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Nanotechnologies for the study of the central nervous system

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ABSTRACT

The impact of central nervous system (CNS) disorders on the human population is significant, contributing almost €800 billion in annual European healthcare costs. These disorders not only have a disabling social impact but also a crippling economic drain on resources. Developing novel therapeutic strategies for these disorders requires a better understanding of events that underlie mechanisms of neural circuit physiology. Studying the relationship between genetic expression, synapse development and circuit physiology in CNS function is a challenging task, involving simultaneous analysis of multiple parameters and the convergence of several disciplines and technological approaches. However, current gold-standard techniques used to study the CNS have limitations that pose unique challenges to furthering our understanding of functional CNS development.

The recent advancement in nanotechnologies for biomedical applications has seen the emergence of nanoscience as a key enabling technology for delivering a translational bridge between basic and clinical research. In particular, the development of neuroimaging and electrophysiology tools to identify the aetiology and progression of CNS disorders have led to new insights in our understanding of CNS physiology and the development of novel diagnostic modalities for therapeutic intervention. This review focuses on the latest applications of these nanotechnologies for investigating CNS function and the improved diagnosis of CNS disorders.

Contents

Abbreviations: Aβ1-42, amyloid-β peptide; AD, Alzheimer's disease; anti-IGFBP7, anti-insulin-like growth factor antibody; BAM10, amyloid-β monoclonal antibody clone; BBB, blood–brain barrier; BRAIN, brain research through advancing innovative neurotechnologies; Cd^{2+}, cadmium heavy metal ions; CdSe:Mn/ZnS, cadmium selenide:manganese/zinc sulphide; CLIONs, cross-linked iron oxide nanoparticles; CNS, central nervous system; CNT, carbon nanotube; CT, computer tomography; CVD, chemical vapour deposition; DAQ, data acquisition card; DOX, doxorubicin; DSC-CBV, dynamic susceptibility contrast perfusion imaging of cerebral blood volume; EAE, experimental autoimmune encephalomyelitis; Gd, gadolinium; GWAS, genome wide association studies; MB, microbubbles; MEA, microelectrode array; MEP, magnetoelectroporation; MNPs, magnetic nanoparticles; MNP-PBP, iron oxide nanoparticle contrast agent targeted towards p-selectin; MRI, magnetic resonance imaging; NIR, near-infra red; PAMAM, polyamidoamine; PBCA, poly(n-butyl cyanoacrylate); PEG, polyethylene glycol; PET, positron emission tomography; PLGA, polylactide-co-glycolide; PMMA, polymethylmethacrylate; QD, quantum dots; SCINEs, solid-conductor intracellular nanoelectrodes; Si-NWFET, silicon nanowire field-effect transistor; SiO_2, silicon dioxide; SPIONs, superparamagnetic iron oxide nanoparticles; SS-CBV, steady-state cerebral blood volume maps; TAT, trans-activator of transcription; Tf, transferrin; USPIONs, ultra-small superparamagnetic iron oxide nanoparticles; VCAM, vascular cell adhesion molecule; VSPIONs, very-small superparamagnetic iron oxide nanoparticles.

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1. Introduction

The impact of central nervous system (CNS) disorders on the human population is significant, covering hundreds of diseases with profoundly debilitating behavioural, social and cognitive deficits. Disorders in brain function are particularly insidious because they affect emotion, learning ability, and memory which unfold and regress as an individual gets older. The limited capacity of self-repair mechanisms in the brain can result in irreversible functionality, establishing disorders that not only have a major impact on individual lives, but also place a severe strain on healthcare resources. An extensive study by the European Brain Council estimated the total cost of disorders of the brain across Europe at €798 billion in 2010 (Gustavsson et al., 2011). Direct healthcare costs were shown to account for 60% of the total cost, while the remaining 40% was associated with loss in production. The breakdown in costs for brain disorders detailing number of persons, cost per person and total costs is summarised in Table 1.

Despite the significant impact of these disorders on society, advances in drug development remain limited, with treatments for diseases such as depression and schizophrenia still largely based on substances identified in the 1950s (Brandon and Sawa, 2011). Most currently available drugs merely delay disease onset or alleviate symptoms, and the lack of progress in the development of blockbuster drugs has led to several big pharmaceutical companies dropping or shrinking research for neural diseases (Abbott, 2011). The length of time for clinical trial and approval phases of CNS drugs is longer than for any other therapeutic area; 10 years for CNS *vs.* 7.8 and 7.6 years for cardiovascular and antineoplastic therapies respectively (Kaitin, 2010). Across the board, CNS drug discovery and development is associated with a longer and riskier development process and given the high rate of attrition for pharmaceutical research into neural disorders, there is a clear and present need to identify innovative approaches for more sensitive diagnosis and efficacious treatment (Muglia, 2011).

Table 1
Number of persons, cost per person and total cost of brain disorders.

Brain disorders	No. of subjects (Millions)	Total cost per patient (€PPP 2010)	Total costs (million €PPP 2010)
Mood disorders	33.3	3406	113,405
Dementia	6.3	16,584	105,163
Psychotic disorders	5	18,796	93,927
Anxiety disorders	69.1	1077	74,380
Addiction	15.5	4227	65,684
Stroke	8.2	7775	64,053
Headache	152.8	285	43,514
Mental retardation	4.2	10,334	43,301
Sleep disorders	44.9	790	35,425
Traumatic brain injury	3.7	8809	33,013
Personality disorders	4.3	6328	27,345
Child/adolescent disorders	5.9	3595	21,326
Somatoform disorder	20.4	1037	21,169
Multiple sclerosis	0.5	26,974	14,599
Parkinson's disease	1.2	11,153	13,933
Epilepsy	2.6	5221	13,800
Neuromuscular Disorders	0.3	30,052	7726
Brain tumour	0.2	21,590	5174
Eating disorders	1.5	559	827
Europe	379.9		797,764

Adapted from Gustavsson et al. (2011).

Due to the substantial heritability of many CNS disorders, research has intensified on identifying targets involved in disease aetiology rather than symptomology and choosing to pursue genetic risk factors as targets for diagnostic intervention. The ability to sequence the human genome and characterise patterns of variation among populations and subpopulations has made it possible to conduct high resolution and large scale genome-wide association studies (GWAS) (Hall et al., 2010), and more recently whole genome sequencing studies. Such hypothesis-free approaches have generated novel insights into the underlying molecular aetiology of neuropsychiatric and neurodegenerative disorders (Engle, 2010; Lin et al., 2009; Mitchell, 2011); for example, identifying shared genes involved in both circuit dysfunction of neurodegenerative disorders and calcium-channel dysfunction in psychotic disorders (Cross-Disorder Group of the Psychiatric Genomics et al., 2013). While such genetic analysis data has redirected efforts in the research community to develop novel targets for future drug developments, these studies have also revealed that genetic data alone cannot build a functional model of disease pathology, as there are further layers of complexity caused by the interaction of functional gene expression with environmental stimuli. Thus, the key challenge for modern medicine is to further identify mechanisms behind brain function, from gene expression to physiological behaviour, and determine their implications in the aetiology and progression of CNS disorders (Stoeckli, 2012). The purpose of this review is to discuss how existing approaches presently limit our ability to meet these challenges effectively and highlight how modern nanotechnological advances could provide the keys to unlocking the complex nature of our brains for clinical intervention.

Current methods for characterising brain development and maturation involve the convergence of several diverse diagnostic methods, ranging from single-neuron observation at the intracellular level, to monitoring the principal activity of millions of neurons in synchrony. One fundamental modality for characterising brain development and activity has been the use of *in vivo* neuroimaging, a powerful technique for observing structural, biochemical, and functional changes within the brain. Significant advances in our ability to image the CNS at increasingly higher resolutions have elucidated several functional components of neuronal activity (Tropea et al., 2010). In addition, the aetiology of disease pathologies has been better characterised, improving our understanding of these disorders and highlighting novel pathways for targeted treatment (Corvin et al., 2012). Nonetheless, there are limitations in current approaches to neuroimaging such as artefact interference, lack of sensitivity, reduced half-life after intravenous administration, and decreased permeation across the endothelial barrier surrounding the brain (Nunes et al., 2012). In parallel, electrophysiological studies have also been critically important for understanding the functional connectivity of neuronal populations (Brown and Hestrin, 2009), combining several layers of CNS

function from the molecular, to the cellular and behavioural. By using metal or silicon electrodes to record electrical signals caused by ion fluxes across neuronal membranes, electrophysiological approaches allow researchers to probe the mechanics of signal propagation and synaptic plasticity at a high signal-to-noise ratio. However, as with neuroimaging, there remain drawbacks to current electrophysiological studies mainly associated with difficulties in multi-parametric studies of physiological models of brain development (Zhao et al., 2008).

Given the struggles of these current approaches and techniques to the study of functional brain activity, the recent and rapidly advancing field of nanotechnology presents a unique opportunity to confront these challenges and provide a platform for innovation in the drug development process for neural diseases. The biomedical applications of nanoscale structures, materials, and devices are expansive and their diverse physical and chemical characteristics demonstrate a capacity for potentially novel technologies. These materials typically consist of solid colloidal particles on the nanometre scale (1–100 nm) composed of insoluble polymers. Within a biological context, they are comparable in size to viruses (20–300 nm) and significantly smaller than cells (30–300 μm). The small size of nanoparticles in relation to the size of cells allow them to be designed for many specific functions, including binding to cell membranes, delivery of agents into cells or across anatomical and physiological compartments, and monitoring of physiological events (Provenzale and Silva, 2009). These advantages provide potential enhancements in neuroimaging techniques through higher contrast and better targeted molecular imaging probes. Additionally, nanomaterials can be implemented into advanced biosensor systems for probing the principles of circuit physiology within the brain. By exploiting the unique and often improved physical, chemical, and biological properties of nanomaterials, current methods for diagnosing debilitating CNS disorders can be significantly enhanced, and new insights into brain physiology can be applied to develop novel therapeutic strategies (Silva, 2006). This review offers a unique opportunity to identify the areas of convergence between nanotechnology and neuroscience, in addition to highlighting current applications in the development of novel "neurotechnologies" for the translation of basic research into clinical application (Table 2).

2. Nanodiagnostic neuroimaging

The ability to visualise the brain represents one of the most powerful methods for the study of CNS disorders and is a key step in establishing new insights into better treatment strategies based on improved diagnosis. In particular, whole-brain imaging has the ability to capture the structural and functional dynamics of neuronal communication in the intact nervous system and provide greater understanding of the patterns of neural activity involved in experience-dependent structural plasticity (Trachtenberg et al., 2002). These observations are critical in diagnosing the clinical progression of many neurodegenerative (McGhee et al., 2014) and psychiatric (Bansal et al., 2012) disorders. However, current whole-brain imaging techniques have several constraints that limit the amount of information that can be gained, including lack of sensitivity to specific clinical diagnostic biomarkers, reduced half-life after intravenous administration, and reduced blood–brain barrier (BBB) penetration. Additionally, several whole-brain imaging techniques only measure surrogate markers of brain activity and do not reflect direct activity within the brain (Vaghela et al., 2010).

