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Recent developments of functional magnetic resonance imaging research for

drug development in Alzheimer's disease

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Abstract

The objective of this review is to evaluate and synthesize recent developments in functional magnetic resonance imaging (fMRI) research in Alzheimer's disease for development of therapeutic agents. The basic building block underpinning cognition is a brain network. The measured brain activity serves as an integrator of the various components, from genes to structural integrity, that impact the function of networks underpinning cognition. Specific networks can be interrogated using cognitive paradigms such as a learning task or a working memory task. In addition, recent advances in our understanding of neural networks allow one to investigate the function of a brain network by investigating the inherent coherency of the brain networks that can be measured during resting state. The coherent resting state networks allow testing in cognitively impaired patients that may not be possible with the use of cognitive paradigms. In particular the default mode network (DMN) includes the medial temporal lobe and posterior cingulate, two key regions that support episodic memory function and are impaired in the earliest stages of AD. By investigating the effects of a prospective drug compound on this network, it could illuminate the specificity of the compound with a network supporting memory function. This could provide valuable information on the methods of action at physiological and behaviourally relevant levels. Utilizing fMRI opens up new areas of research and a new approach for drug development as it is an integrative tool to investigate entire networks within the brain. The network based approach provides a new independent method from previous ones to translate preclinical knowledge into in the clinical domain.

resting state; drug development, biomarker development

1.0 Introduction

In this review we will present a synthesis of recent research in functional neuroimaging and Alzheimer's disease (AD) research that would potentially be useful for the development of pharmaceutical agents for the treatment of AD. The functional neuroimaging results will be focused on recent developments using functional magnetic resonance imaging (fMRI) to measure brain activity with the results from studies including healthy subjects and patient populations. In addition we will review studies that have utilised pharmaceutical agents to investigate changes in brain function. The review article is organized as follows: initially we will describe recent findings on the basic structure of the brain, followed by how the structural architecture of the brain is reflected in the resting state networks of the brain. For the resting state networks we will focus on the default mode network (DMN) as this is the main network investigated in AD-related research because it includes the medial temporal regions and posterior cingulate regions, two regions that are impacted by AD at the earliest stages of the disease. We will then review recent developments utilizing cognitive paradigms that would be useful for the development of pharmaceutical agents and this section would be followed by presentation of evidence from fMRI studies of pharmaceutical agents. There will be a focus on the utility of resting state networks for the development of pharmaceutical treatment in AD.

The network approach reviewed here should prove to reliably detect and track subtle, but functionally relevant state-changes of cognitive networks, they will be an extraordinarily useful biomarker that could not only be used for early diagnosis and prediction of clinical outcome but also for rapid compound labelling and as a secondary outcome endpoint in phase II/III clinical trials. The potential benefits of such a biomarker for the development of disease modifying drugs could potentially be

tremendous: Only a small number of subjects would be required to rapidly test if a compound candidate (in phase IIa) does positively affect a cognitively relevant network or not. In case of no effect, large and costly phase IIb and phase III trials could be avoided, potentially saving billions of costs. On the other hand, regulatory institutions (Food and Drug Administration, European Medicines Agency) will increasingly require the use of biomarkers as secondary endpoints in pivotal clinical trials (Koch et al. 2010). A functional network marker may be of great help in this case and provide the ability to detect functionally relevant changes to distinct cognitive networks from earliest pre-symptomatic stages to clinical stages of AD.

2.0 Network Architecture of the Brain

Brain function is thought to be organized along complex neuronal networks that represent an optimal trade-off between organization and viability. This underlying organization principle may account for the ability of the brain to compensate for progressive and profound focal structural damage and to maintain cognitive functions despite ongoing deterioration of their underlying substrate (Noppeney et al. 2004). In AD, deficits in a cognitive domain are typically linked to metabolic and structural abnormalities in the corresponding brain regions, and the pattern of these abnormalities in AD predicts the manifestation of cognitive loss in this disease. For example, hippocampal atrophy and functional changes is associated with deterioration in memory in AD (Desgranges et al. 1998; Desgranges et al. 2002; Chetelat et al. 2003; Gilboa et al. 2005; Celone et al. 2006; Rombouts et al. 2009; Bokde et al. 2010), while metabolic decline of the parietal cortex and posterior cingulate is evident at the earliest stages of the disease as well as associated with visual-spatial

