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REVIEW

Neuromodulation: selected approaches and challenges

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Abstract

The brain operates through complex interactions in the flow of information and signal processing within neural networks. The 'wiring' of such networks, being neuronal or glial, can physically and/or functionally go rogue in various pathological states. Neuromodulation, as a multidisciplinary venture, attempts to correct such faulty nets. In this review, selected approaches and challenges in neuromodulation are discussed. The use of water-dispersible carbon nanotubes has been proven effective in the modulation of neurite outgrowth in culture and in aiding regeneration after spinal cord injury *in vivo*. Studying neural circuits using computational biology and analytical engineering approaches brings to light geometrical mapping of dynamics within neural networks, much needed information for stimulation interventions in medical practice. Indeed, sophisticated desynchronization approaches used for brain stimulation have been successful in coaxing 'misfiring' neuronal circuits to resume productive firing patterns in various human disorders. Devices have been developed for the real-time measurement of various neurotransmitters as well as electrical activity in the human brain during electrical deep brain stimulation. Such devices can establish the dynamics of electrochemical changes in the brain during stimulation. With increasing application of nanomaterials in devices for electrical and chemical recording and stimulating in the brain, the era of cellular, and even intracellular, precision neuromodulation will soon be upon us.

Keywords: carbon nanotubes, connectivity of networks, deep brain stimulation, desynchronization, neurochemistry.

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Abbreviations used: AD, Alzheimer's disease; ATP, adenosine triphosphate; CFM, carbon-fiber microelectrode; CNF, carbon nanofiber; CNS, central nervous system; CNT, carbon nanotube; CR, coordinated reset; DBS, deep brain stimulation; EEG, electroencephalography; HF, high-frequency; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MWCNT, multi-walled CNT; NASA, National Aeronautics and Space Administration; PABS, poly-*m*-aminobenzene sulphonic acid; PD, Parkinson's disease; PEG, polyethylene glycol; PEI, polyethyleneimine; SCI, spinal cord injury; STDP, spike timing-dependent plasticity; STN, subthalamic nucleus; SWCNT, single-walled CNT; TQ, tinnitus questionnaire; VDCC, voltage-dependent Ca^{2+} channel; WINCS, wireless instantaneous neurotransmitter concentration sensing.

Neuromodulation is a bold and 'heady' concept. It is one thing to interact with the nervous system by making a lesion to mitigate the effects of a seizure focus, for example, or even to place an electrode with a stimulation effect that mimics a carefully placed lesion. It is another 'one thing' to give a drug that interacts with receptors throughout the brain, hoping that the beneficial effects (on a person's depressed mood, for example) outweigh the side effects (which might even include suicidal tendencies). One technique is an electrical sledgehammer, and the other is a pharmacological tsunami.

It is quite something else to persuade a suboptimally performing nervous system to abandon its counter-productive habits and resume the finely-orchestrated concert of electrochemical events that make for a productive, fully functional human being, physically and emotionally. Neuromodulation in its ideal form attempts just that.

For neuromodulation to succeed in that ambitious goal of electrochemical persuasion requires knowledge of all aspects of neuroscience. One must combine knowledge of both neurons and glia, in health and disease, in infancy and dotage. The nuances of nervous system communication (both electrical and chemical, both in the 'contact neighborhood' and many synapses away) must all be understood. Neuromodulation is the pre-eminent multidisciplinary challenge.

The 'Neuromodulation Brainstorming Retreat' held in Carmel, California, March 23–25, 2012, was an initial attempt to bring together various experts in very disparate aspects of nervous system structure and function, but each of them with a keen interest in developing neuromodulation principles in the laboratory for eventual clinical practice. Levels of analysis ranged from the cellular to the organism, while disciplinary coverage induced mathematics, physics, engineering, biology, and medicine. The new and exciting results presented at that retreat prompted the present review, which considers selected approaches for neuromodulation as well as the challenges and future directions in this rapidly evolving field.

Several areas relevant to a broad definition of neuromodulation are not considered here. Although we discuss some new and exciting potential ventures in neuromodulation using carbon nanotubes (CNTs) as advanced materials, our main goal is to address the shortcomings of contemporary deep brain stimulation (DBS) and present a scenario for neuromodulation that restores dysfunctional neural activity to a functional state using permanently implanted devices. This is not to say that techniques to investigate brain electrical and chemical activity (e.g., magnetoencephalography and functional magnetic resonance imaging) and techniques to non-invasively stimulate the brain (e.g., transcranial electrical stimulation and transcranial magnetic stimulation) are not relevant. However, such techniques are not feasible (at the present, at least) for long-term use in clinical settings. One might use the analogy of hybrid versus plug-in electric automobiles. If a neuromodulatory device to control refractory seizures required constant (or even daily) transcranial magnetic stimulation, the patient would either need to go to 'Transcranial Magnetic Stimulation Charging Station' every day or carry around a portable transcranial magnetic stimulator as a constant companion (and be able to use it appropriately to ensure effectiveness). We feel that for more life-threatening conditions such as refractory epilepsy and advanced mood disorders, the neuromodulatory device must be self-contained and, ideally, totally implanted; for other debilitating but not life-threatening disorders such as tinnitus, an external device would be acceptable.

