell my parents and I might need to have some tests done with the doctor.		
I know that I don't have to take part in this study and that I can stop taking part at any time. I can tell my parents, or the researchers and I will be taken out of the study.		

Participant

Name _____

Signature: _____

Date: _____

Caregiver/Parent

Name _____

Signature: _____

Date: _____

To be completed by the <u>RESEARCHER</u>	**Yes**	**No**
I have fully explained the purposes and nature of this research to the participant in a way that s/he can comprehend, and I have invited him/her to ask questions about the study.		
I have explained the potential for risks and benefits to the participant in a way that s/he can comprehend.		
I confirm that I have provided the participant and his/her parent/guardian with copies of the information leaflets and consent forms.		

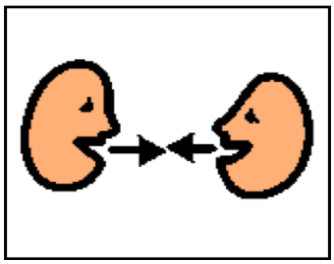
Name _____

Signature: _____ Date: _____

PIL and Consent (Picture Based Version)

Study Title: The impact of social cognition and reward processing on mental health and behaviour in Prader-Willi Syndrome

	Hello! My name is Sarah ☺
	I work in a research group in Trinity College. We are doing a research study on PraderWilli Syndrome.
	I would like to meet you and learn more about what it is like to have PWS
	I would like to find out about your strengths.

	I also want to know more about things you find difficult and are still working on.
	I would like to ask someone who knows you very well to answer some questions about you and to do some other activities
	I would like for you to do some computer games in Trinity with me
	I would like you to wear this special cap while you're playing computer games so I can see how your brain works
	I would like you to take a blood sample from you so I can learn all about your genes!

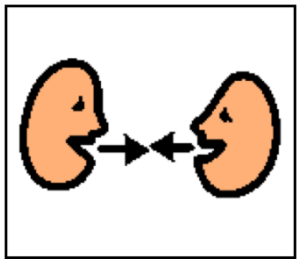
	Everything you do will be kept top secret and all your notes will be locked in the office and on a computer.

	My research group can use this information to see how we can help improve the care people with PWS receive.

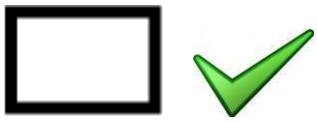
	Would you like to take part in this study?

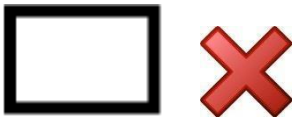
	You can say yes or no. It's ok to say no. It is your choice.

	If you say no that is no problem at all. If you want to leave the study that is ok too.

	Do you want to take part in this study?

PARTICIPANT CONSENT

YES 

NO 

Participant:

Name: _____

Signed: _____ Date: _____

Researcher

Name: _____

Signed: _____ Date: _____

Phone Script for Contacting Participants who Consented to Hear About Study

Introduction

- Hello, this is XX ringing from Trinity College. Is this _____?
- I'm a member of Prof. Louise Gallagher's research team who research PWS. My colleague Sarah may have been in touch with you before. I'm contacting you as you had previously said you would be interested in here more about a PWS study that we are running the group. Is this a suitable time for a brief chat?
- We previously had to postpone our research during COVID which was unfortunate as we just started to contact people about the study. We have spent the last few months adapting the study so we could carry out in a way that is safe for our participants and their families and our own team. We now have approval from the COVID safety committee in Trinity to restart the study and data collection.
- I'm ringing to see if you would be interested in hearing more about it.

The Study

- To give you a bit of background to the study:
- People with PWS can sometimes have autism like behaviours, especially in terms of social communication. For instance, we know they find it difficult to recognise emotions or to figure out what people might be thinking of feeling. As hyperphagia emerges in PWS, we start to see these autism like behaviours increase. So we want to figure out if some of the complex behaviours we see in PWS are related the hyperphagia.
- Phase 1 will involve zoom interviews with you.
- Phase 2 will involve coming to St James's to do eye tracking and EEG with PPE. Do you think you'd be able to come to St James's?
- Phase 3 will involve some online questionnaires.
- There is a phase 4 but at the moment we think it is not possible to do it while wearing a mask and social distancing, so we have postponed this phase (ADOS).
- Are you still interested/happy to participate in this study?

Next Steps

- The first thing we will arrange to do is to send you the participant information leaflet. This is a detailed overview of the study and what is involved. You can read this and then if you have any more questions, you can email or phone me
- If you are happy to take part, you can sign the consent form I will send and then we will arrange the first meeting.

- Do you mind me asking, what age is your child with PWS?
    - ***(If over 18, say we can send them the info too, if they have email address)***

Newsletter

- The last thing, we have started to do a newsletter every six months to keep families and other researchers informed of what we're up to. We just published the first newsletter. Would you like a copy of it emailed to you?

  (check contact details)

End

- I appreciate you taking the time to speak with me today. After this call I will send you an email with the participant information leaftlet and the consent form. You can take your time to go through it. Then if you're happy to take part, you can sign the consent form.
- Please do not hesitate to call or email me if you have any questions about any part of the study.
- Thank you very much for your time today. Thank you and bye for now.

Appendix 2: Study Protocols

Table A.1: Protocol for Chapter 4

Purpose	Respondent	Measure	Duration
Autism Diagnosis	Caregiver	Autism Diagnostic Interview (ADI-R)	2 - 3 hours
	Participant	Autism Diagnostic Observational Schedule (ADOS-II)	60 mins
Autism Behaviours	Caregiver	Social Communication Questionnaire (SCQ)	10 mins
Cognitive Assessment	Participants > 6 years	Wechsler Abbreviated Scale of Intelligence Second Edition (WASI-II)	45 mins
		or	
	Participants < 6 years and non-verbal participants	The Mullen Scales of Early Learning (MSEL)	60 mins
Complex Behaviour	Caregiver	Aberrant Behaviour Checklist (ABC)	5 mins
Adaptive Functioning	Caregiver	Vineland Adaptive Behaviour Scales (VABS-II)	60 mins
Eye tracking	Participant	Face Pop Task	10 mins

Table A.2: Protocol for Chapter 5

Purpose	Measure	Duration
Eye Tracking Premeal	Satiety Labelled Intensity Magnitude Scale (SLIM) = premeal version	5 mins
	FAB task – premeal condition	10 mins
Lunch	Standardised lunch (sandwich, fruit, water)	30 mins
Eye Tracking Postmeal	Satiety Labelled Intensity Magnitude Scale (SLIM) = postmeal version	5 mins
	FAB task – postmeal condition	10 mins
Post Eye Tracking Measures	Honesty Questionnaire	2 mins
	Food stimuli rating scale	2 mins
Demographics	Demographics Questionnaire	2 mins
	Height and weight	5 mins