impairments (Duara et al. 1986; Meguro et al. 2001; Alexander et al. 2002; Drzezga et al. 2003; Ishii et al. 2005). However, this association of cognitive deficits with regional brain pathology is limited because cognitive functions are usually not underpinned by a single cortical region but by a network of regions forming so called 'degenerate systems' which can fully or partially sustain the cognitive function even when one region or part of the network has been damaged (Noppeney et al. 2004). The ability to compensate for functional deficits is a strength of the complex network architecture of the brain. The complex interplay between dysfunctional and adaptational processes in the brain was shown in functional activation neuroimaging studies in older healthy subjects (Grady et al. 1994; Grady 1996; Grady 2002; Grady et al. 2002; Grady et al. 2003; Damoiseaux et al. 2008; Grady et al. 2008), in subjects at high risk to develop AD (Dickerson et al. 2005; Rombouts et al. 2005; Bokde et al. 2006; Teipel et al. 2007; Bartres-Faz et al. 2008; Bokde et al. 2008; Bosch et al. 2010) or with already manifest AD (Horwitz et al. 1995; Pihlajamaki et al. 2008; Rosenbaum et al. 2008; Dickerson et al. 2009; Rombouts et al. 2009; Bokde et al. 2010; Bokde et al. 2010). A large array of functional activation studies performed with a variety of multimodal activation paradigms revealed a high level of heterogeneity across experiments and study groups, making it very difficult to create generally valid interpretations of these imaging data. Systematic reviews and meta-analyses on this subject tried to integrate these heterogeneous findings by creating theoretical models that would explain these findings. In sum, these models proposed that functional activation within a damaged area of the brain depends on the degree of damage, the functional capacity of this area and the impact of functionally associated distinct areas, which may either modulate the activity in the damaged area or which may take over some processing load (degenerate systems) (Prvulovic et al. 2005; Lee

et al. 2006; Alstott et al. 2009). While different regional brain defects may result in similar cognitive deficits, the association of regional brain pathology with a specific cognitive problem may not allow the reverse conclusion that this brain region is specifically responsible for the particular function.

Therefore, functional activation studies may not represent the ideal tool to investigate the functional integrity of brain systems and to track specific disease progress or therapy effects. Importantly, the interaction between different brain areas rather than changes in a single region may best characterize network activity related to a specific cognitive task (Buchel et al. 1999). The integrity of a network can be measured for example, with functional and/or effective connectivity, which may provide substantial advantages over the analysis of activation patterns because it doesn't rely on brain-wide activation patterns but on the state of neural coordination within any network of interest (McIntosh 2004; Sporns et al. 2005; Bokde et al. 2009; Bullmore and Sporns 2009; Rubinov and Sporns 2010). Functional connectivity refers generally to the coherent activation between regions of a brain network, and it usually calculated as the linear correlation coefficient between the brain activity in two regions (Friston et al. 1993; Bokde et al. 2001; Bokde et al. 2006; Buckner et al. 2009; Honey et al. 2009). This approach does not allow for a directional causal interpretation of the influence among different brain regions, but is purely correlational in nature. Effective connectivity refers to the influence of one brain region onto the other where that direction of influence can be explicitly modelled, using approaches such as structural equation modelling (SEM) (McIntosh and Gonzalez 1994), autoregressive correlation, or dynamic causal modelling (Friston et al. 2003). Thus for example using SEM Horwitz, McIntosh and colleagues demonstrated that there were different

interactions among the regions of the visual neural system as a function of the stimuli, with face stimuli activating a network along the ventral visual pathway and location stimuli activating the dorsal visual pathway (Horwitz et al. 1992; McIntosh et al. 1994; McIntosh et al. 1996). In many psychiatric and neurological disorders pathological impairment of cognitive function may be due to abnormally altered connectivity of brain activation within a respective neuronal network (Uhlhaas and Singer 2006). An approach utilizing functional magnetic resonance imaging (fMRI) provides a tool that allows for measuring changes or alterations in the brain networks that underpin cognitive function.