Alternatively, molecular imaging techniques have been highly effective in probing the more specific mechanisms of neuronal function caused by synaptic processes that arise from specific molecular interactions. Due to increased sophistication in animal models of neural diseases, *in vivo* optical fluorescence microscopy has been extremely valuable in revealing many of the diverse processes of disease pathology at single neuron resolutions, as well as evaluating the effectiveness of experimental therapies on specific neuronal subpopulations (Svoboda and Yasuda, 2006). Overall, due to its non-invasive nature, good sensitivity, and use of readily available and relatively inexpensive instruments – when compared to other imaging modalities – molecular neuroimaging has become a fundamental technique for studying the function of living organisms. Yet, there remain significant drawbacks to effectively studying mechanisms of neuronal development, such as artefact interference and phototoxicity caused by the instability of fluorescent probes (Misgeld and Kerschensteiner, 2006).

The shortcomings of current neuroimaging techniques have led to new technological developments that improve on the state-of-the-art. The use of synthetic nanoparticles with diverse surface chemistries and superb optical features represent a versatile diagnostic tool for combating the challenges of *in vivo* neuroimaging techniques. Over the last decade, a variety of nanoparticles have been investigated for use in biomedical imaging due to an appreciation of their unique physical, chemical, and optical properties at the nanometre scale (Nune et al., 2009). Recent advances in the synthesis, engineering, and functionalisation of various nanoparticles have led to the proliferation of novel diagnostic imaging modalities. These present nanoscale probes as important platforms for the visualisation, characterisation, and quantification of biological processes at multiple imaging levels within living systems (Nowostawska et al., 2011).

2.1. Whole brain imaging

The introduction of iodinated contrast agents into the circulating system to create a contrast between blood vessels and the surrounding parenchyma was a major revolution to the visualisation of the central nervous system. Computer tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) have been pivotal instruments to our understanding of CNS structure and function. Additionally, the development of functional MRI to exploit haemodynamic properties of brain tissue at the molecular level has provided even more precise extrapolation of CNS activity. Despite these advances, current whole brain imaging agents present limitations such as permeability across the BBB, limited contrast-agent half-life, lack of specificity and sensitivity to the tissue location, in addition to issues around toxicity. In recent years, several nanotechnological approaches have been established to resolve these problems and in the field of MR-neuroimaging studies, significant advances have been gained (Winer et al., 2011). Among these, magnetic and paramagnetic nanomaterials have been extensively investigated as contrast agent enhancers. In particular, iron oxide nanoparticles have shown significant promise, compared to standard gadolinium-based approaches, as effective and relatively inexpensive multi-layered neuroimaging platforms (Fig. 1).

2.1.1. Iron oxide nanoparticles

The potential uses of iron oxide nanoparticles as MRI contrast agents has been an area of intense interest over the last two decades and there has been an extensive drive towards the implementation of these nanomaterials for human use, hastened by the FDA approval of ferumoxytol, the first iron oxide conjugate for clinical use (McCullough et al., 2012). This has seen a proliferation of studies investigating the use of minimally invasive nanoparticles for diagnostic and therapeutic applications. In particular, the use of multifunctional magnetic iron oxide nanoparticles has gained significant interest for clinical applications

Table 2
Nanotechnology studies of CNS function by disease model, route, results, primary feature(s) and potential clinical application.

Nanotechnology	Description	Disease model	Route	Results	Primary feature(s)	Potential clinical application	Refs.
Iron oxide nanoparticles	Iron oxide nanoparticles (ferumoxytol)	Patients with malignant brain tumours	Intravenous	Better signal resolution of brain tumour lesions than compared to gadolinium.	Improved image resolution Lower cost	Enhanced neuroimaging contrast agent for evaluating tumour lesions.	Neuwelt et al. (2007) Neuwelt et al. (2004)
	Iron oxide nanoparticles (ferumoxytol)	Patients with malignant brain neoplasms	Intravenous	Improved contrast-to-noise ratios and decreased motion artefacts, presenting higher resolution images compared to gadolinium based contrast agents.	Improved image resolution Improved image processing Lower cost	Differential diagnosis, tumour grading, and evaluation of early treatment responses in longitudinal studies of brain pathology.	Varallyay et al. (2013)
	Transferrin-conjugated iron oxide nanoparticles	Rat glioma model	Intravenous	Significant tumour contrast enhancement up to 48 h post injection.	Sensitivity to disease area of interest Lower cost	Targeted MRI contrast agent for visualising brain glioma.	Jiang et al. (2012)
	Dextran-coated iron oxide nanoparticles	Mouse glioblastoma multiforme model	Intravenous	Contrast enhancement between healthy *vs.* diseased tissue.	Sensitivity to disease area of interest Lower cost	Diagnostic neuroimaging of CNS malignancy and potential monitoring of therapeutic interventions.	Tomanek et al. (2012)
	Doxorubicin conjugated iron oxide nanoparticles loaded with microbubbles	Rat glioma model	Intravenous	Successful development of drug nano-carrier with improved imaging properties.	Sensitivity to disease area of interest Lower cost	Theranostic platform by image-guided delivery in brain tumour treatment.	Fan et al. (2013)
	Iron oxide nanoparticles (ferumoxytol)	Patients with CNS inflammatory lesions	Intravenous	Comparable visualisation of CNS inflammation between ferumoxytol and gadoteridol, with safer use for patients with renal insufficiency	Low toxicity profile Lower cost	Clinical diagnosis of inflammation in patients with CNS disorders	Farrell et al. (2013)
	PEGylated iron oxide nanoparticles	Mouse model of Alzheimer's disease	Intravenous	Improved BBB permeability and visualisation of amyloid plaques.	Increased BBB permeability Sensitivity to disease area of interest Lower cost	Longitudinal monitoring of amyloid plaque development and disease progression.	Wadghiri et al. (2013)
	Near-infrared maghemite nanoparticles conjugated to Aβ monoclonal antibody clone (BAM10)	Rat model of Alzheimer's disease	*Ex vivo*	Nanoparticles selectively labelled Aβ40 fibrils and had a significant inhibitory effect on Aβ40 fibrillation kinetics.	Sensitivity to disease area of interest Lower cost	Multifunctional theranostic platform for Alzheimer's Disease treatment.	Skaat et al. (2013)
	Iron oxide nanoparticles (ferumoxytol)	Patients with ischaemic Lesions	Intravenous	More consistent signal enhancement among all patients than compared with gadolinium.	Improved image resolution Lower cost	Diagnostic marker for cellular inflammation in stroke and other CNS disorders.	Saleh et al. (2004)
	P-Selectin targeted iron oxide nanoparticles	Mouse model of cerebral ischaemia	Intravenous	Enhanced imaging of endothelial activation following cerebral ischaemia.	Sensitivity to disease area of interest Lower cost	Detection of early stages of neuroinflammation for improved diagnosis of ischaemic injury.	Jin et al. (2009)
	VCAM-targeted iron oxide nanoparticles	Mouse model of cerebral artery occlusion	Intravenous	Visualisation of the early inflammatory processes of ischaemic lesions.	Sensitivity to disease area of interest Lower cost	Diagnostic tool for patient evaluation, acute monitoring of therapy and design of specific treatment strategies.	Hoyte et al. (2010); Fréchou et al. (2013)
	Iron oxide nanoparticles (Sinerem)	Patients with acute relapse of multiple sclerosis	Intravenous	Comparable visualisation of macrophage inflammation with gadolinium.	Sensitivity to disease area of interest Lower cost	Detection of the dynamic processes of lesion formation in multiple sclerosis.	Dousset et al. (2006)
	Iron oxide nanoparticles	Rat experimental autoimmune encephalomyelitis model	Intravenous and *ex vivo*	Efficient labelling of rat monocytes with iron oxide nanoparticles and successful implementation for *in vivo* monocyte migration in rats with EAE.	Sensitivity to disease area of interest Lower cost	Diagnostic neuroimaging of monocyte migration during inflammatory response.	Engberink et al. (2010)
	Iron oxide nanoparticles	Mouse experimental autoimmune encephalomyelitis model	Intravenous	Nanoparticles accumulated in the choroid plexus and spinal cord meninges of asymptomatic mice prior to the onset of clinical signs and in the absence of overt inflammation.	Sensitivity to disease area of interest Lower cost	Diagnostic marker providing a complementary tool to conventional MRI for monitoring early cellular changes in inflammation.	Millward et al. (2013)

Table 2 (*Continued*)

Nanotechnology	Description	Disease model	Route	Results	Primary feature(s)	Potential clinical application	Refs.
Organic nanoparticles	PLGA nanoparticles conjugated to an opiod glycopeptide (g7) and near-infra red probe	Wildtype mouse brain	Intravenous	Optical imaging of nanoparticle conjugates showing increased permeability across BBB.	Increased BBB permeability	CNS targeting for diagnostic neuroimaging.	Tosi et al. (2011)
	Biodegradable PBCA nanoparticles	Mouse model of Alzheimer's disease	Intravenous	Visualisation of amyloid plaques using traditionally non-BBB permeable reagents.	Increased BBB permeability Sensitivity to disease area of interest	Biodegradable nano-carrier system to deliver BBB-impermeable targeted molecular probes into the brain for diagnostic neuroimaging.	Koffie et al. (2011)
	Polyamidoamine denrimers	Wildtype mouse brain	Intra-parenchymal	BBB permeable showing low toxicity, low immune response and diffusion ability.	Increased BBB permeability Low toxicity profile	Diagnostic nanomaterial for delivery into the CNS with minimal toxicity.	Albertazzi et al. (2013)
Quantum dots	TAT-conjugated quantum dots	Wildtype rat brain	Intravenous	Quantum dot-loaded brain tissue could be visualised, showing improved BBB permeability.	Increased BBB permeability Limited to pre-clinical studies	Molecular neuroimaging platform for pre-clinical studies.	Santra et al. (2005)
	Hydrophilic quantum dots	Wildtype mouse brain	Intravenous	High signal-to-noise ratio and extended circulating half-life within brain vessels.	Improved image resolution	Enhanced neuroimaging platform for evaluation of CNS disorders.	Palui et al. (2012)
	Quantum dots within a core of wheat-germ agglutinin-conjugated PEG-PLA nanoparticles	Nude mouse brain	Intranasal	Targeted high-capacity delivery into several brain regions.	Increased BBB permeability	Targeted diagnostic platform for studying specific CNS disorders.	Gao et al. (2008)
	Quantum dot-doped silica nanoparticles	Wildtype mouse brain	Intracerebral	Visualisation of brain tissue with no toxicity associated with acute administration.	Low toxicity profile	Non-toxic use of quantum dots for diagnostic neuroimaging.	Bardi et al. (2010)
	Liposome-conjugated quantum dots	Nude mouse brain	Intravenous	Greater fluorescence intensity compared to free quantum dots.	Improved image resolution	Evaluation of a non-toxic theranostic nano-carrier platform.	Wen et al. (2012)
	Hydrophilic quantum dots	Wildtype mouse brain	Intracerebral	Exclusive localisation to the brain microglia visualising interaction of microglia and neurons independently.	Improved image resolution Sensitivity to brain region of interest	Selective imaging and delivery of therapeutic agents for CNS disease models.	Minami et al. (2012)
Nanoscaffolds	Nanoscale topographical cues	Wildtype mouse brain	*In vitro*	Control of neurite outgrowth for neuronal network formation and synapse development.	Sensitivity to physiological function Limited to pre-clinical studies	Modelling of neuronal circuit physiology for pre-clinical studies.	Hill et al. (2005), Johansson et al. (2006), Gomez et al. (2007)