Table A.3: Protocol for Chapter 7

Purpose	Respondent	Measure	Duration
Hyperphagia	Caregiver	Hyperphagia Questionnaire (HQ)	5 mins
	Caregiver	Food Related Problems Questionnaire (FRPQ)	5 mins
Eye Tracking Premeal	Participant	FAB task – premeal condition	5 mins
Lunch	Participant	Standardised lunch (sandwich, fruit, yoghurt, bottle of water)	30 mins
Eye Tracking Postmeal	Participant	FAB task – postmeal condition	5 mins
Post Eye Tracking Measures	Caregiver	Food stimuli rating scale	2 mins
	Participant	Food stimuli rating scale	2 mins
Demographics	Caregiver	Demographics Questionnaire	2 mins
	Participant	Height and weight	10 mins

Appendix 3: COVID -19 Lab Protocols

Return to research protocol for Autism and Rare Neurodevelopmental Disorders Research Group

Research Group: The Autism and Rare Neurodevelopmental Disorders Research Group, Department of Psychiatry, School of Medicine

PI: Prof Louise Gallagher

Our current research studies involve characterisation of genotypes and phenotypes in neurodevelopmental conditions such as autism, and rare genetic conditions including NRXN1 deletions and Prader-Willi Syndrome (PWS). We also collect data from typically developing individuals for comparison. All of our studies include recruitment of children, adolescents and adults.

Prior to COVID restrictions all measures were collected in person over 1-3 visits to our research lab at the TCD Health Sciences Centre St. James's or at TCIN on campus. We have adapted some of the protocol for remote testing (At home) to allow for data collection during COVID restrictions, and to reduce face-to-face testing once restrictions are lifted. Most of our protocol requires face-to-face testing (at either neurocognition lab, TCIN, or St. James's CRF).

In preparation for face-to-face testing with participants, we will follow the safety protocol developed by the SOM. In addition, all researchers conducting face-to-face testing with participants will be vaccinated.

General Procedures

- The team will complete the local COVID-19 Induction training programme delivered before returning to research activities
- All members will complete the "return to work" questionnaire day before arriving at work
- An activity log for the neurocognition lab will be set up and each team member will document time of entry, exit, equipment used, along with contact details of any interactions during the day where social distance was not preserved.
- The team will comply with the safety requirements and capacity constraints of all common areas and services both inside and outside of the school
- In line with the SOM designated occupancy capacity based on social distancing requirements. Only three people will be allowed in the Neurocognition Lab or four people, where two of them are from the same household e.g. two research participants from the same household and two researchers.

- The SafeZone app will be installed and used at all times by all members of the research team

Participant Entry Procedures

Procedures outlined under Section 8.7.2 "**Participant Entry Procedures"** by the SOM will be followed for all participants coming for research study visits which include:

- Participants will be required to complete a pre-trial COVID screening form (Appendix B) a minimum of 48 hrs prior to arrival at the facility.
- Completed screening forms will be returned to facility PI via email.
- If completed screening form is acceptable, contact details and date/time of planned campus entry will be forwarded to E&F manager.
- Staff will arrive on site a minimum of 30 minutes prior to planned arrival time of participant in order to prepare workstation for participant arrival.
- Staff hands will be sanitised, and appropriate PPE donned.
- COVID screening form completed, and staff temperature taken.
- Arrival time logged in activity log (Appendix C).
- Workstation and equipment will be disinfected, calibrated and prepared for use.
- ·At the designated time of planned campus entry, participant will be met at the Lincoln Place Security Hut.
- Researcher will supervise hand sanitisation and donning of facemask by participant.
- Hard copy of COVID screening form will be checked and participant temperature will be taken.
- If screening form and temperature are satisfactory, participant will be admitted on campus and accompanied to the designated research facility.
- If screening form or temperature are unsatisfactory, participant will not be admitted on campus and recommendations regarding social isolation and contact of family GP will be provided.

Participant Exit Procedures

Procedures outlined under Section 8.7.3 "**Participant Exit Procedures"** by the SOM will be followed for all participants coming for research study visits which include:

- Social distancing of 2m between researcher and participant will be maintained throughout study protocol, where possible.

- In cases where direct contact is required, skin site will be disinfected prior to contact.
- Upon completion of all trial procedures, participant will be accompanied back to the Lincoln Place Security Hut for exiting the campus.
- Single-use PPE will be removed and disposed of, and the researcher will again supervise hand sanitisation.
- Researcher will disinfect equipment and workstation with 70% ethanol solution, complete and file the activity log for the session, clean and store reusable PPE, dispose of all single-use PPE, sanitize hands before exiting the facility.

High Risk Activities: Biological Sample Collection

Research activities which have been designated high risk by the SOM include capillary blood sampling and venous cannulation as they require direct human contact and the exposure to bodily fluids. For our research, biological samples will be collected and include both blood and saliva samples. These are collected from participants and family members for whole genome sequencing.

Sterile aseptic techniques are already standard practice for both procedures, including alcohol swabbing of sampling site, wearing of gloves and disposal of all biohazardous material in appropriate receptacles. Procedures outlined by the SOM will be followed for mitigating risk of transmission for these high-risk procedures including the following:

- Isolation of test procedure to confined space via use of physical barrier such as Perspex.
- The use of fluid resistive gown/lab coat by operator throughout the procedure.
- The use of latex gloves by operator throughout the procedure.
- Use of protective face mask and goggles/visor by operator throughout the procedure.
- Use of protective face mask by participant in procedures where respiration rate or tidal volume is not a limiting factor.
- Disinfection of skin contact site via alcohol swabbing prior to contact.
- Disposal of non-reusable test equipment in appropriate marked biohazard bins upon completion of procedure.
- Disinfection of all reusable test equipment upon completion of procedure.
- Washing of hands with alcohol gel upon arrival and departure from facility.
- Disinfection of test site by operator upon completion of each data collection session.

Families can provide blood samples in the following ways:

- By a research nurse in the Clinical Research Facility (CRF), St. James's Hospital (Age 16+ years only). Guidelines specified by the CRF will be adhered to during visits which include pre- screening questionnaire for COVID symptoms and masks during visit. A member of the research group will wear a mask when accompanying the family to and from the CRF, and mask and gloves when returning the samples to Trinity Translational Medicine Institute (TTMI) for processing and storage.
- By a trained phlebotomist/clinician at our neurocognition lab and will follow SOM procedures.
- By a family GP. A member of the research group will wear a mask and gloves when delivering materials and collecting samples from the GP for processing and storage in TTMI.