3.0 Coherent Fluctuations within Networks

Until recently research into particular networks of the brain, such as memory and attention, utilized an activation paradigm, where a person performed a specific task and brain activity during the task would illuminate the network of interest. It has recently been discovered that the apparently random signal within the measured fMRI signal during a time period of no cognitive task (i.e. resting state) is not random but has a spatial-temporal structure and it is referred to as resting state networks (Fox et al. 2005; Damoiseaux et al. 2006). There are various ways to measure the resting state networks, for example by calculating the functional connectivity of a reference voxel to all other voxels in the brain, and the regions that are functionally part of the same network as the reference voxel will be correlated at a statically significant level (see pioneering work of Bharat and colleagues (Biswal et al. 1995)). This approach has been utilized for example to examine the posterior cingulate as reference region (among may reports see (Andrews-Hanna et al. 2007; Zhang et al. 2009; Koch et al. 2010)) and one sees significantly correlated time series in lateral parietal regions,

medial temporal regions and medial frontal regions. Thus for example one may use multiple reference voxels across the brain to investigate the functional organization of the brain, such as Buckner and colleagues did (Buckner et al. 2009), and found that there are cortical regions that are more highly functionally connected that others. The regions that are more highly interconnected may be key in integrating neural activity in the brain, but may also serve as a key point in the cascade leading to brain disease such as AD (Buckner et al. 2009).

Another approach is to use independent component analysis, which searches for statistically significant patterns of co-activation, and with the results similar to the previous approach (Damoiseaux et al. 2006; Calhoun et al. 2008; Esposito et al. 2008). An advantage of using an ICA approach is that the multiple resting state networks can be detected without any a-priori reference points in the data whereas the functional connectivity approach requires a-priori determination of the reference voxel to detect specific resting state networks. A study compared both approaches for discriminating between AD patients and healthy subjects and found that the functional connectivity approach performed slightly better than the ICA but combining both approaches gave significantly diagnostic power compared to each method alone (Koch et al. 2010).

The spatial temporal structure extends throughout the brain and has been found also in non-human primates and the resting state networks have been hypothesized to reflect the architecture of the human brain (Vincent et al. 2007). The findings of Vincent and colleagues suggest that fluctuations of spontaneous activity across anatomically interconnected brain regions constitute a fundamental principle of brain organization.

Such an interpretation is supported by the fact that organized patterns of brain activity are present in both humans and non-human primates. One of the possible functions of the networks may be a tool to reinforce or maintain the integrity of the networks by reinforcing the synaptic connections that are critical for the network operations. The results in this area of research has also generated many more questions such as the number of networks that can be quantified using resting fMRI, or what criteria define a network, and what is the association of these networks with each other but also with networks that are activated during a cognitive task. Some initial answers have been obtained and will be discussed later in this review. In addition, it has generated new issues when examining brain activation due to a cognitive task, such as the relationship between the task-associated network and the resting networks in the brain (Greicius and Menon 2004; Buckner and Vincent 2007).

In addition to investigating the underlying network architecture of the brain using fMRI, this underlying structure has also been investigated using *structural* measurements, such as cortical thickness to measure correlations across the brain (He et al. 2007). The underlying structure found had short range and long range connections and were consistent with the known neuroanatomical structure of the brain using diffusion tensor imaging (DTI). Of note, the short range anatomical correlations were more robust as they were insensitive to the correlation thresholds. In AD patients the structural correlations were different from the healthy subjects, indicating that the anatomical brain structure network was aberrant (He et al. 2008). The findings suggest that the coordinated patterns of cortical morphology are widely altered in AD patients, thus providing structural evidence for disrupted integrity in large-scale brain networks that underlie cognition. Further evidence for these changes

have been found using DTI, showing large scale changes in the structural integrity of the white matter tracts in AD patients (Teipel et al. 2007).