We deliver our selected topics starting with the discussion of basic discoveries that have the least translational promise at present and then transition to practical applications used in clinical work. First, we discuss modulation of neurite outgrowth in neuronal cell culture by using CNTs as water-dispersible agents. This approach is instrumental in a translational application, that is, it can be used to aid regeneration after spinal cord injury *in vivo*. We also discuss a potential use of CNTs for the modulation of morpho-functional characteristics of astrocytes. The sobering reality, however, is that CNT applications are not ready for the prime time in terms of clinical applications due to lack of chronic toxicity data. We then discuss the dynamics of connectivity between neural circuits at intercellular level along with a presentation of its geometric mapping. This computational biology approach is important in the treatment of dysfunctional neural circuits in various human pathologies and can be readily applied in translational approaches. We then present applications that are used in clinical practices. Hence, we discuss novel and sophisticated mathematical models of stimulation (broadly defined as electrical, mechanical, and potentially pharmacological) used to coax 'misfiring' neuronal circuits to resume productive firing patterns. Finally, we discuss the incorporation of neurotransmitter data into our understanding of the effects of traditional electrical stimulation of the nervous system.

Water-dispersible carbon nanotubes as modulators of neuronal and astrocytic growth

Carbon nanotubes have emerged as one of the promising nanomaterials that can be used in neuroprosthetics. We focus our discussion on CNT applications as water-dispersible agents, an approach that has been used to modulate neurite outgrowth in cell culture and also to aid regeneration after spinal cord injury *in vivo*; they can also modulate morpho-functional characteristics of astrocytes in culture. An emerging picture is that CNTs are neural-compatible injectable agents which may find future utilization in medicine.

The first evidence for the applicability of CNTs as water-dispersible modulators of neuronal growth came from the work on cultured neurons (Ni *et al.* 2005). Here, a strategy was to generate CNTs that are dispersible in aqueous media of extracellular space of the brain, so that they could be delivered as a diffusive agent to affect neurite outgrowth. Single-walled (SW)CNTs were functionalized with either polyethylene glycol (PEG) or poly-*m*-aminobenzene sulphonic acid (PABS) to render their dispersability in aqueous media. At physiological pH of extracellular space ($\sim$7.4), SWCNT-PEG and SWCNT-PABS graft copolymers are neutral and zwitterionic, respectively.

Neurons, obtained from dissociated hippocampi of 0- to 2-day old Sprague–Dawley rats, were plated onto glass coverslips pre-coated with polyethyleneimine (PEI), a cationic polymer commonly used to grow neural cells in culture. As expected, PEI was a permissive planar substrate for neuronal growth. Neurons grown on PEI substrate were treated with SWCNT-PEG and SWCNT-PABS. Using calcein-loaded hippocampal neurons and fluorescence microscopy (Fig. 1a–c), it appeared that neurons treated with either form of dispersible SWCNTs showed a reduced number of neurites and growth cones when compared with control (sham treated with the vehicle) neurons. Coincidentally, neurons treated with water-dispersible SWCNTs also showed longer neurites than controls (Fig. 1d). A mechanism underlying this enhancement of selected neurite outgrowth was through SWCNT-PEG action on reducing Ca^{2+} influx from the extracellular space through voltage-dependent Ca^{2+} channels (VDCCs). This was determined by using the intracellular calcium indicator fluo-3 and fluorescence microscopy. Neurons were depolarized with high extracellular potassium ions (50 mM) to open voltage-dependent Ca^{2+} channels, which allowed Ca^{2+} entry from the extracellular space into the cytosol. When compared with control, neurons treated with SWCNT-PEG had reduced cytosolic Ca^{2+} accumulation due to depolarization-dependent Ca^{2+} entry. An increase in neuronal intracellular Ca^{2+} levels can regulate plasma membrane/vesicular recycling, which has been implicated to play a role in the rate of neurite elongation (Zakharenko and Popov 2000). Consequently, Malarkey *et al.* (2008) examined whether SWCNTs could affect membrane recycling using the fluorescent dye *N*-(3-triethylammoniumpropyl)-4-(4-(dibu-