Families can provide saliva samples in the following ways:

- At our neurocognition lab. PPE will include mask and gloves worn by the researcher collecting these samples from participants and storing them.
- At home. PPE will include mask and gloves worn by the researcher collecting these samples from participants and storing them.
- Mailed to the neurocognition lab. PPE will include mask and gloves worn by the researcher processing and storing these samples on receipt in the lab.

Moderate Risk Activities: EEG and Eye tracking Data Collection

Research activities which have been designated moderate risk include electroencephalography (EEG), and eye tracking. Our EEG and eye tracking data collection will take place in the neurocognition lab. In all cases, the EEG procedure is designated moderate risk due to unavoidable human-to-human contact. For EEG, this contact can involve palpation of bony landmarks, preparation of recording site and placement of electrodes on the skin. Procedures outlined by the SOM will be followed for mitigating risk of transmission for these moderate risk procedures including the following:

- Use of protective face mask and goggles/visor by operator throughout the procedure.
- Use of protective face mask by participant in procedures where respiration rate or tidal volume is not a limiting factor.
- Disinfection of skin contact site via alcohol swabbing prior to contact.
- Hand sanitization with alcohol gel after any direct skin contact

- Disposal of non-reusable test equipment in appropriate marked biohazard bins upon completion of procedure.
- Disinfection of all reusable test equipment upon completion of procedure.
- Hand sanitization with alcohol gel upon arrival and departure from facility.
- Disinfection of test site by operator upon completion of daily work.

Moderate Risk Activities: MRI Data Collection

We will follow the guidelines for testing participants using the MRI suite at TCIN approved by the college (see attached). MRI testing represents a medium/moderate risk activity due to the necessity for close contact (less than 1m) between researcher and participant at the beginning and potentially at the end of the testing session, and between the radiographer and participant to facilitate entry and exit of the participant from the scanner - it requires adjusting pads around the participant's head. All PPE will contain non-ferromagnetic component parts for MRI safety, as per guidelines for MRI testing laid out in Appendix 11 of the TCIN document. Safety of Study Participants & Research Staff Procedures and Facility Procedures outlined in the document for TCIN will be adhered to for all participant MRI testing sessions.

Low Risk Activities: Clinical Data Collection

Research activities which have been designated low risk include height and body mass assessment, and cognitive assessments (WASI). In all cases, the procedure is designated low risk due to the lack of direct human-to-human contact. Procedures outlined by the SOM will be followed for mitigating risk of transmission for these moderate risk procedures including the following:

- Use of protective face mask by operator throughout the procedure.
- Use of protective face mask by participant in procedures where respiration rate or tidal volume is not a limiting factor.
- Disposal of non-reusable test equipment in appropriate marked biohazard bins upon completion of procedure.
- Disinfection of all reusable test equipment upon completion of procedure.
- Hand sanitization with alcohol gel upon arrival and departure from facility.
- Disinfection of test site by operator upon completion of daily work.

Appendix 4: Thesis and Publication

Publication License
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TERMS AND CONDITIONS

Feb 10, 2023

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Licensed Content Volume	64
Licensed Content Issue	2
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A profile of mental health and behaviour in Prader–Willi syndrome

S.-M. Feighan,[1] M. Hughes,[2] K. Maunder,[1] E. Roche[3] & L. Gallagher[1]

1 *Department of Psychiatry, Trinity College Dublin, Dublin, Ireland*
2 *Prader-Willi Syndrome Association of Ireland, Dublin, Ireland*
3 *Department of Paediatrics, Trinity College Dublin, Dublin, Ireland*

Abstract

Background Prader–Willi syndrome (PWS) is a neurogenetic syndrome with an associated behavioural phenotype and a high incidence of behaviours of concern and psychiatric co-morbidity. These associated behaviours and co-morbidities are not well addressed by existing interventions, and they impact significantly on affected individuals and their caregivers.

Methods We undertook a national survey of the needs of individuals with PWS and their families in Ireland. In this paper, we report on the parent/caregiver-reported mental health, behavioural and access to services.

Results Over 50% of individuals with PWS in this survey had at least one reported psychiatric diagnosis, the most common diagnosis was anxiety. The most commonly reported behaviours in children were skin picking, repetitive questioning, difficulty transitioning and non-compliance. The same four behaviours were reported by caregivers as being the most commonly occurring in adolescents and adults in addition to food-seeking behaviours. Increased needs for mental health services were also reported by caregivers. Individuals with PWS had an average wait of 22 months for an appointment with a psychologist and 4 months for an appointment with a psychiatrist. *Conclusion* This study highlighted high levels of psychiatric co-morbidities and behavioural concerns in individuals with PWS in Ireland. The findings of this study suggest that there is an urgent need to provide specialist psychiatric and behavioural interventions to manage complex mental health and behavioural needs to better support individuals with PWS and reduce caregiver burden.

Keywords behavioural phenotype, mental health, Prader–Willi syndrome, psychiatric disorders

Correspondence: Ms Sarah-Marie Feighan, Department of Psychiatry, Trinity Centre for Health Sciences, St. James's Hospital, James's Street, Dublin 8, Ireland (e-mail: sm.feighan@gmail.com).

Introduction

Prader–Willi syndrome (PWS) is a neurogenetic syndrome with a characteristic behavioural phenotype, a high incidence of maladaptive behaviours and psychiatric co-morbidities. PWS has a birth incidence rate of about 1:25000 (Smith *et al*. 2003; Vogels *et al*. 2004b; Whittington *et al*. 2001) and a population prevalence in the UK of about 1:50000 (Whittington *et al*. 2001). It is the first recognised disorder related to genomic imprinting in humans, a process whereby genes are programmed to be silent or expressed depending on parental origin of the chromosome. PWS is due to a failure of paternal expression of maternally imprinted genes at the 15q11–13 region, due to (1) deletion of the 15q11–13 region on the paternal chromosome (DEL), (2) maternal uniparental disomy of chromosome 15 (mUPD) or (3) imprinting centre defects or translocations (IC) (Cassidy *et al*. 2012). In the literature on PWS, the proportion of cases in each of the genetic subtypes are usually given as (approximately) 70% DEL, 25–40% mUPD and 3– 5% other (IC or translocations) (Cassidy *et al*. 2012). However, recent studies have shown an increase of a greater proportion (50%) of those with the mUPD subtype in younger children and have suggested that an increase in maternal age may be driving this changing proportion (Whittington *et al*. 2007; Lionti *et al*. 2015).