Carbon nanotubes	Electrodes coated with carbon nanotubes	Wildtype rat brain	*In vitro*	Carbon nanotube coated electrodes can interface between neurons and electronic systems.	Sensitivity to physiological function Limited to pre-clinical studies	Assessment of neuronal network damage and investigating network repair.	Shein et al. (2009)
	Electrodes coated with multi-walled carbon nanotube pillars	Wildtype rat brain	*In vitro*	Superior charge injection limits than compared to standard platinum electrodes.	Sensitivity to physiological function	Neural prosthesis for recording neural activity and possible therapeutic intervention.	Wang et al. (2006)
	Carbon nanofibre-modified electrode arrays	Wildtype rat brain	*In vitro*	Stimulation and extracellular recording of spontaneous and evoked electrical activity in models of neuronal plasticity.	Sensitivity to physiological function Limited to pre-clinical studies	Evaluation of neural circuit processing at single-cell and intracellular level in pre-clinical studies.	Yu et al. (2007), Yu et al. (2012)
	Electrodes coated with PEGylated single walled carbon nanotubes	Wildtype rat brain	*In vitro*	Promotes the outgrowth of neurons, characteristic with a decrease in the number of growth cones and an increase in body area.	Sensitivity to physiological function	Implantable neural prosthesis for recording neural activity and possible therapeutic intervention.	Malarkey et al. (2009)
	Carbon nanotube-coated microelectrode arrays	Wildtype mouse brain	*In vitro*	Higher signal-to-noise measurements and temporal resolution than standard microelectrode arrays.	Improved signal resolution Sensitivity to physiological function Limited to pre-clinical studies	Preclinical studies of neural diseases and the screening of potential therapies against different neuropathological conditions.	Suzuki et al. (2013)
	Flexible carbon nanotube-coated microelectrode arrays	Chick Retina	*In vitro*	Measured clear spontaneous activity waves and observed electrical response following stimulation protocols.	Improved signal resolution Sensitivity to physiological function	Improved flexibility with better microelectrode recording capacity for *in vivo* neuronal applications.	David-Pur et al. (2014)
	Carbon nanotube-coated electrodes	Rat and monkey brain	Intracranial	Improved recording capabilities, enabling simultaneous measurements of local field potentials and spike activity from the same electrode site.	Improved signal resolution Sensitivity to physiological function Implantable	Implantable neural prosthesis for recording neural activity and therapeutic intervention.	Keefer et al. (2008)
	Carbon nanotube-coated microelectrode arrays	Feline brain	Intracortical	Similar capabilities to internal platinum wire controls over several months of intracortical implantation and microstimulation.	Sensitivity to physiological function Implantable	*In vivo* studies of neuronal activity and potential applications in chronic recording and microstimulation for neural prosthetic devices.	Parker et al. (2012)
	Wafer-scale carbon nanotube microelectrodes	Wildtype rat brain	*Ex vivo*	Demonstrated a fabrication process that supported electrode electrochemical performance and mechanical durability during insertion into the brain.		Innovative approach towards developing neural probes for high-resolution neural recording and stimulation.	Musa et al. (2012)

Table 2 (*Continued*)

Nanotechnology	Description	Disease model	Route	Results	Primary feature(s)	Potential clinical application	Refs.
Nanowires	Nanowire-modified electrodes	Wildtype rat brain	*In vitro*	Improved recording capabilities of intracellular neuronal activity permitting extensive recording of chronic activity from intact neural tissues and mammalian brains.	Improved signal resolution Sensitivity to physiological function	Nanoelectrode platform well suited to *in vivo* studies of neural tissue.	Ferguson et al. (2012)
	Solid-Conductor Intracellular NanoElectrodes (SCINEs)	Wildtype rat brain	*In vitro*	SCINEs could record action potentials as well as slower, subthreshold neuronal potentials without altering cellular properties.	Improved signal resolution Sensitivity to physiological function	Development of less invasive neuronal recording tools for long-term intracellular neurophysiological studies.	Angle and Schaefer (2012)
	Nanowire Field-effect transistors (NWFETs)	Wildtype rat brain	*In vitro*	High resolution recording of action potential signals from individual neurites and the ability for efficient multiplexed recordings from single neurons.	Improved signal resolution Sensitivity to physiological function	Preclinical studies of neurophysiology and the screening of potential therapies against different neuropathological conditions.	Patolsky et al. (2006)
	Silicon nanowire field-effect transistors (Si-NWFETs)	Wildtype rat brain	*In vitro*	Dual-mode recording of electrical signals and imaging of individual cell bodies to identify areas of healthy neurons at both upper and lower tissue surfaces.	Sensitivity to physiological function	Preclinical studies of neurophysiology and the screening of potential therapies against different neuropathological conditions.	Qing et al. (2010
	Free-standing Si-NWFETs	Cardiomyocytes	*In vitro*	Submicron precision of target cells and real-time recording of neuronal action potential amplitude and temporal properties.	Improved signal resolution Sensitivity to physiological function	Novel nanoelectronic probes with broad applications in electrophysiology and implantable bioelectronics.	Qing et al. (2014)

(Prina-Mello et al., 2013). Superparamagnetic iron oxide nanoparticles (SPIONs) are typically composed of a highly superparamagnetic iron oxide core and biocompatible polymers, such as dextran and PCS (polyglucose sorbitol carboxymethyl ether), used as a coating material (Sun et al., 2008). SPIONs can be further classified into cross-linked iron oxide nanoparticles (CLIONs), ultra-small superparamagnetic iron oxide nanoparticles (USPIONs) and very-small superparamagnetic iron oxide nanoparticles (VSPIONs). Due to their strong magnetic moments, these nanomaterials are well suited as T2-weighted MRI contrast agents and present an attractive neuroimaging platform due to their potential to permeate an intact BBB, improved half-life within the vascular system, minimum adverse effects, and clearance by phagocytic cells (Winer et al., 2011). In recent years, there have been a multitude of studies involving both animal models and human trials comparing the effectiveness of MRI between functionalised iron oxide nanoparticles and gadolinium across several brain disorders.

In clinical trials of patients with malignant brain tumours, intravenously administered ferumoxtran-10 or ferumoxytol, showed better signal resolution of brain tumour lesion when compared to MRI scans with gadolinium. The results indicated a likely enhancement of imaging properties due to the prolonged plasma half-life of the iron oxide nanoparticles as well as better CNS targeting and accumulation (Neuwelt et al., 2004, 2007). Further clinical enhancement was demonstrated by increased effectiveness over gadolinium in image visualisation techniques, such as steady-state blood volume maps, which complement conventional MRI. In a clinical study of sixty-five patients with CNS pathologies, the feasibility of high-resolution steady-state cerebral blood volume maps (SS-CBV) using ferumoxytol, was investigated and compared with dynamic susceptibility contrast perfusion imaging of cerebral blood volume (DSC-CBV) using a gadolinium-based contrast agent (gadoteridol). Their results displayed higher image resolution with SS-CBV compared to DSC-CBV as indicated by improved contrast-to-noise ratios and decreased motion artefacts, presenting in higher resolution images and comparable relative CBV values to the DSC technique. The authors concluded that the superior SS-CBV map resolution by ferumoxytol could make the technique clinically relevant, permitting differential diagnosis, tumour grading, and evaluation of early treatment responses in longitudinal studies (Varallyay et al., 2013). Given these advantages shown in patient studies, better functionalised iron oxide nanoparticles have also been explored for targeted tumour imaging in animal models. Jiang et al. investigated the preparation and characterisation of transferrin-conjugated superparamagnetic iron oxide nanoparticles (Tf-SPIONs) for glioma detection. Rats were injected with 12 mg/kg body weight nanoparticles *via* the tail vein and the time course of nanoparticle distribution in the rat brain was monitored by T2-weighted spin echo sequence. Their results showed significant contrast enhancement of brain glioma up to 48 h post-injection and they postulated that Tf-SPIONs could be used as potential targeting-MRI contrast agents for visualising glioma in the brain (Jiang et al., 2012). A recent study focused on dextran coated SPIONs, conjugated to an anti-insulin-like growth factor antibody (anti-IGFBP7), as an MRI contrast agent in a mouse model of glioblastoma multiforme. T2 relaxation time was used to measure the accumulation of the contrast agent within the tumour and their results indicated a statistically significant difference between SPION localisation in healthy *vs.* diseased tissue. They concluded that the developed antibody conjugated nanoparticles selectively bind to abnormal vessels within a glioblastoma for improved diagnosis and detection of tumour lesions and potential monitoring of therapeutic interventions (Tomanek et al., 2012). Another recent study proposed the use of SPIONs conjugated to doxorubicin (DOX) loaded onto microbubbles (MB) as a combined novel MRI and drug

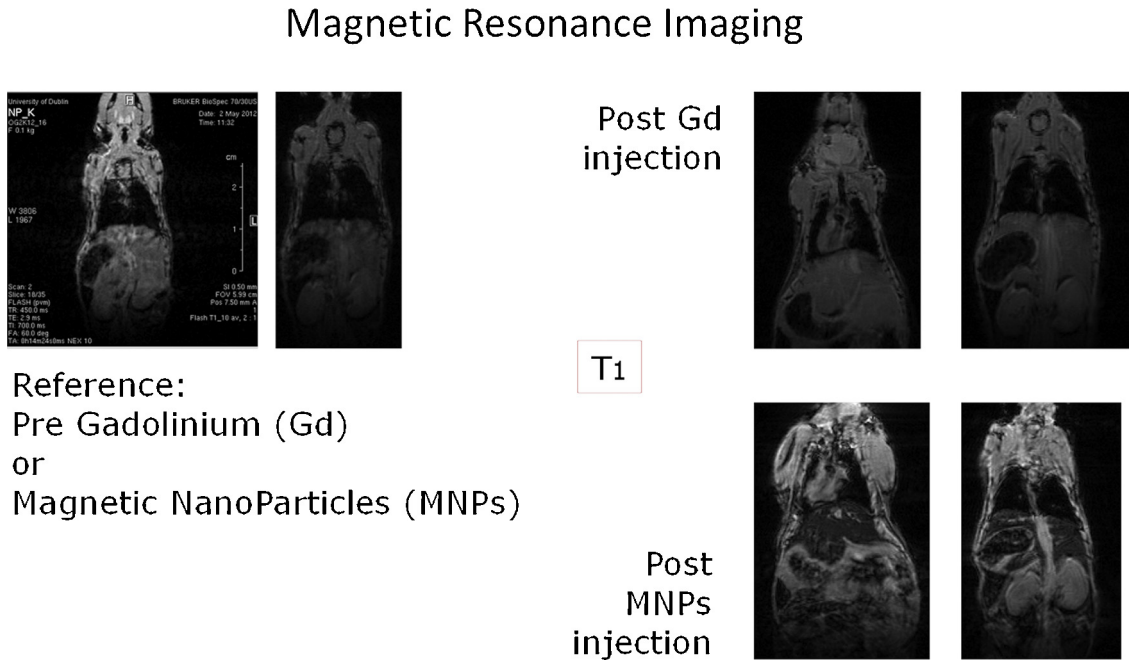

Fig. 1. Magnetic resonance full body T1-weighted images of reference mice (left panel) and injected mice with gadolinium (Gd) or magnetic nanoparticles (MNPs, right panels). Images were recorded using a Bruker BioSpec 7T system. Reference scans: all mice were imaged before injection. Mice were injected with 100 μl of gadolinium (a 1 in 3 dilution of MAGNEVIST injection) or MNPs (1 mg/ml) through the tail-vein. Mice were imaged again at 24-h (right panel).
(Courtesy of Dr. Oliviero Gobbo, Trinity College Dublin under MULTIFUN project – project ref. 242943)

delivery nanoplatform. The nanoparticle conjugates were delivered *in vivo* and were monitored by T2 weighted MRI in a rat glioma model. They found that the DOX-SPION-MBs were stable and provided significant superparamagnetic/acoustic properties for imaging. They concluded that their platform could improve the clinical therapeutic efficacy of chemotherapeutic agents by image-guided drug delivery for brain tumour treatment (Fan et al., 2013).