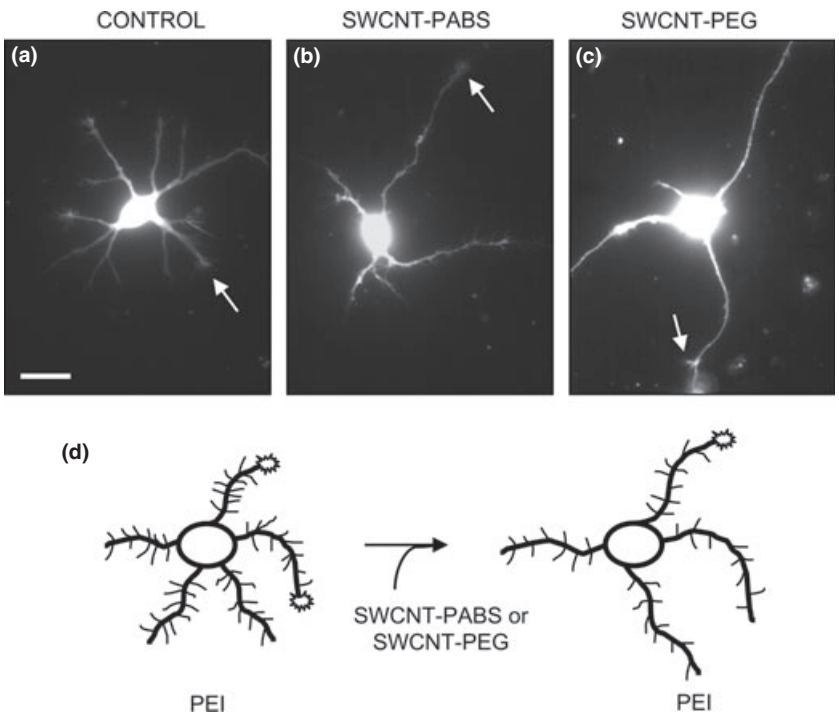

Fig. 1 Chemically functionalized water-dispersible single-walled carbon nanotubes (SWCNTs) added to the culture medium modulate neurite outgrowth. Fluorescence images of live hippocampal neurons, accumulating the vital stain calcein. Neurons grown on polyethyleneimine (PEI)-coated glass coverslips (a; control, sham treated) can be treated with water-dispersible CNTs, either SWCNT-poly-*m*-aminobenzene sulphonic acid (PABS) (b) or SWCNT-polyethylene glycol (PEG) (c) to affect their growth characteristics. Arrows indicate growth cones. Scale bar, 20 μm. (d) Drawing summarizing the effects of water-dispersible SWCNTs on neurite outgrowth and growth cones. Water-dispersible SWCNT-PABS or SWCNT-PEG graft copolymers, when added to the culturing medium of neurons grown on PEI substrate, increased the length of selected neurites and reduced the number of growth cones. PABS, poly-*m*-aminobenzene sulphonic acid; PEG, polyethylene glycol; PEI, polyethyleneimine. Modified from Ni *et al.* (2005).

tylamino)styrl)pyridinium dibromide (FM 1-43) which is taken up by cells through endocytosis. They found no significant differences in the dye load in neurons at rest that were exposed to the different concentrations of SWCNT-PEG or sham treated, indicating that constitutive membrane recycling was not affected by SWCNT-PEG. However, SWCNT-PEG inhibited depolarization-dependent load of the dye (Malarkey *et al.* 2008), with subsequent experiments indicating that such inhibitory action was preferentially affecting regulated endocytosis. Therefore, the exocytotic incorporation of vesicles into the plasma membrane was not balanced by the endocytotic retrieval, in the presence of SWCNTs. This could effectively cause the increase in neurite length observed by Ni *et al.* (2005), while SWCNTs' effect on the reduction of the number of neurites could be then a compensatory cellular mechanism to keep the cell surface/volume relatively constant. The reduction of depolarization-dependent Ca^{2+} accumulation (Ni *et al.* 2005) and the inhibition of regulated endocytosis (Malarkey *et al.* 2008) both showed concentration-dependence on SWCNTs, which corresponded well to SWCNT-PEG concentrations affecting neurite numbers and outgrowth (Ni *et al.* 2005). Taken together, these results suggest the exciting possibility that water-dispersible SWCNTs could be delivered locally to the site of CNS injury to enhance neurite outgrowth, which might increase the probability to overpass the site of injury and aid in the process of regeneration.