Prader–Willi syndrome presents with a complex and changing developmental profile. Infants are born with hypotonia and have poor suck, feeding problems, failure to thrive and developmental delay. Motor milestones and language development are delayed, and all individuals have some degree of cognitive disability. Children with PWS can experience a range of endocrinological problems affecting the thyroid, adrenal and gonadal axes. Growth hormone insufficiency or dysfunction is common, leading to short stature. Obesity occurs after a characteristic period of failure to thrive and is associated with extreme food-seeking behaviour and hyperphagia in early childhood (Cassidy *et al*. 2012). Hyperphagia is related to an impaired satiety response and a high reward value of food in PWS (Hinton *et al*. 2006; Miller *et al*. 2007). This extreme drive for food is a life-long stressor for affected individuals and their carers due to the necessity to significantly limit overeating and impacts significantly on their health and well-being.

Individuals with PWS have an increased risk for specific co-morbid behavioural and psychiatric difficulties (Whittington and Holland 2018). A recent study found that 89% of patients with PWS over 12 years of age had at least one psychiatric disorder (Shriki-Tal *et al*. 2017). The core behavioural phenotype of PWS is characterised by temper tantrums, mood lability, repetitive and ritualistic behaviours and severe skin picking, seen in all genetic subtypes. However, the mUPD genetic subtype has a strikingly higher prevalence of psychosis, which has been estimated at a 60–100% lifetime prevalence, compared with the deletion subtype that has a similar prevalence to adults with intellectual disabilities in the wider population (Boer *et al*. 2002;

Hinton *et al*. 2006; Soni *et al*. 2007, 2008; Verhoeven *et al*. 2003; Vogels *et al*. 2004b). Autism spectrum disorder (ASD) diagnosis occurs in 12–25% of individuals with PWS, with the mUPD genetic subtype having a significantly increased risk of ASD (Bennett *et al*. 2015). Additionally, clinically impairing ASD symptoms occur in both the mUPD and DEL genetic subtypes (Bennett *et al*. 2015; Dykens *et al*. 2017). Psychiatric co-morbidities rank highly as factors negatively affecting the quality of life of individuals with PWS and are reported as the most difficult aspect of the condition to manage by their caregivers (Lanfranchi and Vianello 2012). In a survey study of children with neurogenetic syndromes, including PWS, fragile X, Williams syndrome and 22q11.2 deletion syndrome, having a behavioural/psychiatric condition was a significant predictor of negative families outcomes across all syndromes (Reilly *et al*. 2015)

Because of the range of problems and variability of symptom severity across individuals with PWS, clinical management is age dependent, multidisciplinary, targeted at symptoms and tailored to the individual. As such, there is no specific treatment for PWS-specific behavioural disturbances. Applied behaviour analysis interventions, a treatment approach that is commonly used in autism, showed efficacy in some small case studies for treating skin picking and food-related behaviours, but research is limited (Page *et al*. 1983; Maglieri *et al*. 2000; Stokes and Luiselli 2009). Treatment with numerous psychotropic medications such as antidepressants, antipsychotics and appetite suppressants have shown very little effectiveness in controlling hyperphagia and behaviours related to the phenotype, although these may also be used to treat psychiatric co-morbidity (Bonnot *et al*. 2016).

The combination of severe hyperphagia, psychiatric co-morbidities, challenging behaviours and lack of effective treatments creates unique challenges in caring for persons with PWS. Managing and treating these features has become a critical issue in the clinical care of people with PWS (Schwartz *et al*. 2016). Higher levels of family stress were found in families of children with PWS compared with other genetic syndromes such as Down syndrome, fragile X and Williams syndrome (Lanfranchi and Vianello 2012). Similarly, caring for an individual with PWS also negatively impacted carer's romantic relationships, ability to work, sleep and mood (Kayadjanian *et al*. 2018).

This study was the result of a collaboration between the Prader-Willi Syndrome Association of Ireland (PWSAI), Trinity College Dublin and Tallaght University Hospital Dublin to undertake a national survey in Ireland to identify the physical, mental health/behavioural and service needs of individuals with PWS and their families to inform policy developments. The aim of the present study is to report the findings from the sections on mental health and behaviour, access to clinical services and the impact of caring for an individual with PWS.

Methods

Community and participant engagement was an important component of the development of this research. A panel of clinicians, researchers and parent advocates were involved in the survey design, which was informed by literature review and clinical consensus. The survey was informant based and targeted at caregivers, with quantitative and qualitative elements, and focused on early life and development, physical health, mental health and behaviour, education and employment, residential and respite support needs, and caregiver impact. The survey was revised based on feedback from the PWSAI committee and expert medical and behavioural clinicians. The revised survey was piloted with parents of individuals with PWS, which informed further minor revisions. The final version was approved by the PWSAI. Ethics approval for the survey was provided by the Tallaght University Hospital/St. James's Hospital Joint Research Ethics Committee.

The mental health and behaviour section included parent-reported diagnoses of psychiatric disorders, the date of first diagnosis, current and past psychotropic medication, dates commenced and duration of treatment. For the purpose of this study, the term psychiatric disorder encompassed 'Anxiety
Disorder', 'Bipolar Disorder', 'Depression', 'Obsessive Compulsive Disorder' and 'Psychosis'. There was also an 'Other' option where participants could name any other psychiatric diagnosis. In this section, participants were also asked to report if they had a diagnosis of 'Autism Spectrum Disorder'. Two sub-scales of the Behaviour Problems Inventory – Short Form (Rojahn *et al*. 2012) – provided measures of self-injurious behaviours (eight

items) and aggressive–destructive behaviour (10 items). Caregivers were asked to rate frequency ('never', 'monthly', 'weekly', 'daily' and 'hourly') and severity ('no problem', 'mild', 'moderate' and 'severe'), and subtotals for each were calculated. In consultation with the family association, we decided that only caregivers of individuals with PWS over the age of 4 years would be asked to complete the mental health and behaviour sections of the questionnaire.

We incorporated the Hyperphagia Questionnaire (Dykens *et al*. 2007), a 13-item instrument that measures the presence and severity of food-related preoccupations and problems in PWS on a 5-point scale (1 = not a problem to 5 = severe and/or frequent problem). The scale provides a total score and three subscores: behaviour, drive and severity.