These studies have provided insight into the structural and functional organization of the brain and have raised some intriguing approaches for investigating neural networks that one may wish to target during drug development. In addition, it provides for a methodology that would be relatively simple to apply to investigate functional changes in the brain in AD patients independently of their cognitive impairment. Given that this approach does not require active participation (in the cognitive domain) from the participant it opens up fMRI studies on moderately cognitively impaired patients and it allows one to investigate a range of networks simultaneously, such as networks involved in memory, attention, motor function, and perceptual function.

4.0 Default Mode Network

The most investigated network among the resting state networks is the default mode network (DMN), see Figure 1, which is of particular interest for AD research because the network includes the medial temporal regions and the posterior cingulate; two key areas supporting memory function. It is hypothesized that the DMN is active as long as a person does not perform a goal oriented task and during this period the activity in the DMN reflects undirected, spontaneous, conscious mentation or monitoring of the external environment (Shulman et al. 1997; Gusnard et al. 2001; Raichle et al. 2001). Consistent with the proposed hypothesis of the DMN is that this network during cognitive tasks deactivates, for example, the DMN needs to be disengaged or deactivated during performance of a memory task (Daselaar et al. 2004; Miller et al.

2008; Stevens et al. 2008; Wang et al. 2010) (For a more detailed review of the DMN, please see (Buckner et al. 2008)). The tern deactivate refers to the fact that the activity in the DMN during cognitive tasks compared to baseline is negative – that is – during performance of a cognitive task the DMN regions are likely to show negative magnitude activation. It has been found that the DMN does not only show stage dependent deactivation deficits (Lustig et al. 2003) but that functional connectivity between core DMN regions is significantly impaired even at pre-symptomatic stages of the disease (Hedden et al. 2009). Further functional impairments of the DMN have been reported in AD patients (Lustig et al. 2003; Greicius et al. 2004; Rombouts et al. 2005; Wang et al. 2006) and in MCI subjects (Rombouts et al. 2005; Sorg et al. 2007). It has been found that AD patients have decreased activation in the medial temporal areas and posterior cingulate compared to healthy controls (HC) while the MCI subjects have decreased activation in medial temporal areas. Recently, an approach has been suggested to derive diagnostic information from these alterations of the DMN with sensitivity and specificity levels ranging between 85 to 95% for the discrimination between AD patients and healthy elderly subjects (Koch et al. 2010). In addition, a recent study found that the DMN was more sensitive in discriminating between carrier and non-carriers of the APOE e4 allele, a risk factor for AD, than the activation pattern during a memory encoding task (Fleisher et al. 2009). Given that the DMN includes the medial temporal lobes and the posterior cingulate, two areas key in memory and disrupted in AD patients, it offers the possibility to investigate medial temporal lobe function in AD patients without the use of a cognitive paradigm. In particular, measurement of the default mode network requires no active participation by the patient in a task, such as memory or attention, thus offering a unique opportunity to investigate moderately severe cognitively impaired patients.

The data acquisition takes approximately 5 to 7 minutes and it requires that the patients remain awake during measurement time.

An important prerequisite for the use of the DMN as a potential marker of disease progression and intervention effects is the stability of networks within individuals over time. In a study on 18 healthy young subjects the DMN was reproducible within a single imaging session as well as between imaging sessions twelve hours and one week apart (Meindl et al.). Size and location was most reproducible within session and between sessions for anterior and posterior cingulate, followed by the lateral parietal regions and then medial frontal regions. The numbers of voxels found activated were not significantly different between sessions across subjects, but the coefficient of variation of numbers of activated voxels ranged between 6% in anterior cingulate and 35% in superior frontal gyrus. This variation suggests that the power to detect effects of disease or intervention on DMN activity would vary between different parts of the networks and will also be dependent on the method of analysis. The latter aspect is illustrated by a study in healthy elderly subjects on DMN connectivity where multivariate independent component analysis showed an effect of age on posterior cingulate activity that was not detected by univariate signal time course correlation analysis (Koch et al. 2010). Still, the reproducibility of the DMN over a relatively short interval of time of one week matches the level of reproducibility of activation in motor and visual tasks (Meindl et al. 2009). Future studies will have to assess the stability of DMN connectivity over longer period of times.