A possible therapeutic intervention using water-dispersible SWCNTs has been initiated for the treatment of spinal cord injury (SCI) (Roman *et al.* 2011). Traumatic SCI causes tissue damage resulting in the formation of a cavity that inhibits axonal re-growth. Filling this cavity with a growth-inducing agent, such as SWCNT-PEG, could promote regeneration and repair. Here, SCI was caused by complete transection of the spinal cord at the thoracic 9 vertebrae of adult female Sprague–Dawley rats. One week after injury, the epicenter of the lesion was injected with either SWCNT-PEG at various concentrations or the vehicle. Behavioral tests were conducted before injury, before treatment, and once a week until 28 days after treatment. At this juncture, the rats were killed and spinal cord tissue was submitted to histological examination. The addition of SWCNT-PEG was found to decrease lesion volume and promote neurite outgrowth, the latter seen as an increase in neurofilament-positive fibers; there was no effect on reactive gliosis. In addition, SWCNT-PEG treatment offered a modest improvement in hindlimb locomotor recovery without inducing hyperalgesia. These data suggest that injectable water-dispersible, and biologically non-degradable, SWCNT-PEG hold promise as a neurite outgrowth-promoting agent with prolonged actions in the treatment after SCI.

The mechanisms underlying biological actions of the above chemically functionalized SWCNTs are likely mediated simply by physical-chemical characteristics of materials. One caveat of such an approach is that it may lack specificity that neural cells utilize in intercellular interactions. Consequently, it should be important to engineer CNTs that are functionalized with biological molecules that possess ligand-receptor specificity. As a first step toward such a goal, Matsumoto *et al.* (2007) functionalized CNTs using endogenous ligands in the CNS, neurotrophins. Here, nerve growth factor or brain-derived neurotrophic factor was covalently attached to multi-walled (MW)CNTs. The effects of these hybrid materials on neurite outgrowth were studied after dispersing them in culturing media of neurons isolated from dorsal root ganglion of 8-day-old chick embryos. Nerve growth factor -multi-walled CNT (MWCNT) or brain-derived neurotrophic factor-MWCNT prompted neurite outgrowth, which was comparable to that caused by the respective soluble neurotrophins. Thus, neurotrophins covalently attached to MWCNTs retained their bioactivity. Future experiments will have to be carefully designed to assess whether CNTs co-functionalized with neurotrophins and organic compounds like PEG could, as multi-hybrid nanomaterials, prove advantageous by offering additive promoting effects on neurite outgrowth in culture and *in vivo*.

Thus far, we have only discussed the use of water-dispersible CNTs to affect neuronal growth. However, neurons in the brain are accompanied by glial cells. The total quantity of neural cells in the brain of higher primates, including human, is not known precisely. However, it is likely that the human brain contains as many as several hundreds of billions (10^{11}) of neurons, with similar or higher numbers of glial cells. Thus, the effects that water-dispersible CNTs might have on various types of glial cells are critically important to understand not only because they roughly represent half of the brain but, also because there are manifold bidirectional interactions between neurons and glia (Hatton and Parpura 2004; Parpura and Haydon 2009) that could represent a fertile ground for medical intervention. Recently, it has been shown that SWCNT-PEG and SWCNT-PABS modulate morphological and functional, that is, biochemical, characteristics of astrocytes (Gottipati *et al.* 2012). When added to the culturing medium, SWCNTs were able to make live cortical astrocytes, grown on PEI-coated glass coverslips and loaded with calcein, larger and stellate/mature, as determined by quantitative assessment of the area, perimeter and form factor (a measure of the roundness of an object/cell). Astrocytes treated with SWCNTs showed elongated cell bodies along with an increased extension of processes, which was evidenced as an increase in the area and perimeter values along with a decrease in the form factor. These changes are consistent with morphological maturation of these glial cells. In addition to this morphological plasticity, astrocytes treated with SWCNTs showed change in their functionality based on the increased levels of the astrocyte-specific marker glial

fibrillary acidic protein, as determined by immunocytochemistry (Gottipati *et al.* 2012). It is then tempting to speculate that SWCNTs could be used to affect the course of, for example, familial Alzheimer's disease (AD). A recent study on a transgenic mouse model of a hereditary form of AD showed that astrocytes in the entorhinal cortex of these animals undergo atrophy at the very early ages (Olabarria *et al.* 2010; Kulijewicz-Nawrot *et al.* 2012; Yeh *et al.* 2012). Thus, a conceivable therapeutic strategy to slow down the progression of the AD could be to use SWCNTs on the brain during the early onset of AD.