We assessed caregiver impact using the Brief Family Distress Scale (Weiss and Lunsky 2011) to evaluate current level of crisis experienced by the caregiver/family on a 10-point scale. Each point was grounded in a statement describing a point along a scale from no stress ('0') to complete crisis ('9'). We also asked caregivers to rate the impact of caring for an individual with PWS on the family on a scale of 1–4 (1 = 'no impact', 2 = 'small negative impact', 3 = 'significant negative impact' and 4 = 'extreme negative impact').

Study information was e-mailed to PWSAI members, and hardcopies were given to patients and carers attending the PWS Specialist Medical Clinic at Tallaght University Hospital who contacted the study team if they wished to participate. Further information regarding the study was discussed by telephone. Information sheets, consent form and surveys were sent via post. On the advice from PWSAI, caregivers of children less than 4 years of age (n = 8) did not complete the behaviour and psychiatric sections, as it was thought that this might burden them unduly.

Seventy-one participants provided their contact details to the research team, of whom 65 were successfully contacted and 61 of which returned completed surveys (response rate: 94%). We estimate that the respondents represented approximately 60% of the total known PWS population in Ireland, based on estimates of diagnoses from the National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin. However, this does not include individuals with PWS who are undiagnosed in Ireland or who received a diagnosis from abroad. We present demographic, mental health and behaviour, and caregiver impact data analysis in the following section.

Results

Sixty-one caregivers of individuals with PWS participated in this study; 82% of respondents were the biological mother of the person with PWS, 13% were the biological father and 5% were a sibling. The age of the individuals with PWS ranged from 11 months to 52 years with a mean age of 16.3 years
(SD = 11.3). Within the sample, 58% were female (n = 35) and 42% were male (n = 26). Based on caregiver reporting, 43% (n = 25) had the deletion subtype, 26% had the mUPD subtype (n = 17), 3% had an imprinting centre defect (n = 2) and 28% were unsure of the genetic subtype (n = 18). Recognising that developmental and behavioural needs change across lifespan, we subset the results into three age groups based on education stage: children (primary school, aged 4–12 years), adolescents (secondary school, 12–18 years) and adults (>18 years).

Psychiatric disorders

Table 1 shows the prevalence of psychiatric disorders in participants in both the adolescent and adults groups (n = 38). No participants under the age of 12 had been diagnosed with a psychiatric disorder. Fifty per cent of participants over the age of 12 years had been diagnosed with a psychiatric disorder (Table 1). Anxiety was the commonest diagnosis in adolescents followed by obsessive–compulsive disorder (OCD). Anxiety was also the commonest diagnosis in adults. Adults had more diagnoses of depression, psychosis and bipolar disorder compared with adolescents (Table 2). The average onset of a psychiatric disorder was 16 years (SD +4.9, range 6–23). The commonest co-morbid diagnoses were anxiety with OCD, followed by anxiety with depression.

Psychotropic medication

No participants less than 12 years old were prescribed with psychotropic medication. Forty-two per cent of participants over 12 years old were currently prescribed with psychotropic medication (Table 3). Selective serotonin reuptakes inhibitors (SSRIs) were the

commonest prescribed medications in adolescents and antipsychotics the commonest in adults (Table 4). Antipsychotic medication was significantly more likely to be prescribed in the mUPD subtype compared with the DEL subtype (Fisher's exact test: p <0.01, odds ratio = 23.1, 95% confidence interval = 2.0, 768.9). Although 80% of mUPD participants were prescribed with antipsychotic medication, none were reported as having a clinical diagnosis of psychosis.

Behaviours

Skin picking was the most prevalent reported behaviour on the self-injury scale in children, adolescents and adults, reported in 76% of cases. Skin picking was particularly common in adolescents (93%) (Table 5). Teeth grinding was the second most prevalent self-injurious behaviour, highest in the adolescent group (Table 5). Aggressive behaviours

Table 2 Prevalence of psychiatric diagnoses across age group (adolescents and adults), gender and genetic subtype

		Age group		Gender		Genetic subtype			
	Total	Adolescent (12–17 years)	Adults (≥18 years)	Male	Female	DEL	mUPD	IC	Unknown
	N = 38	*N* = 17	*N* = 21	*N* = 14	*N* = 24	*N* = 17	*N* = 5	*N* = 2	*N* = 14
	%	%	%	%	%	%	%	%	%
Anxiety disorder	37	29	43	36	38	29	80	100	21
ASD	5	12	0	7	4	6	0	0	0
Bipolar disorder	8	0	14	7	8	6	20	50	0
Depression	24	6	38	14	29	18	20	100	21
OCD	16	18	14	14	17	6	20	100	14
Psychosis	16	6	19	14	17	18	0	100	7

% is prevalence within each group.

ASD, autism spectrum disorder; OCD, obsessive–compulsive disorder; DEL, deletion subtype; mUPD, maternal uniparental disomy; IC, imprinting centre defect.

Table 1 Number of psychiatric diagnoses received by participants over the age of 12 years

Number of diagnoses	Participants ≥12 years	
	N	%
0	19	50
1	8	21
2	6	16
≥3	5	13
Total	38	100

differed across age groups. 'Hitting others' was the most endorsed item in children, whereas 'verbal abuse' was the commonest in adolescents and adults. On the PWS specific sub-scale, repetitive questioning was a highly prevalent reported behaviour in all three age groups, reported by 100% of caregivers of children and adolescent 67% of adults (Table 5). Stealing food and money and lying, associated with the core hyperphagia phenotype, were prevalent behaviours; however, non-compliance, difficulty transitioning, and obsessions/compulsions were more frequently reported by caregivers across all age groups.

Hyperphagia occurred in 81% of participants over the age of 4. The average age of onset of hyperphagia

Table 3 Number of psychotropic medications prescribed for participants over the age of 12 years

Number of medications	Participants ≥12 years	
	N	%

Table 4 Prevalence of psychotropic medication usage across age group (adolescents and adults), gender and genetic subtype

	Total	Age group		Gender		Genetic subtype			
		Adolescent (12–17 years)	Adults (≥18 years)	Male	Female	DEL	mUPD	IC	Unknown
	N = 38	*N* = 17	*N* = 21	*N* = 14	*N* = 24	*N* = 17	*N* = 5	*N* = 2	*N* = 14
	%	%	%	%	%	%	%	%	%
On medication	42	35	48	36	46	35	100	100	21
Antipsychotic	24	6	43	14	29	12	80	100	7
Mood stabiliser	11	6	14	14	8	6	20	50	7
SSRI	26	29	24	21	29	29	40	0	21
Another	8	6	10	7	8	6	0	100	0

% is prevalence within each group.
SSRI, selective serotonin reuptake inhibitor; Other, alprazolam, biperiden, lorazepam and sodium valpraote; DEL, deletion subtype; mUPD, maternal uniparental disomy; IC, imprinting centre defect.