An important issue in the application of resting state networks as secondary outcomes in intervention studies is the question of the underlying substrate of these changes that will have to be linked to the proposed mode of action of a novel compound. It has been suggested that apparent resting state networks can partly be explained by fluctuations in physiological parameters, such as CO_2 levels (acting as a vasodilator) due to variations in depth of breathing. These effects would be most prominent in grey matter areas with high blood volume partly overlapping with the key areas of the DMN (Birn et al. 2006). Removing breath volume effects from the regression across regions during resting state led to reductions of intracortical correlations although the overall DMN remained preserved. Evidence, however, is accumulating that DMN connectivity rests on a morphological basis. Two studies have shown that the functional coupling between selected key areas of the DMN from resting state fMRI data is correlated with the strength of fiber tract connectivity based on DTI data (van den Heuvel et al. 2008; Greicius et al. 2009). These region-selective findings were extended by a data driven approach, where the functional connectivity between hippocampus and posterior cingulated resting state signal recovered the spatial extent of the entire posterior DMN in DTI data of subcortical fiber tracts. In addition, a multivariate analysis showed a significant correlation between the DMN functional connectivity and a similar spatial pattern of fiber tracts derived from DTI data (Teipel et al. 2010).

5.0 Memory Network

The majority of fMRI research in AD patients has focused on memory. In a recent study in MCI and AD patients (Celone et al. 2006), it was demonstrated that there is a non-linear change in brain activation within the memory network, which includes

medial temporal lobes, posterior cingulate, inferior parietal area and dorsolateral prefrontal cortex. It was found that a large pattern of brain regions were temporally synchronous, supporting the hypothesis that a specific set of large-scale distributed brain networks mediates the process of associative encoding. What was also fascinating given the previous discussion of the resting networks, is that this study found evidence for a strong reciprocal relationship between the degree of memory-related activation within the hippocampus and deactivation of medial and lateral parietal regions. Subjects at the very mild end of the cognitive impairment continuum demonstrated evidence of paradoxically increased activation not only in the hippocampus and functionally connected neocortical regions, but also increased deactivation in the default network compared with controls. Subjects at the more impaired end of the MCI continuum showed significantly decreased hippocampal activation and reduced deactivation in default regions, in a pattern similar to patients diagnosed with mild AD.

In addition, activation of a task-related network leads to deactivation of the DMN in HC but only to partial deactivation in AD patients (Rombouts et al. 2005; Pihlajamaki et al. 2008). Successful deactivation of the DNM has been found to be necessary for successful encoding in HC (Daselaar et al. 2004). It was found that the level of deactivation in the medial parietal areas (part of the DMN) was correlated with memory performance (Pihlajamaki et al. 2008) with less deactivation correlated with less successful encoding in AD patients. Thus the initial evidence is that DMN plays an important role in memory performance and that a compound targeting the DMN could also potentially affect memory performance.

6.0 Visual Perception

Earlier work has shown that visual perception impairments are correlated with a higher cognitive impairment in AD patients. Recent work has also investigated visual perception in MCI subjects (Bokde et al. 2006; Bokde et al. 2008) and AD (Bokde et al. 2010). It has been found that there is a differential change along the ventral and dorsal visual pathways to the presence of the putative AD neuropathology. The activation patterns between the MCI and HC groups were not different but there was a significant difference in the functional connectivity of the right fusiform gyrus to frontal areas, and inferior and superior parietal areas. The right fusiform gyrus is a key area for processing of faces. There are alterations in the visual processing of stimuli and these alterations affect also cognitive performance, as more resources are utilized for basic perceptual tasks. The differentiation between ventral and dorsal pathways was also done through examination of the coherent fluctuations (Fox et al. 2006). As with the results with the resting state networks, the level of activation in the right fusiform gyrus in the MCI and HC was linearly correlated with the grey matter density along the ventral and dorsal visual pathways, with the correlation higher in the MCI than in the HC group (Teipel et al. 2007). The structural correlates of the networks also affect activation levels in the brain, and both are associated to one another. In terms of AD patients, memory, attention and visual perception have been the networks most investigated in AD patients.