Beside the above presented body of work on CNT usage, CNTs can also be used in electrodes for neural interfaces (Lee and Parpura 2009) and as a tool for targeted drug delivery (Klumpp *et al.* 2006). However, at present, it is unclear how CNTs would find ways to clinical applications due to lack of safety and effectives data for chronic implantation of CNTs in the brain. An overview on contradicting reports dealing with CNT biocompatibility and toxicity found in the literature is available elsewhere (Kaiser *et al.* 2011). Briefly, most of the CNT toxicity studies were done using non-neural cells or cell-lines *in vitro* reporting that the exposure to CNTs increased oxidative stress and cell death (Cui *et al.* 2005; Jia *et al.* 2005; Manna *et al.* 2005; Monteiro-Riviere *et al.* 2005; Bottini *et al.* 2006; Magrez *et al.* 2006). It has been proposed that these negative effects could be circumvented by using CNTs which surface is grafted with gallic acid, an antioxidant triphenol (Cirillo *et al.* 2011). Furthermore, the SWCNT suspension caused acute toxic effects, evidenced by a reduction in DNA content and number of cells, in primary cultures of glia from the CNS (spinal cord) and peripheral nervous system (dorsal root ganglion), while only peripheral nervous system neurons were affected by CNTs (Belyanskaya *et al.* 2009). In contrast, CNTs were proven biocompatible with neural cells (Dubin *et al.* 2008), their toxicity did not appear to be a concern in systemic applications (Liu *et al.* 2008), and CNTs as biomaterials had comparable basic safety properties to those of tattoo inks (Hara *et al.* 2011). Thus, it is abundantly clear that further evaluation of CNT effects on cells/tissue must take place. However, a more systematic approach will be needed to address acute and long-term effects that CNTs as injectable materials may have on the brain and the whole living organism to establish safety guidance for their use. Toxicity is less likely to be of such grave concern when the carbon nanotubes/nanofibers are attached to electrodes and are coated with a conducting polymer of proven safety *in vivo,* such as polypyrrole (Keefer *et al.* 2008) or polyimide (Chang *et al.* 2012).

As nanotechnology further advances, there should be various additional materials generated that together with CNTs could aid our ability to repair the loss of brain function due to injury. At the present time CNTs, and many other nanomaterials, are mainly under investigation in research laboratories. Widespread commercialization of CNTs is expected in the near future and consequently the exposure of the general populace to this material. However, this must not occur without prior adequate testing to establish exposure guidelines and safety regulations.

Challenges associated with mapping the causal dynamic connectivity of cellular neural networks

It is a generally accepted assumption that complex interactions in the flow of information and signal processing within networks composed of large numbers neural cells, specifically neurons and astrocytes, presumably result in emergent systems-level phenomena responsible for how neural information is represented and processed. Changes in the structure of cellular and higher organizational networks are associated with pathophysiology of several neurological disorders, including for example AD (Grady *et al.* 2001; Delbeuck *et al.* 2003, 2007; Greicius *et al.* 2004) and schizophrenia (Friston and Frith 1995; Friston 1998; Tononi and Edelman 2000). One of the things that is so fascinating about the mesoscale associated with cellular neural networks in the brain is precisely the idea of emergent systems-level phenomena from the often well understood and more deterministic molecular and cellular processes that make up each cellular component in the network. There is now considerable evidence to suggest that from a dynamical systems perspective, the brain is never static in the context of neural activity and cell signaling (see, e.g., McKenna *et al.* 1994; Hoyer 1997; Werner 2007; Beggs 2008; Bullmore *et al.* 2009; Chialvo 2010). The activity of the brain is in a constant state of flux near critical threshold state transitions where dynamical mechanisms such as sensitive dependence on initial conditions, and positive and negative signaling feedback, can quickly, and often unpredictably, shift the state of neural activity, and therefore what the brain is doing any given moment in time. A quantitative analysis of this dynamics in the context of the available experimental data (i.e. analyses and theories that can both explain and predict observable measurements) is the only way we will understand such neural dynamics. In many ways, despite the very rich history and tradition of neuroscience, this is very much in its infancy and we are just beginning to understand the right questions to ask, let alone develop the methods to answer them or arrive at meaningful answers. This will necessarily require pursuits at the intersections between neuroscience, mathematics, physics, and engineering. This has implications not just for understanding how the brain works, but also for understanding how it breaks down in disease and what we can do to correct it. Approaches such DBS and nanotechnologies such as recording and stimulation with carbon fibers as discussed below in this article will, in different ways, depend on this.