0	22	58
1	6	16
2	4	10
≥3	6	16
Total	38	100

was 3.8 years (±1.6 months, range 1–7 years). Hyperphagic drive, hyperphagic behaviour and hyperphagic severity did not differ based on gender, genetic subtypes or age group (Table 6). A small correlation was observed between age and hyperphagic behaviour (r = 0.28, p <0.07) in children/adolescents. Thirty-six per cent showed little variability in food preoccupation, 49% reported occasional variability and 14% showed high variability in food preoccupation.

Access to services

All sixty-one participants were asked to answer questions about their access to clinical services.

Ninety-two per cent of participants had attended speech and language therapy. The average time participants had to wait for their first speech and language therapy session was 11 months, and at the time of this study, 4% of participants were on waiting lists to attend speech and language therapy. In a smaller proportion of participants, 75% were receiving occupational therapy. The average waiting time for the first occupation therapy was 12 months (Fig. 1). Fewer participants (67%) had attended psychology services; however, there was a notable increase in waiting times for this service (22-month wait for an appointment). Twenty-eight per cent of participants had attended a psychiatrist, with an average waiting time of 4 months for the first appointment.

Impact on families

A significant impact of caring for an individual with PWS on caregiver employment was reported by a large proportion of respondents. Seventy-five per cent reported that either they or their partner had stopped working entirely, and 16% reported that they reduced their working hours. Only 9% reported no impact on employment.

Family impact was measured on a scale of 1–4 (1 = 'no impact', 2 = 'small negative impact', 3 = 'significant negative impact' and 4 = 'extreme negative impact'). Seventy-five per cent reported significant or extremely negative emotional impact on the family related to caring for their relative with PWS relative. Emotional impact was reported as less severe in caregivers of individuals with PWS who lived in assisted accommodation. Negative physical impact and impact on family relationships was highest in the adolescent group, and financial impact was highest in the adult

group. Caregivers were also asked to rate their perceived level of crisis. Thirty-two per cent said, 'everything is fine/sometimes a little stressful', 52% said 'things are very stressful' and 16% said they are 'in crisis and cannot cope'.

Discussion

This study summarises parent/caregiver reports of psychiatric co-morbidities, behaviours of concern, service needs and impact of caring in families of an individual with PWS. The study highlighted the complexity and challenges associated with the behavioural phenotype and the high prevalence of reported psychiatric co-morbidities. Challenges were reported across childhood, adolescence and adulthood, although these were observed to differ with age. In the context of the psychiatric and behavioural challenges, the study also identified delay in accessing behavioural support services in particular for individuals with PWS in Ireland and the significant impact that caring for an individual with PWS can have on the caregiver.

The Irish PWS population exhibit a similar psychiatric profile to previous studies reported in PWS: high levels of anxiety appear in adolescence, followed by the onset of affective disorders and psychosis in adulthood. The prevalence of psychiatric diagnosis in participants over the age of 12 years in our study was lower than a recent study from Israel in which the majority (89%) had a psychiatric diagnosis. This may be explained by the reliance on parentreported psychiatric diagnoses in the current study as opposed to the use of direct clinical assessment. Anxiety has previously been found to be the commonest diagnosis in a study looking at neuropsychiatric diagnosis of adults with PWS in residential care (Manzardo *et al*. 2018). Notably, anxiety is one of the most significant predictors of psychosis in 22q11.2 deletion syndrome, a genetic syndrome that is also associated with high rates of psychosis (Tang *et al*. 2017). More research is needed to investigate predictors of psychosis in PWS, especially in the mUPD genetic subtype who are more at risk. Thirteen per cent of participants had received three or more comorbid psychiatric diagnoses, which emphasises the complex nature of psychiatric disorders in PWS. A recent review of the psychiatric conceptions of mental and behavioural disorders in PWS discussed how psychiatric disorders in PWS are different to those

Table 5 Item-level prevalence of challenging behaviours from the sub-scales of the Behaviour Problems Inventory – Short Form (self-injurious and aggressive/destructive) and survey (PWS specific) across total sample and across age groups

		Total	Age categories		
		(4–52 years)	Children (4–11 years)	Adolescents (12–17 years)	Adults (≥18 years)
		N = 53	*N* = 15	*N* = 17	*N* = 21
Sub-scale	Item	%	%	%	%
Self-injurious behaviours	Self-biting	11	21	13	6
	Head hitting	4	7	7	0
	Body hitting	12	14	27	0
	Pica	11	14	12	11
	Inserting objects	16	7	24	21
	Hair pulling	11	7	18	11
	Teeth grinding	36	40	53	21
	Skin picking	76	71	93	72
Aggressive destructive behaviours	Hitting others	27	40	29	21
	Kicking others	17	7	29	17
	Pushing others	34	33	59	24
	Biting others	7	7	13	5
	Grabbing/pulling others	23	33	29	16
	Scratching others	13	20	18	5
	Verbally abusive	39	33	59	37
	Pinch others	16	27	23	6
	Destroying things	29	20	41	26
	Bullying	20	20	35	11
PWS specific behaviours	Stealing food	45	53	50	47
	Stealing money	16	7	23	24
	Lying	53	40	71	40
	Repetitive questioning	78	100	100	67
	Obsessions/compulsions	44	67	64	22
	Non-compliance	62	80	71	56
	Difficulty transitioning	50	87	64	22

% is prevalence within each group.

PWS, Prader–Willi syndrome.

observed in the general population; however, there are overlaps in symptoms (Whittington and Holland 2018). As psychiatric disorders in PWS are atypical, it may be difficult to classify them within existing psychiatric

diagnoses, which may explain why some participants in the current study had three or more diagnoses.