7.0 Clinical Drug Development

Generally studies that have examined drug action using fMRI have combined administration of a drug with a cognitive task to investigate the drug induced modulations of the task-related activation pattern if the brain – with the main focus

being the magnitude of activation in the brain. These types of designs may have placebo and verum arms in the same subject or more typically, subjects being randomized into either verum or placebo arm. For example fMRI study examined the impact of a single dose of a cholinesterase inhibitor in a group of Mild Cognitive Impaired (MCI) subjects, and mild AD patients during a memory encoding task and found that activation in fusiform gyrus (recruited for visual processing of stimuli) was increased compared to activation pattern before drug dose (Rombouts et al. 2002; Goekoop et al. 2004). Another very similar investigation also examined modulation due to single dose and also after 5 days treatment, where the brain activation pattern during memory recognition was examined, and drug treatment led to modulations in brain activity in visual cortex, prefrontal cortex and hippocampus. Consistent with the above acute or short treatment period, other studies have found effects in visual cortex were significant over a variety of cognitive tasks such as perceptual comparison task (Bokde et al. 2009).

The modulation due to AD –related drug mechanisms have also been investigated by using anticholinesterase physotigmine on healthy subject populations during tasks of working memory, emotion processing, visual attention and found that cholinesterase inhibitors may through modulation of activation in visual areas (increased) and frontal areas (decreased) lead to enhancement of stimulus processing and selectivity improve cognitive performance (Furey et al. 2000; Bentley et al. 2003; Bentley et al. 2004; Furey et al. 2008). In addition, similar approach has been utilized in studies comparing healthy subjects with AD patients during a visual attentional task where activation pattern was investigated during placebo and physotigmine-induced modulation of the activation (Bentley et al. 2008). In this study it was reported that in

the AD group there was physotigmine-induced increased activation in extra-striate cortices that are affected by AD such as precuneus and parahippocampal cortex, while in fusiform gyrus, a region not affected by AD, showed decreased activation after physotigmine infusion. The pattern in the fusiform gyrus was similar in the pattern seen in healthy controls which may reflect a region-specific loss of functional cholinergic cortical inputs in AD (Bentley et al. 2008). Further investigation found that physotigmine lead to enhanced memory performance in healthy subjects which correlated with the increased activation during the task in fusiform gyrus while in AD patients there was enhancement of memory performance but the activation magnitude in fusiform gyrus was increased non-selectively (Bentley et al. 2009).

Given that recent research in neuroscience has moved towards examination of neural networks, there have been a few initial studies that have examined the effects of drugs on the connectivity pattern of the DMN. For example in mild sedated subjects the connectivity of the posterior cingulate was decreased compared to normal rest which the authors interpreted as being associated with the decreased consciousness of the subjects during sedation (Greicius et al. 2008). As pointed out above, even though in AD-related research the DMN is the main area of focus of investigation with resting state, other resting state networks may be used to investigate pharmaceutical agents. For example – the motor cortex in young healthy subjects – after a single dose of levodopa the functional connectivity in motor pathways between putamen and cerebellum and brainstem was increased, as well as between inferior ventral striatum and ventrolateral prefrontal cortex, and it disrupted the functional connectivity of the ventral striatal and dorsal caudate with the DMN (Kelly et al. 2009). These results

were consistent with previous studies that have utilized motor tasks and demonstrate that utilization of the resting state networks can be sensitive to pharmaceutical agents.