Iron oxide nanoparticles have also been investigated in models of neuroinflammation, implicated in the pathophysiology of several CNS disorders such as Alzheimer's disease, multiple sclerosis and stroke. In a clinical study of twenty patients with presumptive or known CNS inflammatory lesions, the enhancement properties of ferumoxytol were assessed in comparison to gadoteridol. Conventional T1- and T2-weighted imaging was acquired before and after contrast administration in all patients, as well as perfusion MRI for relative CBV obtained in patients receiving ferumoxytol. Their results indicated comparable visualisation of CNS inflammation between ferumoxytol and gadoteridol, with ferumoxytol highlighted for potential use in improved targeting of surgical biopsy, avoidance of nephrogenic systemic fibrosis (caused by gadolinium administration to patients with renal insufficiency), and improved correlation between CBV and CNS inflammation. Their results suggested a beneficial role for iron oxide nanoparticles in the clinical diagnosis of inflammation in patients with CNS disorders (Farrell et al., 2013). A recent animal study investigated the use of USPIONs in the detection of amyloid plaques in Alzheimer's disease (AD). Wadghiri et al. developed bi-functional USPIONs chemically coupled to the amyloid-β peptide (Aβ1–42) to image amyloid plaque deposition in the AD transgenic mouse brain. The nanoparticles were conjugated with polyethylene glycol (PEG) to improve BBB permeability and injected intravenously into the right femoral brain, followed by magnetic resonance microimaging. The amyloid plaques were detected by T2-weighted imaging, forming a 3D gradient multi-echo sequence with a 100 μm isotropic resolution. Their results showed the uptake of USPION-PEG- Aβ1–42 conjugates through intravenous administration and the detection of amyloid plaques without the need to co-inject a further BBB permeability agent. They concluded that this technique could aid the visualisation of plaque development and the longitudinal monitoring of disease progression in clinical studies of CNS disorders (Wadghiri et al., 2013). Another recent study focused on using nanoparticles to observe potential therapeutic applications of passive anti-Aβ immunotherapy, based on the administration of anti-Aβ monoclonal antibodies to delay Aβ aggregation in the brain. The study reported immobilisation of the Aβ monoclonal antibody clone (BAM10) to near-infrared fluorescent (NIR) maghemite nanoparticles for the inhibition of $A\beta_{40}$ fibrillation kinetics and specific detection of $A\beta_{40}$ fibrils. T2-weighted MR imaging of *ex vivo* whole rat brains indicated that conjugation of BAM10 to NIR fluorescent iron oxide nanoparticles selectively labelled $A\beta_{40}$ fibrils and had a significant inhibitory effect on $A\beta_{40}$ fibrillation kinetics. Their study highlighted how immobilisation of the anti-Aβ monoclonal antibodies to dual-modal nanoparticles could contribute to the development of multifunctional agents as theranostic indicators of Alzheimer's treatment (Skaat et al., 2013).

In a study by Saleh et al. on stroke diagnosis, an open-label clinical Phase II pilot trial was conducted using USPION-enhanced MRI in ten consecutive patients with early ischaemic stroke. In comparison to gadolinium they observed consistent USPION-related signal changes in all ten patients, whereas gadolinium enhancement occurred in only three patients, suggesting that USPION-enhanced MRI could provide a novel *in vivo* surrogate marker of cellular inflammation in stroke and other CNS disorders (Saleh et al., 2004). More recent studies on animal models have sought to improve efficacy and expand the parameters for iron oxide nanoparticle-based MRI stroke imaging. Jin et al. developed a novel magnetic resonance molecular imaging method using an iron oxide nanoparticle contrast agent targeted towards P-selectin (MNP-PBP) to image endothelial activation following cerebral ischaemia. This study demonstrated the feasibility of *in vivo* imaging of endothelial activation in a mouse model of acute stroke

and the rapid accumulation of the MNP-PBP conjugates within the brain microvasculature. By targeting endothelial activation through P-selectin, their platform allowed for the detection of early stages of neuroinflammation providing a comparative measure of pathophysiological findings between lab and clinical studies (Jin et al., 2009). In a similar study, Hoyte et al. monitored inflammatory responses to cerebral ischaemia by the specific imaging of vascular cell adhesion molecule (VCAM)-1 expression in a mouse model of middle cerebral artery occlusion. Their results showed that using ligand targeted contrast agents could determine the early inflammatory processes of ischaemic lesions *in vivo*; providing a potentially valuable clinical diagnostic tool for patient evaluation, acute monitoring of therapy, and the design of specific treatment strategies (Hoyte et al., 2010). More recently, Fréchou et al. developed an USPION contrast agent targeting VCAM-1 (P03011) to detect the role of the vascular inflammatory response following cerebral ischaemia. In animals receiving P03011, both *in vivo* and ex *vivo*, T2-weighted MRI performed 24 h after ischaemia onset showed hypointense foci corresponding with USPION target binding. Further histological analysis was seen to associate with co-localisation of the targeted USPIONs and VCAM-1 and they concluded that iron oxide nano-enhancement of MRI following stroke could be an interesting clinical tool to characterise ischaemic lesions in terms of vascular damage (Fréchou et al., 2013).

Iron oxide nanoparticles have also found useful applications in studying the role of neuroinflammation in human and animal studies of multiple sclerosis. A clinical phase II pilot study by Dousset et al. investigated CNS infiltration of macrophages in acute relapse of multiple sclerosis. They compared tissue lesion enhancement by gadolinium with USPIONs in ten patients and found comparable *in vivo* visualisation of macrophage activity as a distinct cellular and inflammatory event in the dynamic process of multiple sclerosis lesion formation (Dousset et al., 2006). As a result, several studies have focused on using the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), to increase the detection capabilities of iron oxide nanoparticles (Engberink et al., 2010; Oude Engberink et al., 2010). In a study by Engberink et al., magnetoelectroporation (MEP) was used to label monocytes with SPIONs and injected into rats induced with EAE. Their results demonstrated efficient labelling of rat monocytes with iron oxide nanoparticles and successful implementation for *in vivo* monocyte migration in rats with EAE. They suggest that monocyte labelling with iron oxide nanoparticles for clinical diagnostics could facilitate bench to bedside translation of novel therapeutics by targeting monocyte recruitment in the inflammatory response (Engberink et al., 2010). Additionally, Millward et al. investigated the use of VSPIONs for the early detection of subtle inflammatory events that may be missed by gadolinium-based MRI studies. Using encephalitogenic T-cells to induce EAE in mice, they demonstrated that VSPIONs accumulated in the choroid plexus and spinal cord meninges of asymptomatic mice prior to the onset of clinical signs and in the absence of overt inflammation. Their study suggested that the choroid plexus involvement in early inflammatory processes of autoimmune inflammation and postulated the use of VSPIONs as a complement to conventional MRI approaches in monitoring early cellular changes in inflammation (Millward et al., 2013).

2.2. Molecular imaging

The structural design of nanomaterials for biomedical imaging has sought to expand on the intrinsic advantages of multi-photon optical imaging by enhancing the properties of the fluorophores used to illuminate cellular activity (Fig. 2). In recent years, a variety of nanoparticle carriers have been investigated to explore their potential use in molecular imaging, with emerging tools increasingly focused on novel approaches to improve the study and potential diagnosis of CNS disorders. There are several key parameters required for the effective delivery of optically active nanoparticles to the desired target. The most important of these include particle size, shape, charge, hydrophilicity and bioconjugation, with a plethora of nanomaterials under investigation for their molecular imaging properties, including solid lipid nanoparticles, liposomes, micelles, nanotubes, metallic nanoparticles, polymeric nanoparticles, dendrimers and quantum dots (Nune et al., 2009). Several recent articles have been published detailing the use of a variety of nanoparticles as *in vivo* detection tools for optical fluorescence imaging in the CNS and these are discussed below.

2.2.1. Organic nanoparticles

Tosi and co-workers described the development of targeted nanoparticles for optical *in vivo* brain imaging. Their study conjugated polylactide-co-glycolide (PLGA) nanoparticles with an opioid glycopeptide (g7) and a near-infra red (NIR) probe. Surface modification of the PLGA nanoparticles with the opioid g7 peptide sequence enhanced the ability of polymeric nanoparticles to cross the BBB and deliver previously impermeable molecular agents. The NIR-labelled nanoparticles were injected intravenously into mice tail veins and observed *via in vivo* time-domain optical imaging. Their results demonstrated that the nanoparticle conjugates could be easily visualised by optical imaging techniques due to the NIR probe, and permeability across the BBB could be enhanced by the g7 glycopeptide, thus presenting a novel CNS-targeted *in vivo* tool for improving diagnostic neuroimaging capabilities (Tosi et al., 2011). Another study looked at the use of biodegradable polysorbate 80-coated poly(n-butyl cyanoacrylate) (PBCA) nanoparticles to explore *in vivo* diagnostic imaging of dynamic CNS processes in living animals. In this work, Koffie et al. investigated the potential of PBCA nanoparticles to deliver BBB-impermeable molecular imaging contrast agents into the brain of mice for *in vivo* multi-photon microscopy. They validated their approach through the visualisation of amyloid plaques in an *in vivo* mouse model of Alzheimer's disease using (traditionally) non-BBB permeable reagents. Their resultant 4-dimensional, real-time, two-photon imaging revealed PBCA nanoparticle uptake into the brain parenchyma and highlighted the potential use of a biodegradable nano-carrier system to deliver BBB-impermeable targeted molecular probes into the brain for diagnostic neuroimaging (Koffie et al., 2011). Albertazzi and colleagues describe the use of modified polyamidoamine (PAMAM) dendrimers to cross the BBB *in vivo* and penetrate living neurons without inducing apoptotic cell death or activating microglia. Dendrimers are highly branched molecules synthesised with a precise, well-defined structure and low polydispersity (Tomalia et al., 2007). Due to their variable size, large numbers of reactive sites and hollow interior, dendrimers have proved to be a promising platform for neuroimaging. Their study investigated the diffusion kinetics of PAMAM dendrimers into the brain parenchyma using two-photon microscopy in living animals after direct intraparenchymal injection. Due to the low toxicity, low immune response, and diffusion ability, PANAM dendrimers were suggested by the authors to be potentially viable diagnostic nanomaterial for *in vivo* delivery to the CNS (Albertazzi et al., 2013).