In our study, 42% of the sample over the age of 12 years were prescribed with at least one psychotropic medication. SSRIs were the commonest in adolescents and antipsychotics in adulthood and were highly correlated with comorbid psychiatric diagnosis. SSRIs were also the commonest prescribed psychotropic medication in a separate review of psychotropic medication usage in PWS (Bonnot *et al*. 2016). In the present study, the mUPD genetic subtype was found to be more likely to take antipsychotic medication than the deletion subtype. This probably reflects the known increased risk of psychosis in the mUPD genetic subtype (60– 100% prevalence) compared with the deletion subtype, where prevalence rates are similar to

Table 6 Factor means, standard deviations and ranges of the Hyperphagia Questionnaire across age groups

	Behaviour			Drive			Severity		
	Children *N* = 15	Adolescents *N* = 17	Adults *N* = 21	Children *N* = 15	Adolescents *N* = 17	Adults *N* = 21	Children *N* = 15	Adolescents *N* = 17	Adults *N* = 21
Mean	14.4	13.5	12.8	7.7	7	5.3	4.9	4	3.6
SD	3.4	3.9	5.2	2.8	1.8	2.9	1.6	1.9	2
Range	10–21	7–20	0–20	1–12	3–10	0–10	1–7	1–7	0–6

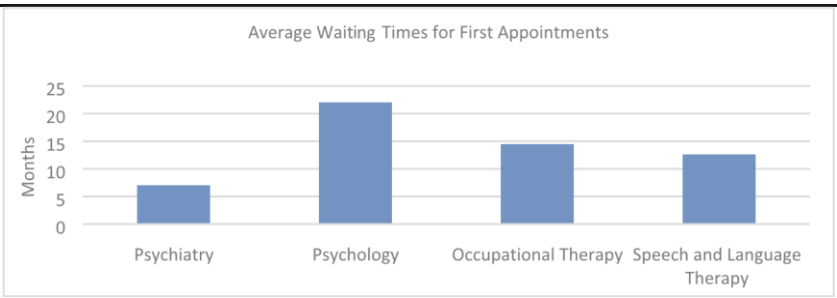

Figure 1. Average waiting times for access to services [Colour figure can be viewed at wileyonlinelibrary.com]

individuals with intellectual disability more generally. It has previously been shown that those with mUPD are more likely to have been prescribed with psychotropic medication and to have tried a larger number of psychotropic medications, possibly due to a poor response to medication (Soni *et al*. 2007). Although 80% of mUPD participants were prescribed with antipsychotic medication, none were reported as having a clinical diagnosis of psychosis. It is possible that the individuals may have been prescribed with antipsychotic medication for other symptoms such as irritability. A weakness of the current study is that we did not ask respondents to specify what exact symptom medication was being used to target. We also relied on caregiver reports of psychiatric diagnosis that may explain the discrepancy between medication usage and psychiatric diagnosis.

The current study identified a pattern of aggressive behaviours in PWS using the aggressive– destructive behaviour sub-scale of the behavioural problems inventory (Behaviour Problems Inventory – Short Form). This is the first study in PWS to characterise aggression at an item level. 'Hitting others' was the most frequently rated aggressive destructive behaviour in children, and 'verbal abuse' was the most frequent and severe behaviour in adolescents and adults. Although research characterising aggression in PWS is limited, recent studies have highlighted the prevalence of disruptive behaviour disorders (DBDs) such as oppositional defiant disorder (ODD) and conduct disorder (CD). A 2-year longitudinal follow-up study of psychiatric disorders in children and adolescents with PWS identified ODD in 20% of participants and identified 'arguing with parents' as a common feature (Lo *et al*. 2015). DBDs were also commonly reported in individuals with PWS over 12 years in the Israel national cohort, 50% were diagnosed with ODD and 17% with CD (Shriki-Tal *et al*. 2017). A very small minority of caregivers in the present study reported that their child had a diagnosis of a DBD. The very low prevalence rates of DBDs in the current study may be related to the self-report nature of the study or the underuse of these diagnostic labels in Ireland. Further research is needed to understand how specific features of the PWS behavioural phenotype such as hyperphagia may explain the high prevalence of DBDs. For example, lying is a criterion for diagnosis of CD; however, this may only be relevant in the context of food in PWS. It would be interesting to see how the use of direct clinical assessment would impact on the prevalence of DBDs in the current study.

Skin picking was extensively reported by caregivers in all age groups, most notably in 93% of adolescents. Skin picking is a widely recognised feature of the PWS behavioural phenotype (Morgan *et al*. 2010). It is a matter of debate if skin picking is a characteristic of self-harm, an obsessive–compulsive behaviour or a direct consequence of loss of the necdin gene (Whittington and Holland 2018). The latter association is suggested by the observation of a skinpicking phenotype in necdin knockout mice (Muscatelli *et al*. 2000). It was previously found that skin picking was related to DBDs but not OCD in PWS, suggesting that skin picking is not representative of an obsessive–compulsive behaviour (Shriki-Tal *et al*. 2017). This is further supported by a factor analysis study of behaviour in PWS, which found that skin picking did not load on to the same factor as compulsions (Holland *et al*. 2003). Further research is needed to better understand the processes driving skin-picking behaviour in PWS so that better approaches can be developed to address this behaviour.

Other commonly reported behaviours in the present study included repetitive questioning, difficulty transitioning, non-compliance, food stealing and obsessive–compulsive behaviours. Repetitive questioning, difficulty transitioning and obsessive–compulsive behaviours overlap with behaviours seen in ASD. In addition to the occurrence of ASD traits in PWS, there are also potentially overlapping genetic susceptibilities. The PWS critical region 15q11–13 is epigenetic
'hotspot' for ASD susceptibility genes (Dykens *et al*. 2011). 'Insistence on sameness' in ASDs has been associated with one of several $GABA_a$ receptors within the PWS critical region (Shao *et al*. 2003). ASD diagnosis occurs in 12.3–25% of individuals with PWS, mUPD carriers are particularly at an increased risk (Bennett *et al*. 2015; Dykens *et al*. 2017). Some have argued that ASD symptoms become more prevalent over the course of childhood in PWS, although the reasons for this are unclear (Lo *et al*. 2013; Song *et al*. 2015). Further research is needed to improve our understanding of the clinical relationships between PWS and ASD and of the possible shared genetic pathways.

Therapeutic interventions for PWS are needs based and typically start with assessment and early speech and language therapy intervention. In this study, 97% of participants attended speech and language therapy and had timely access to their first appointments. In contrast, participants experienced longer waiting times for psychological services for behavioural interventions, on average 22 months. Management of behavioural problems is most effective if detected early, as multiple studies have shown that difficulties tend to increase with age in PWS. While there is limited research in PWS, targeted behavioural interventions are effective in treating anxiety/obsessive–compulsive symptoms (Ung *et al*. 2015) and self-injury (Peters-Scheffer *et al*. 2011) in children and adolescents with other neurodevelopmental disabilities. Therefore, timely access to behavioural management supports is an important clinical need. Further

research is now needed to modify and test the efficacy of behavioural interventions for anxiety, temper outbursts and social challenges in PWS.