The physiological behavior and outputs of a neural cell network is dependent on both its dynamic topology and the dynamics of the individual cells that make it up (Born and Bradley 2005; Cannon and D'Alessandro 2006; Bokde *et al.* 2009; Oberheim *et al.* 2009; Buibas and Silva 2011; Sporns 2011). Within such a network, there are two topologies, or patterns of connectivity: (i) a static structural, that is anatomical, topology, that describes all the physical connections within the network; and (ii) a dynamic topology, that represents how signals and information propagate through the network's fixed structural topology (however, for examples of structural dynamics of brain connections, see Hatton 2004). The structural topology of a network constrains the range of possible dynamic states and provides computable bounds on the network's dynamics. Put in simple terms, if two cells, that is, nodes, in a network are not 'physically' connected, the two nodes cannot signal each other and no information can possibly flow from one to the other (or in more technical terms if two vertices in the graph theoretic model that represents the network have no edge between them). Note, however, that in neural networks and cellular networks, more broadly the 'physical' connectivity or topology may not necessarily require physical contact between cells. In the classical picture of neuronal networks, this is not the case, in the sense that one assumes that neurons can only communicate with each other if they are chemically (synaptically) or electrically (through gap junctions) connected, that is, communication does require physical contact between neurons. But intercellular signaling in networks of other cell types need not require physical connections between them. Paracrine signaling via the diffusion of a signaling molecule can act as the link between two nodes in the network. For example, this is the case with the diffusion of ATP that underlies intercellular calcium waves in astrocyte and other related glial cell networks. Intracellular calcium transients and resultant waves inside the cell lead to the release of ATP which then diffuses to surrounding astrocytes and propagates the signal (Kim *et al.* 1994; Araque *et al.* 1999; Scemes 2000; Fields and Stevens-Graham 2002; Scemes and Giaume 2006; Macdonald *et al.* 2008; Fiacco *et al.* 2009; Yu *et al.* 2009; Verkhratsky *et al.* 2012). Nonetheless, though, there is an upper bound to the distance such a signaling molecule can diffuse before it becomes so diluted, it has no effect on a downstream cell, and this distance then represents the maximum 'length' a link can take that connects two nodes in the network (Arcuino *et al.* 2002). From an analysis perspective, such networks provide additional complicating theoretical considerations.

In the systems neuroscience literature, a distinction is often made between the functional connectivity of a dynamic neural network and its effective connectivity (Friston 1994; Bullmore and Sporns 2009; Sporns 2011). Functional connectivity implies a statistical correlation between the activation of one cell and another, but not necessarily causation in the sense that one cell is responsible for, or the cause of, the activation of the other. Statistical correlations mathematically do not infer causation, only that two events occur more frequently than would be expected by chance. Beyond cellular networks, functional connectivity is often inferred or proposed at higher anatomical and structural levels of organization at the scale of the whole brain using functional magnetic resonance imaging methods. Effective connectivity, on the other hand, invokes the much stronger statement of causation between cells in a network and implies that one can explain and predict how and when one cell in the network causes a response in another using appropriate mathematical and computational descriptions.

Functional and effective connectivity topologies are subsets of the structural topology and vary depending on the details of how the network is specifically wired up (i.e. the details of its structural connectivity), the internal dynamics of individual cells in the network, and the details about how and when and where the network is stimulated. Just because two cells are 'physically' connected in a network does not mean that one has to necessarily signal the other every time a signaling event propagates through the network or through either one of the cells. Whether one cell causally produces an activation of the second depends on a temporally and geometrically intricate interplay between the speed at which signaling is occurring, the distance and geometry of the 'physical' connection between them, the strength of the connection between them (e.g., synaptic weights), and the instantaneous internal state of each cell. Subtle changes in even one of these variables, with everything else in the network left untouched, can have dramatic effects on the effective connectivity and overall behavior of the network (Fig. 2).

The above considerations present some very challenging conditions for methods that hope to identify or map effective connectivity from experimental data. For example, the signaling events that propagate through a given structural topology to produce a specific instance of effective connectivity may be nearly unique or at least not robustly reproducible even given the exact same stimulus and recording conditions. This means that a specific dynamic network may be observable only once as a signal propagates through it. The effective connectivity or topology may itself be dynamic and change during the course of observation if the network is responding to a perturbation or other stimulus. And noise and unknown variables such as unknown inputs into an open network can severely limit experimental measurements and observation of the dynamic network. Any computational method that aims to map effective connectivity must take these considerations carefully into account, and it must be grounded in appropriate theory beyond simply numerical examples or validation with subsets of data if the method is to be trusted. This is critical because the interpretation of any subsequent results completely relies on the method providing accurate and meaningful mappings,