2.2.2. Quantum dots

Quantum dots (QDs) are fluorescent semiconductor nanocrystals of variable size (1–100 nm), with unique electrical and optical properties (Fig. 3). In particular, they display superior imaging characteristics in comparison to organic dyes and fluorescent proteins, possessing near-unity quantum yields and far greater

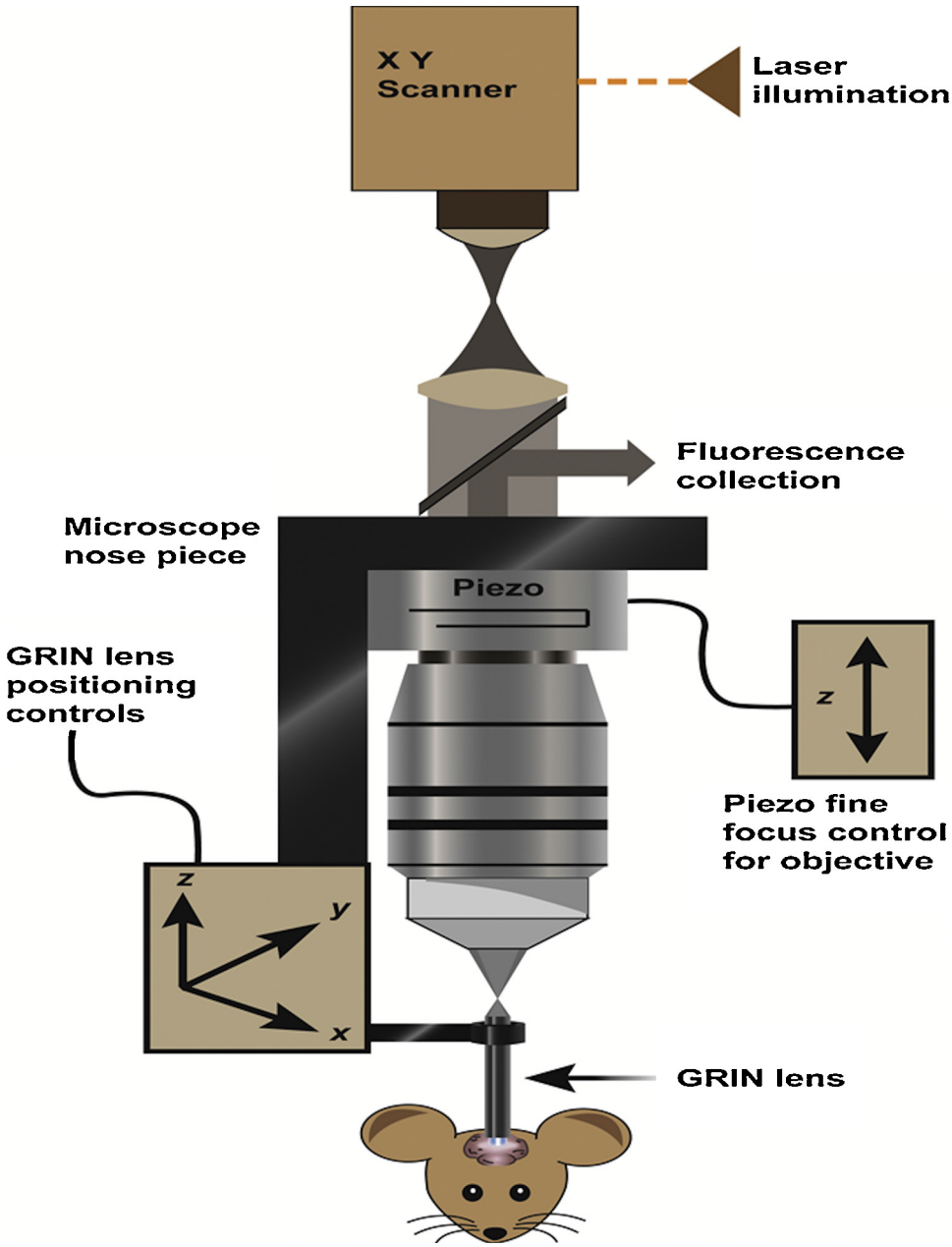

Fig. 2. Multiphoton microscopy of brain tissue with gradient index (GRIN) lenses. Technique for molecular imaging of *in vivo* brain tissue and visualising target neural pathways.
Adapted from Levene et al., 2003.

brightness (Smith et al., 2008). These factors coupled with their broad absorption spectra yet narrow emission spectra characteristics, relatively long fluorescence lifetime, and negligible photobleaching over minutes to hours, make QDs advantageous for biomedical imaging applications after conjugation with specific bioactive moieties (Pathak et al., 2006). By pairing QD imaging with *in vivo* targeting of specific cell populations, an increasing number of studies have validated the potency of bioconjugated QDs for molecular neuroimaging (Provenzale and Silva, 2009).

Santra et al. demonstrated the rapid and effective labelling of brain tissue using TAT (a cell penetrating peptide) conjugated to CdSe:Mn/ZnS (cadmium-selenide:manganese/zinc sulphide) QDs intra-arterially delivered to a rat brain. Their experimental data showed that QD-loaded brain tissue could be grossly visualised (using handheld UV excitation) and cellular analysis of the brain parenchyma could be imaged using fluorescence microscopy. They were able to confirm the conjugation of the TAT peptide as a method to overcome the BBB and demonstrated the possibility of delivering a large amount of QD-based imaging agents into brain tissue (Santra et al., 2005). Additionally, Palui et al. recently developed highly luminescent and hydrophilic CdSe/ZnS QDs of various sizes, showing excellent colloidal stability with optimum spectroscopic properties. They validated the effectiveness of their QD conjugates by performing two-photon fluorescence *in vivo* imaging of mice brain vessels to visualise the brain capillaries of live mice for several hours without apparent adverse effects (Palui et al., 2012). Another recent study reported the selective *in vivo* targeting of microglia by semiconductor QDs. Highly active microglia are the resident immune cells of the brain, continuously probing their vicinity and responding to nearby damage

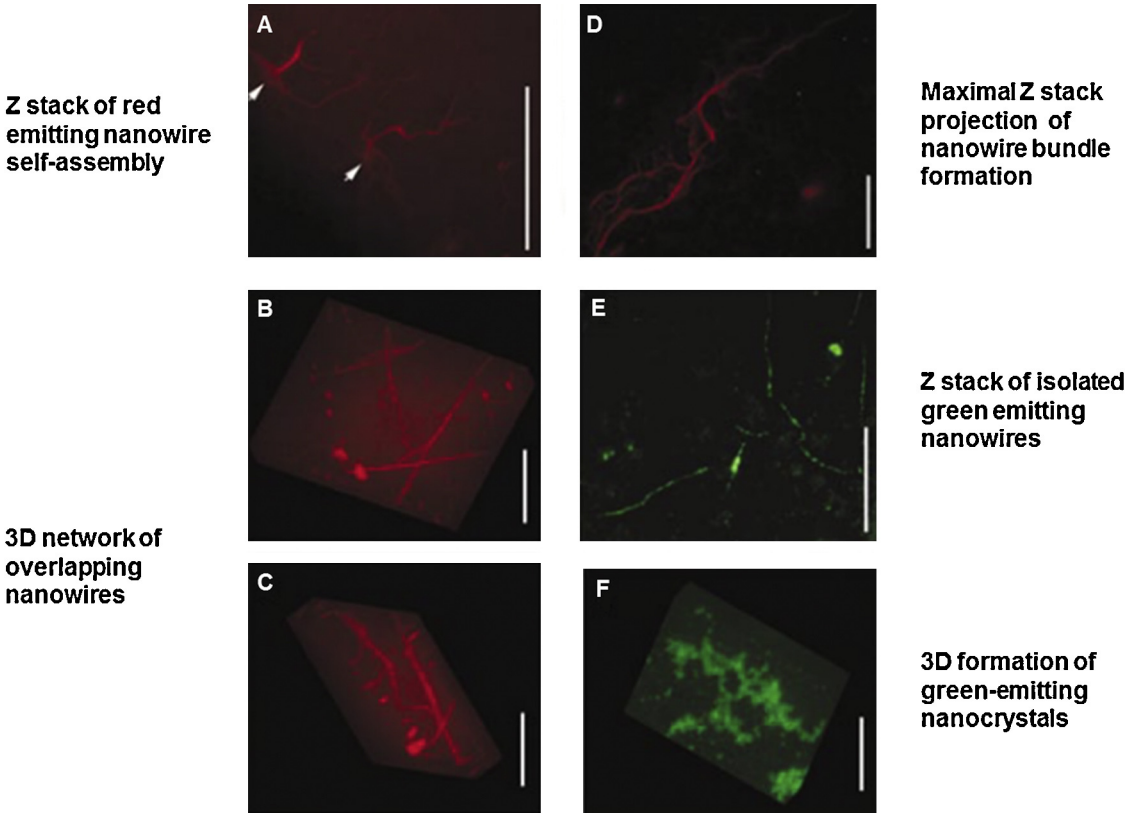

Fig. 3. Confocal microscopy images of QD nanowires self-assembled from red- (a–d) and green-emitting (e and f) CdTe nanocrystals. (a) Early stage of the nanowire formation shown as a Z stack projection. Multiple growing and branching nanowires of varying length emerge from two distinctive nucleation patches, which are shown by arrows. (b and c) 3D network of overlapping nanowires shown by two selected 3D projections of the same Z-plane confocal image stack. (d) Maximal Z stack projection illustrating nanowire bundle formation. (e) Isolated green-emitting nanowires shown as a Z stack projection. (f) Formation of green-emitting nanocrystals aggregating within a 3D volume. Scale bars = 10 μm. (Volkov et al., 2004).

(Nimmerjahn et al., 2005). However, they have also been implicated in brain injury and various neurological disorders, requiring greater knowledge of their behaviour in disease processes. Employing stereotaxic injections into mice hippocampus, Minami et al. demonstrated an almost exclusive localisation of QDs to the brain microglia, providing a means of observing the dynamic interaction of microglia and neurons independently and uncovering new insights into their roles in the pathogenesis of CNS disorders (Minami et al., 2012).

Several new studies have looked into conjugating QDs to other types of nanoparticles in order to improve stability and further enhance BBB permeability. Gao et al. reported the development of a QD-based nanoplatform for brain imaging by encapsulating QDs into the core of poly(ethylene glycol)-poly(lactic acid) nanoparticles and conjugating the agglomerate particles with wheat-germ agglutinin to improve their brain delivery. The resulting nanoparticles displayed high-payload capacity and were water soluble, showing considerable potential for the development of specific imaging agents for various CNS diseases (Gao et al., 2008). Bardi et al. similarly investigated the biocompatibility and imaging capabilities of amino functionalised CdSe/ZnS QD-doped silica nanoparticles administered to mice visual cortex *via in vivo* intracerebral injection. Their results demonstrated no toxicity associated with acute administration and supported their hypothesis for the use of QD-doped nanoparticles for biomedical imaging as a valuable tool for future nanomedicine applications (Bardi et al., 2010). Additionally, QDs have been incorporated into liposomes to eliminate uptake by the liver and enhance brain targeting. Liposome-QD conjugates were injected intravenously through the tail vein of female nude mice to observe the localisation and time-course of fluorescent signals emitted. The results showed improved fluorescence intensity of liposome-QD conjugates in the brain when compared to free QDs and validated their potential use as a novel tool for the diagnosis of CNS disorders (Wen et al., 2012).