The resting state networks could be used to detect the efficacy of the targeting of a drug agent to the desired network – the network would be the one that supports the cognitive domain of interest or where the regions(s) of interest are the target of the drug agent. This would have two primary outcomes. First, in a proof of principle approach a new compound would show its ability to modulate activity in disease relevant networks such as the DMN which in turn is related to a relevant clinical outcome such as conversion into dementia (Figure 2). Additionally, this approach can be used in dose finding studies, where a minimum dose can be identified that modifies network activity as a short term surrogate measure of a long term behavioural outcome. An additional approach for determining the mode of action of a new compound will be the integration of functional connectivity with data on structural connectivity derived from DTI. Multivariate analysis of DTI data has been shown to have a high power to detect typical pattern of morphological brain destruction in AD (Teipel et al. 2007), but also to uncover the underlying morphological substrate of functional connectivity across the DMN (Teipel et al. 2010). The detection of changes in functional connectivity accompanied by alterations of underlying fiber tract integrity would be valuable to discriminate symptomatic from disease modifying treatment effects.

A pilot study investigating the use of resting state network in a animal model of AD demonstrated impaired functional connectivity between the amygdala and hippocampus in rats with amygdala lesions (Sakoglu et al. 2011). The connectivity

between the amygdala and hippocampus are blocked so tasks in which these structures participate such as memory and emotion are impaired (Richter-Levin and Akirav 2000; Akirav and Richter-Levin 2006). In the pilot study it was found that the functional connectivity was decreased in the rat with lesion compared to the sham rat. This initial study demonstrated that resting state functional connectivity may be a useful approach for drug development studies.

A new compound for AD would initially be tested for its' ability to impact a specific network such as the DMN if using a resting fMRI approach or a memory network when utilizing a cognitive paradigm. In the initial stages, a specific network would be like an assay to give an initial evaluation of the effectiveness of the compound. Thus if the compounds affect other networks, this information could provide a window into possible mechanisms that the compound activates. Thus fMRI would provide unique information about the compound very early in the clinical development of a drug and in combination with the traditional information obtained in this phase such as safety, could be used to have an initial impression on the probability of successful development. Better knowledge of the mechanisms of action and the brain networks that it affects, could potentially lead to greater safety in the large scale clinical studies. An advantage of using the coherent fluctuations networks is that it would provide within a short measurement time (5 to 7 minutes) information on a possible drug effect over multiple neural networks. Thus fMRI could potentially be an integrator to examine the specificity of the compound in the brain.

It would be also of great value to utilize activation paradigms for testing neural networks. The coherent fluctuations that define resting networks are limited in

number and may not include the regions of interest for a particular disease. Therefore activation paradigms are important, as a direct connection between network activation and behaviour performance can be made. Activation paradigms are important as they allow us to test specific networks during performance of a task but also provide a more dynamic picture of the changes or neural coordination in the course of the disease. With the use of activation paradigms it has been possible to quantify small changes in cognition as disease severity progresses.

Of high interest to AD related studies, would be the interaction between the memory network and the DMN as both networks include in part the medial temporal areas and the posterior cingulate (Sambataro et al. 2008; Kim et al. 2009). Thus the DMN provides an "assay" to measure hippocampal activity and to investigate the effects of new compounds on this region. Thus if a compound has an effect on this network it may also positively affect memory-related network coordination between those areas, which would imply a benefit for memory function.

8.0 Limitations

There are still issues remaining to be investigated with respect to the resting state networks such as optimal measurement strategy (Fair et al. 2007), analysis approach (Weissenbacher et al. 2009; Koch et al. 2010), the vascular effects on the signal (Fukunaga et al. 2008) and physiological influence on the signal (Lu et al. 2007; Birn et al. 2008). The networks have been found to be strong and consistent across subjects (Damoiseaux et al. 2006; Calhoun et al. 2008; Meindl et al. 2009), but it needs to be expanded to various age groups, and well as longer time periods between measurements, and expanding to multi-center studies differences. In addition, more

detailed investigation of the individual networks needs to be performed such as more detailed characterization of anatomical localizations, how variability in the resting state networks relates to inter-individual cognitive differences (Bosch et al. 2010).