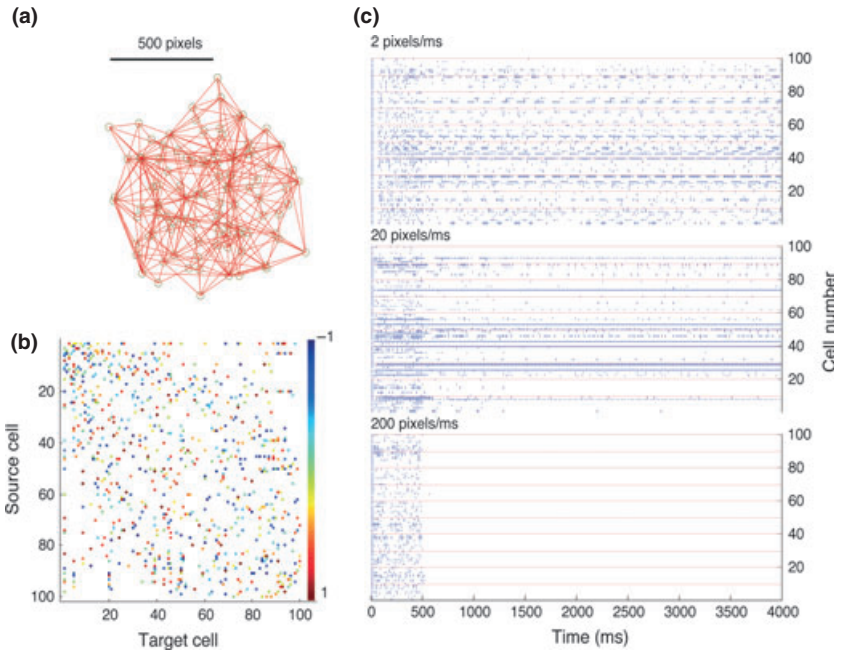

Fig. 2 Effects of signaling speed on network dynamics. (a) The three-dimensional geometric network is assigned random weights uniformly distributed between −1 and 1 on each physical edge. (b) An Izhikevitch model of bursting neurons was used to model the individual vertex dynamics. By varying the speed of signal propagation between cells, signaling delays have a critical impact on the resultant spike dynamics. (c) In recurrent networks, delays serve as a form of signal storage, essentially giving cells time to recover from a refractory period between activations, which in turn maintains recurrent signaling propagation well beyond an initial stimulus (500 ms in this example). If, however, the signaling speeds are too fast, incoming signaling from upstream cells never has an opportunity to activate downstream cells because they are still refractory and do not respond. This leads to signaling in the network quickly dying away and not being sustained without it being driven by an external stimulus, as is the case with speeds of 200 pixels/ms in this example. With more physiological signaling speeds, central pattern generator-like patterns emerge (see the 2 pixels/ms plot). Reproduced with permission from Buibas and Silva (2011).

for example, attempting to explain a neurobiological behavior or output from the network or organism or test a hypothesis based on an analysis of causal effective connectivity. Framed in this context, it quickly becomes evident why so few methods exist to do this and why those that do tend to be in the earliest stages of research. It is hard enough to develop appropriate and validated methods that provide structural connectivity information at the single cell level, that is, the cellular connectome. Attempting to develop validated theoretical and computational methods that can provide the temporal and spatial evolution of causal dynamic connectivity as signals and information flows through structural networks based on single observations seems almost overwhelming. Yet, it is hard to see how systems neuroscience and experimentally testable theories about how the brain works can move forward in their absence.

Desynchronization of neural circuits: an approach to the treatment of brain disorders

Abnormal neuronal synchronization severely impairs brain function. In fact, pathological synchrony is a hallmark of several neurological and psychiatric diseases. We provide a review of coordinated reset (CR) neuromodulation. Based on an explanation of the CR principle, pre-clinical and clinical results are presented.

A number of brain diseases, for example, movement disorders such as Parkinson's disease (PD), are characterized by abnormal neuronal synchronization (Nini *et al.* 1995; Llinas *et al.* 1999; Hammond *et al.* 2007). DBS of the subthalamic nucleus (STN) is nowadays an established therapy for late stage PD (Krack *et al.* 2003; Deuschl *et al.* 2006). Classical DBS is a permanent high-frequency (HF) (> 100 Hz) periodic pulse train stimulation, which is delivered through depth electrodes that are chronically implanted in target areas (Benabid *et al.* 1991). HF DBS protocols were empirically developed (Volkmann *et al.* 2006), and the mechanism of action of HF DBS is still not fully understood (Benabid *et al.* 2005).

To overcome the limitations of standard HF DBS, such as side effects or limited therapeutic efficacy (Kumar *et al.* 2003; Volkmann 2004; Rodriguez-Oroz *et al.* 2005), a model-based approach to desynchronizing DBS was developed (Tass 1999). Consecutively, a number of desynchronizing

stimulation techniques have been developed (Tass 2001, 2003a,b; Rosenblum and Pikovsky 2004; Hauptmann *et al.* 2005; Popovych *et al.* 2005; Popovych and Tass 2010), some of them more application oriented than others. We focus here on CR stimulation (Tass 2003a,b), a robust desynchronizing stimulation technique, that aims at a therapeutically modulating synaptic connectivity to unlearn both pathological synaptic connectivity and pathological synchrony (Tass and Majtanik 2006). CR stimulation can be delivered in a closed-loop and an open-loop mode. It requires neither time-consuming calibration, nor technically involved real-time measurements and data processing. CR stimulation, that is, the sequential application of phase resetting stimuli at different sites, counteracts synchronization in the neuronal target population by dividing the entire population into a few mutually phase-shifted subpopulations (Tass 2003a,b). CR stimulation can be realized by different stimulation modalities, for example, electrical stimulation or acoustic stimulation (see below). In this section, the principle of the CR approach and its experimental and clinical applications are reviewed.