The development of functionalised QDs with reduced toxicity profiles provides potentially clinically relevant tools for evaluating specific brain processes. However, investigations of QD applications in diagnostic neuroimaging have been primarily limited to pre-clinical studies. This is mainly due to the varied and contradictory reports on the toxicity profile of QDs resulting from the release of cadmium heavy metal Cd^{2+} ions which has been shown to be cytotoxic to cells (Valizadeh et al., 2012). For QDs to be realised as a valuable diagnostic medical technology, further studies are needed to investigate toxicity in a case-by-case manner, thereby improving study quality and leading to the potential identification a QD formulation that is safe for human use (Tsoi et al., 2013).

3. Nanoscale electrophysiology

Elucidating the mechanisms involved in regulating neuronal synapse dynamics is critical for gaining new insights into the aetiology and progression of CNS disorders, leading to more sensitive diagnosis and potential treatment. Functional brain development requires synchronisation of environmental signals with adaptive neuronal communication; therefore, managing extracellular stimuli from early sensory experiences plays an important role in the ability of neural circuits to modulate synaptic connections and drive modifications required for many aspects of postnatal development (Barnes and Finnerty, 2010). This has led to

an increasing appreciation for the so-called 'critical period' of development, characterised by enhanced plasticity in post-natal life, whereby the mechanisms of neuronal activity and functional plasticity acquired at an early age are recruited for adult processes (Linkenhoker et al., 2005). This phenomenon is identified by physiological and morphological changes which include excitation-inhibition dynamics that may lead to structural rearrangements of dendritic branches and synapse formation or elimination. These synapse dynamics are extraordinarily flexible (Bourne and Harris, 2008) and are regulated by extracellular activity and visualised by variability in the size and number of spine heads at the synapse (Matsuzaki et al., 2004). In neurodevelopmental and neuropsychiatric disorders, synapse dynamics can become impaired, illustrating the pivotal role of functional neuronal communication in the pathogenesis of these human diseases (Cohen and Greenberg, 2008).

Understanding the role of neuronal activity and plasticity in synapse development requires critical examination of how relationships between interacting neurons produce a collective functional output. This involves the study of computations of locally interconnected neurons–microcircuits – and requires the identification of sequences of events that associate experience and learning with activity-dependent synaptic plasticity (Caroni et al., 2012). Detecting the early stage mechanisms in functional synapse development poses unique technical challenges which are difficult to resolve with current technologies. The lack of direct access to the brain has obvious limitations to the study of human brain development and while functional brain imaging studies remain a powerful means of visualising regional brain structures and activity, they lack the resolution required to probe neuronal activity at the microcircuit level. Electrophysiological studies using metal, glass or silicon electrodes have typically been the gold-standard for probing cellular dynamics based on animal models of CNS disorders. Due to the relative ease of experiments and the strong signal-to-noise data capacity, dual-cell recordings have been an influential method for probing synapse connectivity.

In recent years, the development of advanced electrodes using thin-film manufacturing techniques and chemical synthesis has allowed for material properties to become tailored for specific requirements in various biological processes. Given the improved physical properties of materials at the nanoscale, there has been increasing progress in the use of nanotechnology to expand bioelectrical contact with cells within the nervous system (Pancrazio, 2008). These advances in novel technologies are providing increasingly more powerful platforms for bioelectrical devices as high-quality neural interfaces (Kipke et al., 2008). In particular, neural biosensors are being developed to understand how the nervous system encodes information in response to activity-dependent stimulation and elucidate many of the mechanisms underpinning neural dysfunction.

3.1. Neural biosensors

One of the major requirements for studying CNS disorders and discovering potential diagnostic biomarkers is to examine the relationship between cellular function and network structure, *i.e.*, the integration of circuit physiology and synapse dynamics to develop functional CNS structure and activity (Lee and Reid, 2011). Primary neuronal culture has typically been the gold-standard for studying the cellular and molecular mechanisms of functional neuronal activity, permitting easy access to individual neurons for electrophysiological recording and stimulation, pharmacological interventions, and high resolution microscopic analysis. In particular, the introduction of more accurate assays for modelling brain disorders through induced pluripotent stem cell (iPSC) models, have provided innovative approaches for probing molecular mechanisms of neuronal development (Marchetto et al., 2010) and high-throughput screening of novel therapeutic compounds (Lee and Studer, 2011). These humanised disease models are derived from peripheral cells isolated in affected individuals and differentiated into human neurons to study disease specific pathogenesis (Ming et al., 2011). This approach presents a valuable diagnostic tool for understanding many of the properties of CNS function and the development of personalised therapeutic strategies (Chailangkarn et al., 2012). However, classic neuronal culture preparations are not amenable to modelling microcircuit connections that can observe the physiological and morphological modifications regulated by neuronal activity and synaptic plasticity. Firstly, connectivity testing is limited by random and uncontrolled synapse formation within the culture. Secondly, the number of cells available for recording is severely limited by the health of the culture over time and the technical difficulties of simultaneous electrophysiology recordings (Potter and DeMarse, 2001). These restrictions impede understanding of how neurons in a local circuit carry out specific computations and limit the identification of biomarkers that can aid the diagnosis of CNS disorders for therapeutic intervention.

In recent years, neural biosensors have been developed using micro- and nanoscale arrays that go beyond several of the limitations mentioned and reveal added layers to the properties of functional activity within neuronal networks (Fig. 4). While some of these technologies have been suggested to play a therapeutic role (Silva, 2010), we will focus on the development of these biosensors for studying neuronal function and suggest the role they may play in clinical applications for CNS disorders.

3.1.1. Nano-modified MEAs

Microelectrode array (MEA) technology has emerged as a credible tool for the functional analysis of activity-dependent circuit physiology. MEAs are planar glass structures fabricated from standard photolithographic techniques implemented by the semiconductor industry. These biosensors are able to provide unique insight into the spatiotemporal patterns of activity in intact two-dimensional neuronal networks, a concept analogous to having petri dishes with electrodes embedded into the culture substrate and linked to a recording/stimulation system (Fig. 5). They function by forming an electrical interface between neurons grown over electrode-embedded glass substrates. This enables researchers to monitor the circuit physiology of neuronal networks and provides a unique opportunity for multi-parametric analysis of microcircuit plasticity *via* combined electrophysiological and molecular imaging modalities of high specificity and sensitivity (Nam and Wheeler, 2011). Critically, MEAs are non-invasive and offer considerable flexibility in culture preparation, experimental design and high-throughput approaches when compared to conventional electrophysiological techniques (Morin et al., 2005). Spontaneous electrical activity generated by action potentials elicited within the cell bodies, axons, and dendrites of neurons create extracellular field potentials that are within the receptive field of the electrode array (Stegenga et al., 2008). Signal transduction primarily involves microelectrode detection of the local variations in extracellular field potentials created by spontaneous cellular activity and analysis of the spatiotemporal properties of the activity generated. Basic measurements typically assess the rate of action potentials ('spikes') and groups of action potentials ('bursts') recorded from each electrode, as well as the overall network spike and burst rates (Jones et al., 2011). Furthermore, coupling the MEA device to stimulus generators makes it possible to create stimulating electrodes which confer the ability to measure the effects of evoked activity and allow for greater insight into the principles of neuronal activity and plasticity (Hoffman and Bading, 2006).

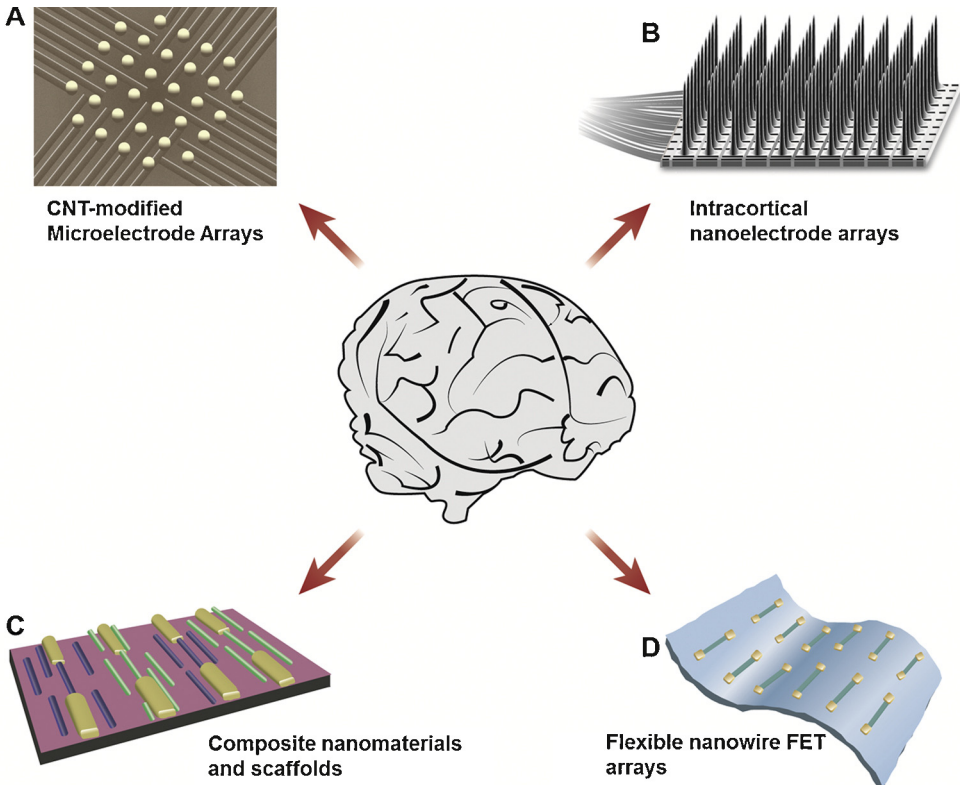

Fig. 4. Schematic of nanoscale neural interfaces for modelling and recording circuit physiology. (A) Carbon nanotube (CNT) films coated onto microelectrode arrays for studying neuronal networks. (B) Intracortical nanoelectrode arrays for *in vivo* recording and stimulation of brain tissue. (C) Assembly of composite nanomaterials and nanoscaffolds on the same interface for controlling neurite outgrowth. (D) Flexible nanowire field effect transistors for intracellular electrical recordings.

Since the development of MEA technology for monitoring neuronal responses (Pine, 1980), several studies have assessed how different activity-dependent stimulation paradigms act on target neuronal networks (Beom Jun et al., 2005; He et al., 2009; Marconi et al., 2012; Scott et al., 2012; Wagenaar et al., 2005), in addition to how mechanisms of transcription-dependent plasticity affect neuronal architecture (Arnold et al., 2005; Liu et al., 2011). These studies have investigated the effects of activity-dependent stimulation on developmental plasticity and demonstrated the benefits of MEA technology for delineating the transcriptional activity of neuronal networks with high spatial and temporal resolution. However, maximising the potential of MEA technology requires the ability to monitor the electrical activity of single neurons within a network and examine development-, activity-, and pharmacology-dependent changes in synaptic network and function (Jun et al., 2007). Several new strategies have been incorporated into the design of MEAs, proposing innovative concepts to improve the interface between microelectrodes and neurons in culture and defining broader applications for MEA based studies. The recent trend in higher density electrode arrays on MEA devices has led to the implementation of novel nanotechnology-based approaches to enhance MEA performance for neuronal recording and stimulation and new features have been developed to enable precise cell growth and guidance on novel nano-enhanced biosensor arrays (Heim et al., 2012).