Finally, our study highlighted negative impacts on the caregiver and the family in terms of their financial circumstances and emotional and physical well-being. A high proportion (75%) gave up or reduced their work, and a small but significant proportion of participants reported an extreme negative financial impact. Change in employment status underscores the loss of income to families directly attributed to caring for PWS individuals/children. Significant emotional and physical impacts were most notable in the adolescent group, which has been related to higher levels of caregiver burden in carers of adolescents and young adults with PWS compared with older adults and younger children (Kayadjanian *et al*. 2018). The increase of psychiatric/behavioural symptoms in adolescents with PWS could explain why the physical and emotional impact is highest with this group. Our study highlights that mental health monitoring and treatment in PWS, and caregiver wellbeing should be priorities for care. Additional social and respite supports are also likely to be required during more challenging periods of the individual's life to protect family relationships and prevent caregiver burnout.

Limitations

A limitation of the present study is the reliance on a single source for both clinical and genetic information, namely, the primary caregiver. We believe that the data are representative of the PWS population in Ireland that is estimated to be 100 cases. However, ascertainment bias cannot be excluded as we may not have detected those with the greatest difficulties. Finally, in future studies, it would be preferable to confirm genetic diagnosis through clinical genetics services.

Conclusion

The mental health and behavioural needs of individuals with PWS in Ireland are significant and illustrate the challenges faced by individuals with PWS and those caring for them. The complexity of the mental health and behavioural needs of PWS individuals requires skilled multi-disciplinary professionals, who can provide appropriate assessment and individual-centred interventions. The development of specialist behaviour support services in Ireland is urgently needed to help manage the complex behavioural phenotype of PWS and help reduce caregiver burden, so we do not rely solely on medication-based approach to mental health management for this medically complex group.

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Conflict of Interest The authors declare no conflicts of interest.

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Appendix 5: Prevalence of Autism Diagnosis in PWS cohort

Table 9.4: Participants who reached the cut-off for autism diagnosis on the ADOS and ADI

	PWS (n=22; 14:8 F:M)	PWS Deletion (n=11; 7:4 F:M)	PWS mUPD (n=11; 7:4 F:M)
ADOS Diagnosis			
Autism	4	1	3
Autism Spectrum	3	1	2
Non Spectrum	15	9	6
ADI Diagnosis			
Autism	5	2	3
Non-Autism	17	9	8
Meets ADOS and ADI criteria	5	2	3
Meets ADOS criteria only	2	0	2
Meets ADI criteria only	0	0	0

Number of participants who met criteria on autism measures; Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview – Revised (ADI-R)

Appendix 6: Food Attentional Bias Task – Self Report Measures

1. Hunger and Mood Rating Scales

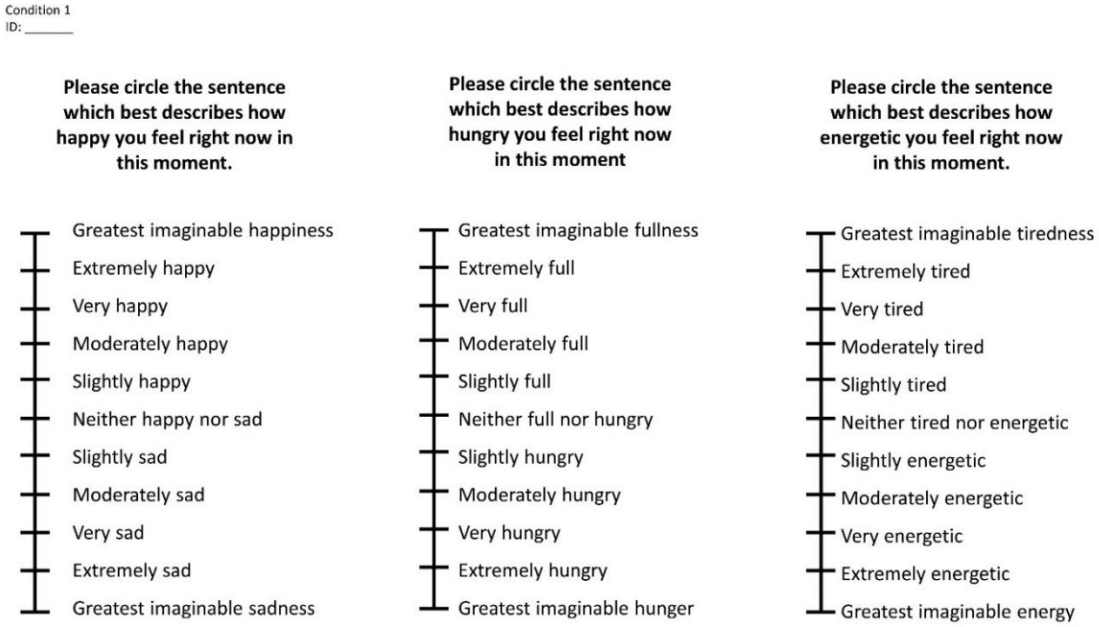

Figure 9.1: Hunger and mood rating scales used to measure satiety before each round of the FAB task paradigm. Two “distractor” scales measuring mood and energy were also included to reduce emphasis on hunger before the eye tracking task.

2. **Closing Questionnaire**

Please answer the following questionnaire honestly.

The study is complete. This questionnaire ensures our study's measures are valid.

Please tick yes or no.

Did you have food in the four-hour fasting period before the study?

(Before you came to do the first eye tracking task)

Yes	**No**

Did you guess what the study was about?

Yes	**No**

Did you alter your behaviour to fit what you thought it was about?

Yes	**No**

What did you do?

_____

_____

_____

_____

_____

_____

_____

Thank You for taking part in our study!!

Appendix 7: Materials for Adapted FAB Task Protocol

Remote Consultation Form – Pre research visit

Contact Information	
Participant's Name	_____
Caregiver Name	_____
Number	_____
Email	_____
Research Visit - Location	
Lab Visit ϒ	Travelling from: _____ Method of Transport: _____ Reminder to get receipts provided: ϒ
Home Visit ϒ	Address: _____ Home Office: ϒ Kitchen: ϒ Other room to use: _____
School Visit ϒ	Name of School: _____ Contact details _____
Research Visit	
Date	_____
Usual breakfast time	_____
Usual lunch time	_____
Arrival time	_____
Finish by	

Meal	
Age:	_____)))))))))))))))))))))))
Gender	_____
Diet Type	Weight Loss ϒ Weight Maintenance ϒ
Usual lunch time	
Lunch order (participant)	Fruit: Sandwich: Yogurt
Lunch order (caregiver)	Fruit: Sandwich: Yogurt

Preparation for Visit
Zoom call/Whatsapp call
Send schedule
Send FAQ
Gift token
Token/Motivators for visit

On the Day

ADOS
WASI
Saliva Sample
Monitor
Timing:

-