One should note that AD and drugs can lead to global non-specific changes in the fMRI signal measured which may potentially confound the differences between patients and healthy subjects or between different drug treatment arms. One approach would be to utilize both activation and network analysis methods for the analysis of fMRI data – methods that examine connectivity among regions of the brain are less likely to be affected by global non-specific changes in vascular reactivity. Another approach to controlling for these effects is to use complementary approaches, for example perfusion imaging (arterial spin labelling) or direct measurements of neuronal activation such as electroencephalography (EEG) and magnetoencephalography (MEG). Arterial spin labelling magnetically labels the blood so that it acts as an endogenous contrast agent, but the challenge with this technique is to obtain measurements of the whole brain (Alsop et al. 2000). With EEG there are MRI compatible EEG systems so that one is able to measure simultaneously fMRI and EEG (Friston 2009; Rosa et al. 2010) and may offer a way to investigate possible vascular effects of a drug compound. The EEG and fMRI signals measured in a study would measure different aspects of brain activity and could offer complementary information in a pharmaceutical study.

9.0 Conclusions

Recent developments in our understanding of the organizational structure of the brain have opened up new strategies for the development of therapeutic agents for the targeting of specific cognitive networks. FMRI opens up new areas of research and a new approach for drug development as it is an integrative tool to investigate entire networks within the brain. It can be used in drug discovery and development to provide valuable insight between drug action and disease phenotypes. The network based approach provides a new independent method from previous ones to translate preclinical knowledge into in the clinical domain.

10.0 Acknowledgements

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Figure Legends:

Figure 1: The default mode network (DMN) has primary network center in the posterior cingulate/retrospinal cortex (PCC/RSP) with this regions strongly functionally connected to left and right inferior parietal lobulus (IPL), ventral and dorsal medial prefrontal cortex (V MPFC and D MPFC), as well as the lateral temporal lobes (LTC). The DMN seems to be composed of a main network (indicated in red) and a sub-system (indicated in blue). In addition, PCC/RSP is strongly connected to all regions of the DMN and is the main functional connector to the hippocampus (HP) and parahippocampal cortex (PHC). The hippocampus and parahippocampus cortex seem to be a sub-system of the DMN because it is not as strongly connected to the rest of the DMN. The black lines indicate the primary functional connectivities in the DMN with the thickness of the black line indicating the relative strength of the functional connectivity. The PCC/RSP is connected to all regions in the hippocampus and parahippocampal cortex whereas the L IPL and V MPFC are less strongly connected to the temporal cortex sub-regions (regions indicated in blue). The 5 regions that have a square outline (PCC/RSP, V MPFC, D MPFC, L IPL, R IPL) have been shown to be connected to both local and distant regions in the brain whereas the HC and PHC are preferentially functionally connected to local regions. As comparison visual, motor, auditory, and somatosensory cortices have preferential functional connectivity to local regions. The DMN graphed in this image is based on data from young healthy subjects. This image based on figure in Buckner et al (2008).

Figure 2: Fast compound labelling using episodic memory sub-network integrity as outcome measure. The effects of various compounds on functional coordination and functional connectivity measures in memory-relevant sub-networks (e.g. between HC and the PCC) can be used to test whether a compound is capable of improving the functional integrity of this particular network (compound A) or not (compound B). In our example, compound B shows even a deterioration of network integrity, which may predict potential adverse effects on cognitive functions.

Article Highlights

- Functional magnetic resonance imaging provides valuable insight between drug action and disease phenotypes.

- Using a neural networks based approach provides a new independent method from previous ones to translate preclinical knowledge into in the clinical domain.

- The default mode network offers the possibility to investigate medial temporal lobe function, key regions supporting memory function, in Alzheimer's disease patients without the use of a cognitive paradigm.

- Development of mathematical and analytical models could provide a significant boost to increasing the efficiency and effectivity of functional magnetic resonance imaging in the development of new compounds.

Abbreviations

AD Alzheimer's disease
DMN Default mode network
DTI Diffusion tensor imaging
EMEA European Medicines Agency
FDA Food and Drug Administration
fMRI functional magnetic resonance imaging
HC Healthy controls
MCI Mild Cognitive Impairment