Feature size has become an important characteristic for developing neural interfaces that more accurately mimic *in vivo* conditions. For network modelling applications, nanostructured scaffolds have been used to increase cell-material interactions providing a significant advantage for the study of basic neurobiology and the development of neural biosensors. In particular, nanoscale grooves developed through photolithographic techniques have been successful in enhancing neuronal interaction with array substrates. Photolithography produces nanoscale and spatially defined topographical patterns on array surfaces, scaffolding neurite outgrowth for the investigation of *in vitro* neuronal networks and identifying developmental mechanisms associated with functional circuit physiology (Seidlits et al., 2008). In a study of axonal outgrowth on nano-imprinted patterns, Johansson et al. developed nanoscale grooves with depths of 300 nm and varying widths of 100–400 nm on polymethylmethacrylate (PMMA)-covered silicon chips. They monitored the growth of mouse sympathetic and sensory ganglia cultured in medium containing 25 ng/ml of nerve growth factor to stimulate axonal outgrowth. Using immunocytochemistry and scanning electron microscopy, they found that axons displayed contact guidance on the patterned surfaces but grew preferentially on ridge edges and elevations in the patterns rather than in grooves. Additionally, they found that grooves less than 100 nm wide were less effective at promoting parallel neurite alignment (Johansson et al., 2006). In a similar study, Gomez et al. combined nanoscale topographical cues with chemical stimulation by nerve growth factor. Their results demonstrated the role of narrower and shallower grooves in the induction of neurites to grow across the grooves and align perpendicular to the long axis of the grooves, whereas wider and deeper grooves resulted in the growth of the neurites parallel to and along the grooves. They concluded that topography primarily dominated polarisation mechanisms, whereas the synergy of immobilised NGF plus topography, dominated axon length (Gomez et al., 2007). Another approach has been to use photo-crosslinking of proteins to focally excite non-cytotoxic photosensitizers that promote protein crosslinking, such as BSA, into matrices having sub micrometre feature sizes. The fabrication of barriers, growth lanes, and pinning structures comprised of cross-linked proteins fabricated under conditions that do not compromise the viability of

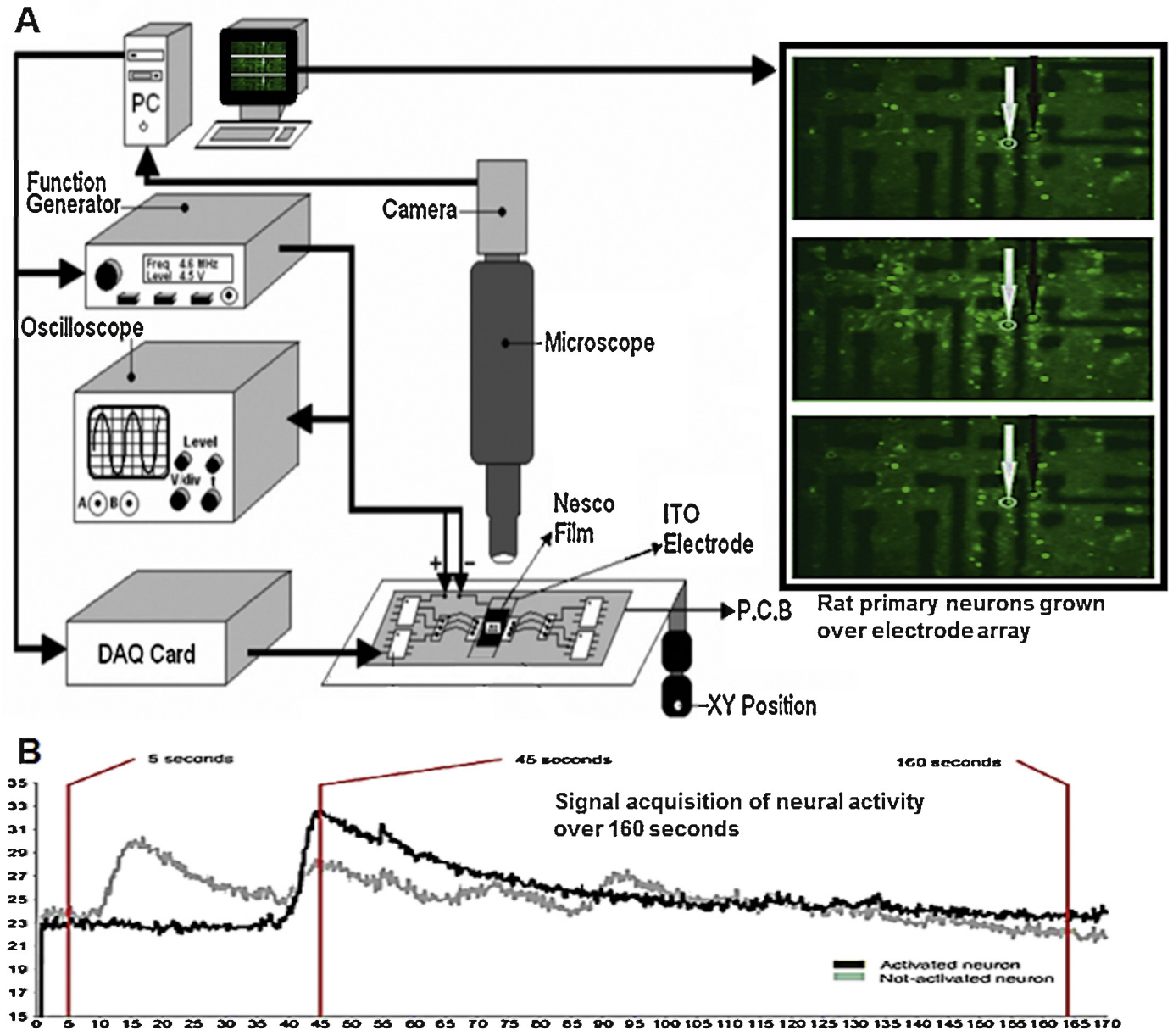

Fig. 5. Rat primary cortical neurons cultured on customised microelectrode arrays. (A) Set-up of MEA experiments consisting a microscope camera, function generator, and data acquisition card (DAQ) (adapted from Jaber et al., 2009). Video output of Ca2+ fluorescent intensities for the sample of neurons at three distinct time points of the experiments. (B) Measurement of Ca2+ fluorescent intensities in two sample neurons (grey and black circles) at baseline after 5 s, 45 s following acetylcholine (20 mM) application and mecamylamine (5 mM) addition at 160 s from beginning of recording.

neurons has been shown to restrict neurite outgrowth within confined structures (Hill et al., 2005; Kaehr et al., 2004).

The development of nanomaterials to improve cell adhesion properties and cell-to-substrate MEA interfacing has also been investigated. This has been demonstrated by direct growth of carbon nanotube (CNT) films, from a thin catalyst layer, onto the electrode using a chemical vapour deposition (CVD) method; with growth conditions determining whether CNTs are deposited in a random manner (Su et al., 2010), or vertically aligned along well defined parameters (Nguyen-Vu et al., 2006). Due to their high surface-to-volume ratio, the growth of neurons on carbon nanotubes as possible substrate enhancements for recording and stimulating neuronal populations has gained popularity (Lovat et al., 2005; Mazzatenta et al., 2007; Zhang et al., 2005), displaying higher capacitance and lower impedance values than when compared to bare metal electrodes (Keefer et al., 2008). A study by Ballerini and co-workers proposed the 'electronic hypothesis' to explain the physical interactions between neuronal cells and carbon nanotubes, illustrating mechanisms by which CNTs affect the collective electrical activity of cultured neuronal networks. Using single-cell electrophysiology, they showed that direct nanotube–substrate interactions with membranes of neurons affect single-cell activity. The group were able to decipher the nanoscale physical interactions between the nanotube and the neuron through the formation of tight contacts with the cell membranes, favouring electrical shortcuts between the proximal and distal compartments of the neuron. Their discoveries suggest an ability to predict or engineer interactions between neurons and carbon nanotubes, presenting CNTs as novel interfaces to improve neural signal transfer while supporting dendrite elongation and cell adhesion (Cellot et al., 2009). Similarly, Shein and co-workers presented novel electrode arrays composed of cell-appealing CNT islands of microelectrodes coated by a layer of dense and entangled CNTs synthesised through the CVD process.

Their result illustrated that the CNT islands strongly attracted and anchored dissociated primary rat neurons to pre-defined locations and enabled the formation of stable sub-networks on electrically active recording sites. They concluded that CNT-coated electrodes were well suited to assist interfacing between electrically active biological cells and conventional electronic systems. In addition they suggested that such CNT based devices could provide a valuable diagnostic tool for studying network damage and investigating network repair (Shein et al., 2009). In another study, Wang et al. presented an MEA neural interface employing vertically aligned multi-walled CNT pillars as microelectrodes. They demonstrated the effectiveness of their platform using hippocampal sliced cultures grown on the CNT-modified MEA device and revealed superior charge injection limits than compared to standard platinum electrodes (Wang et al., 2006). Similarly, Yu et al. tested vertically aligned carbon nanofibre electrode arrays for their potential to record electrophysiological activity and reported the stimulation and extracellular recording of spontaneous and evoked electrical activity in organotypic hippocampal slice cultures. Their results suggested the potential use of such a platform in improving electrophysiological studies of neuronal populations by enabling multimodal recordings at high spatial resolutions (Yu et al., 2007). They further demonstrated the capabilities of their carbon fibre interface for dual-mode recordings of neuroelectrical and neurochemical activity in models of neuronal plasticity, and postulated the use of their device for understanding how neural circuits process information at the single-cell and intracellular level (Yu et al., 2012). In another study Malarkey et al. demonstrated how conductive single-walled CNTs could be used as substrates to modulate neuronal growth. Using variably conductive PEGylated nanotube films sprayed on hot glass coverslips as substrates to culture neurons, they showed that nanotubes in a narrow range of conductivity could promote the outgrowth of neurons, characteristic with a decrease in the number of growth cones as well as an increase in body area (Malarkey et al., 2009). In a more recent study, Suzuki et al. developed CNT-modified MEA sensors for non-invasive and real-time measurements of dopamine neurotransmitter release, action potential spikes and post-synaptic field potentials. They describe successful chronoamperometric measurements of synaptic dopamine release from spontaneous firings in mouse striatal brain slices. The detection of nanomolar concentrations of dopamine was attributed to higher signal-to-noise ratio as a result of increased sensitivity from CNT-modified array electrodes. They further measured action potentials and field synaptic potentials of cultured hippocampal neurons demonstrating high temporal resolution and a 100-fold greater signal-to-noise ratio. They concluded that CNT-modified arrays have broad applicability in both basic neuroscience research and preclinical studies of neural diseases, including studies of network properties in epilepsy in addition to the screening of potential therapies against different neuropathological conditions (Suzuki et al., 2013). Most recently, David-Pur et al. investigated a full CNT-modified MEA for electrical recording and stimulation. These flexible devices were fabricated using standard lithographic processes and CVD for multi-walled CNT deposition onto a polymeric support. In their study, they analysed the electrical activity of embryonic chick retina (day 14) and measured clear spontaneous activity waves. Additionally, they were able to observe an electrical response following stimulation protocols achieved with currents as low as 4 μA and a pulse width of 1 ms. They concluded that flexible CNT-MEA devices could match classical rigid counterparts with added benefits for possible implantable applications. They further suggested their fabrication process for developing entangled yet porous bundles of CNTs make their device particularly optimal for neuronal applications and a promising advancement in MEA technology (David-Pur et al., 2014).

MEAs have also been investigated as high quality, chronic *in vivo* neural interfaces and the nano-modification techniques described for external sensors have found increasing use for improving implantable MEA devices (Kipke et al., 2008). Keefer et al. reported the use of CNTs covalently attached to either amine-functionalised gold electrodes or electropolymerised with the conductive polymer polypyrrole to the electrode surface. Both strategies were used for *in vivo* study of the rat motor cortex and the monkey visual cortex and showed reduced impedance and noise, enabling simultaneous measurements of local field potentials and spike activity from the same electrode site (Keefer et al., 2008). Recently, Parker et al. characterised the long-term performance of a CNT coating applied to a chronic intracortical MEA, used for both microstimulation and recording, in order to determine the enhanced electrical properties and ensure that CNTs do not adversely affect long-term MEA performance. Their results showed that the performance of CNT electrodes, over several months of intracortical implantation and microstimulation, was similar to internal platinum wire controls. They concluded that CNT-based electrodes could be useful for *in vivo* studies of neuronal activity in addition to potential applications in chronic recording and microstimulation for neural prosthetic devices (Parker et al., 2012). A different approach by Musa et al. describes a bottom-up silicon dioxide (SiO_2) fabrication process with integration of wafer-scale CNT microelectrodes in next generation neural probes for both recording and stimulation capabilities. Their results demonstrated a fabrication process that supported electrode electrochemical performance and mechanical durability during insertion into the rat brain and presented an innovative approach towards developing neural probes for high-resolution neural recording and stimulation (Musa et al., 2012).

3.1.2. Nanoelectronic sensors

While we have shown the significant potential of nano-modified MEAs as substrates for studying neuronal activity, they are still limited in their ability to record information from subcellular structures and subthreshold changes in electrical activity. This often results in complicated post-processing in order to identify individual neuronal signals and reduce error (Abdoun et al., 2011). Further bioelectronic devices made with materials entirely in the nanoscale have been developed providing unique advantages over nano-modified MEAs due to their capacity for subcellular recording probes and precise cell-electrode coupling.

Nanowire field-effect transistors (NWFETs) comprising chemically synthesised semiconductor nanowires as functional channels, increasingly represent an effective method for subcellular recording between biosensors and biological systems (Duan et al., 2013). The earliest use of NWFETs for extracellular recording of neural tissue involved the use of surface patterning of poly-L-lysine onto NWFET device sensors. Cultured rat cortical and hippocampal neurons were selectively grown on NWFET surfaces ensuring precise neuron-nanowire coupling and efficient interfacing. The study demonstrated high resolution recording of action potential signals from individual neurites and the ability for efficient multiplexed recordings from single neurons (Patolsky et al., 2006). A similar study by Qing et al. described the development of silicon nanowire field-effect transistor (Si-NWFET) arrays fabricated on transparent substrates which were reliably interfaced to acute brain slices. The NWFET arrays were designed to record across a range of length scales and allowed imaging of individual cell bodies and the identification of areas of healthy neurons at both upper and lower tissue surfaces. Their results suggested that the NWFET array devices could be used as a promising tool for *in vitro* recordings due to the small device feature size, biocompatibility, and nanoscale

morphology, thus promoting better attachment of active neurons (Qing et al., 2010). Recently, Qing and colleagues reported the further development of free-standing kinked nanowire probes as an improvement over standard patch-clamp techniques. They express overcoming the limitations of CNT devices through the capacity for targeted intracellular recording in three dimensions. Their study describes the development of kinked silicon nanowires mounted on free-standing probe structures; allowing for submicron precision of target cells and real-time recording of neuronal action potential amplitude and temporal properties. They suggest their approach could be scaled up to provide novel nanoelectronic probes with a broad range in electrophysiology and bioelectronics (Qing et al., 2014).

Taking a different approach, Ferguson and colleagues developed nanowires grown over microwire electrodes for intracellular recording of action potentials within rat hippocampal slices. Their results indicated improved recording capabilities of intracellular neuronal activity that could allow for extensive recording of chronic activity from intact neural tissues and mammalian brains. They suggested that the development of a nanoelectrode platform with supporting microwire structures could be well suited to *in vivo* studies of neural tissue (Ferguson et al., 2012). Additionally, Angle & Schaefer demonstrated how engineered nanoelectrodes could be used to record neuronal transmembrane potentials in brain tissue without causing physiological disruption. They describe the fabrication of Solid-Conductor Intracellular NanoElectrodes (SCINEs) and their use in recording neuronal potentials in hippocampal tissue. Performing simultaneous whole-cell patch recordings, they showed that SCINEs could record both neuronal action potentials as well as slower subthreshold potentials without altering cellular properties. They suggested a key role for their device as more suitable over chip-based nanotechnologies for *in vivo* electrophysiology and that such a system could lead to long-term intracellular electrical recordings that are free of the fundamental limitations of existing recording methods (Angle and Schaefer, 2012).

4. Future perspectives

In this review we have highlighted some converging applications between nanotechnology and neuroscience with specific focus on how these technologies could further our understanding of CNS function and the progression of CNS disorders. Basic research provides evidence of a common basis of neural circuit damage and/or dysfunction across a wide range of CNS disorders ranging from neurodevelopmental to neurodegenerative. However, it remains unclear how gene function, synapse development, and circuit physiology effectively map onto patient symptomology at a clinical level (Stoeckli, 2012). In order to bridge this gap, researchers require enhanced tools to study functional CNS development both structurally and physiologically in order to identify mechanisms that lead to devastating and debilitating disorders.

Advancements in nanodiagnostic neuroimaging have shown significant potential to revolutionise how the brain is visualised. The ability to track the progression of CNS disorders at cellular and molecular levels has considerable benefit for clinical disease monitoring. We have highlighted the increasing role of iron oxide nanoparticles as front-runners for developing multifunctional nanoplatforms for improved diagnostic whole-brain imaging. We also described several significant improvements of iron oxide nanoparticles over the high-cost, low sensitivity and low-spatial resolution of its more conventional counterparts. In addition, we illustrated the recent developments in molecular imaging techniques using organic nanoparticles and quantum dot applications for visualising *in vivo* molecular pathways. We envision that the combination of these nanoplatforms will significantly enhance how researchers and clinicians can quantitatively monitor the structure and function of CNS activity as well as molecular pathways associated with CNS disorders, pioneering the discovery of novel biomarkers for better diagnosis and more targeted treatment opportunities (Blumling Iii and Silva, 2012). However, the *in vitro* and *in vivo* mechanisms of nanomaterial toxicity related to size, shape, charge and surface chemistry (Aillon et al., 2009) will need to be taken into account, signifying the importance of reliable characterisation of nanomaterial properties as a key element in this endeavour (Hole et al., 2013). Further studies on biodistribution, pharmacokinetics, and local and systemic toxicity will need to be addressed, and while the impulse to continuously pursue new applications is exciting, it must be followed up by detailed toxicity evaluation. Synthesis strategies will need to attain non-toxic, biocompatible and biodegradable nanoparticles with better targeting and increased signal-to-noise ratio. Additionally, nanoparticles must demonstrate large-scale reproducibility with a high value-to-cost ratio. However, even with these reservations in mind, the future of nanoparticles for neuroimaging remains bright with a strong potential for translation into clinical diagnostics.

Comparatively, we also highlighted the role of nanotechnology in the development of next-generation biosensors for neuronal modelling and electrophysiology. We explored how feature size plays a significant role in sensor functionality; from surface properties affecting cell adhesion and network formation, to the development of CNT-based MEAs and flexible nanoelectronic arrays for *in vivo* applications. We highlighted how engineered *in vitro* neuronal networks grown on nanomaterial scaffolds can be used to investigate circuit physiology, focusing on their applications for improving basic research. Additionally, we addressed the development of nanosensors for direct intracellular interaction with CNS tissue, opening up several opportunities for developing interfaces for studying brain development. This capacity for highly sensitive nanoscale probes to record neuronal activity provides a unique opportunity to have a profound impact on basic and clinical research (Duan et al., 2013). The integration of these nanosensors with genome analysis, iPSC disease models and advanced imaging of individual patients may provide an excellent translational approach to explore the dynamics of gene expression with synapse development and circuit physiology. While still in relatively early stages of development, the field is growing fast and it is possible that new next-generation sequencing devices will take advantage of these nanobiotechnology platforms. However, as with neuroimaging, there remain issues with cytotoxicity (Zhao and Liu, 2012), especially as applications move towards implantable devices (Bareket-Keren and Hanein, 2012). Further study into the long-term effects of CNT and nanowire interactions with the biological milieu is necessary for efficient development of safe and effective neurotechnologies. Furthermore, comprehensive studies in animal models followed by clinical trials will be required to allow routine use of nanoelectronic devices for clinical applications.

Over the next decade, nanotechnological approaches will continue to play a vital role in neuroscience, not just in the development of highly specific and sensitive imaging probes and biosensor interfaces but also potential treatment strategies (Silva, 2010). Similar advances in other complimentary techniques such as optogenetics will add further value to the capabilities of neuroscience research. Optogenetics in particular has potential synergistic applications with several nanotechnologies, presenting an alternative approach for sensory and electrical stimulation with the capability to surpass the specificity and versatility of stimulating metal electrodes (Zhang et al., 2007). Light-activated molecules such as channelrhodopsin or halorhodopsin can allow for targeted stimulation of neurons and their compartments with

even greater spatial, temporal and genetic specificity (Scanziani and Häusser, 2009). Specifically, optogenetics provides further advantages to conventional neuronal stimulation by allowing the individual study of activity-dependent expression of distinct neuronal populations on network development (Zhang et al., 2008). Such advantages can allow researchers to precisely determine patterns of connectivity and circuit dynamics of functional neuronal networks and presents a novel avenue for convergence with the modelling and recording capabilities of biosensor-based studies.

By accelerating the growth and application of innovative nanotechnological solutions for neuroimaging and electrophysiology, researchers will be able to fully construct dynamic pictures of brain development and physiological function. This has the potential to open up vast new territories in the search for more effective treatments for CNS disorders driven by multidisciplinary research and a high demand in the neuroscience community for improved tools with better diagnostic precision. The recent announcements of the Human Brain Project and BRAIN Initiative in Europe and the USA respectively, point to an awareness of the need to refocus efforts on utilising such advancements in technology to help understand the fundamental processes underlying brain function (Devor et al., 2013).

There is little doubt that there are still several challenges that must be overcome to fulfil the promise of nanotechnological applications in neuroscience. Investigating the safety and biocompatibility of nanomaterials remains a key research area and care must be taken to understand and avoid any potential hazards. However, there is a growing body of evidence to suggest that the promise of nanotechnologies outweigh their perils and that the next decade will present huge scope for developing and delivering truly transformational technologies.

Conflict of interest

The authors declare no conflict of interest on the content of this review.

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