Principle of CR-induced anti-kindling

In neuronal populations, changes of dynamics and connectivity are strongly linked (see e.g., Yuste and Bonhoeffer 2004). Synaptic weights are up- or down-regulated by the spike timing-dependent plasticity (STDP), depending on the relative timing of the pre- and post-synaptic spikes (Gerstner *et al.* 1996; Markram *et al.* 1997). Even in simple neuronal networks, multistability emerges due to STDP (Tass and Majtanik 2006; Tass and Hauptmann 2007, 2009; Popovych and Tass 2012). Different stable states (i.e., attractors) coexist which differ concerning both fast neuronal dynamics and slow synaptic dynamics (i.e., connectivity pattern). Stable weakly synchronized or desynchronized states with weak mean synaptic connectivity coexist with stable synchronized states with strong mean synaptic connectivity. In computational studies, it was shown that appropriate stimulation may shift a network from one stable state to another, so that the stimulation effects outlast the cessation of stimulation. Desynchronizing CR stimulation (Tass 2003b) shifts a network from a synchronized state with strong synaptic connectivity to a desynchronized state with weak synaptic connectivity (Tass and Majtanik 2006; Hauptmann and Tass 2007, 2009; Tass and Hauptmann 2007, 2009; Popovych and Tass 2012). Put otherwise, due to CR stimulation the network gets reshaped and unlearns both pathological connectivity and synchrony (Fig. 3).

According to the CR stimulation algorithm (Tass 2003a, b), phase resetting stimuli are sequentially delivered to M, say 4, different subpopulations of the neuronal target ensemble (e.g., in the case of electrical stimulation via M different stimulation contacts), optimally with a delay of T/M between subsequent stimuli. The stimulation period T is optimally chosen close to the mean period of the synchronized neurons. Within one cycle of duration T, each stimulation site is activated once. Particularly favorable for desynchronization is a periodic ON–OFF CR neuromodulation protocol with m cycles ON stimulation followed by n cycles OFF stimulation, where, for example, $m = 3$ and $n = 2$ (Lysyansky *et al.* 2011).

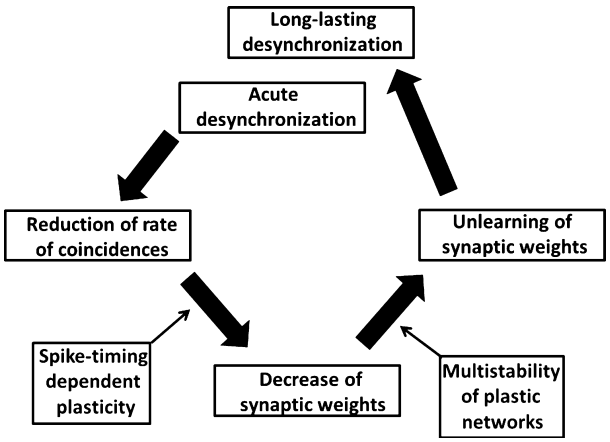

Fig. 3 Schematic illustration of the desynchronization-induced anti-kindling process. Effective desynchronizing stimulation, such as coordinated reset neuromodulation (Tass 2003b), causes an acute desynchronization, that is, a desynchronization during stimulation. Desynchronization reduces the rate of coincidences. Hence, due to spike timing-dependent plasticity, the strength of the synaptic connections decreases. As soon as the neuronal population enters a basin of attraction of a desynchronized stable state (attractor), it spontaneously relaxes into the stable desynchronized state. Desynchronizing stimulation leads to an unlearning of the pathological connectivity and pathological synchrony, so that the neuronal population remains in the desynchronized stable state without further intervention (Tass and Majtanik 2006). In other words, this enables a long-lasting desynchronization after the stimulation is turned off.

In ensembles of spiking and bursting model neurons interacting via excitatory and inhibitory synapses with STDP, it was computationally shown that a reset of neuronal populations as well as the CR-induced desynchronization and the unlearning of pathological connectivity (anti-kindling) can robustly be obtained by means of direct electrical stimulation or by indirect, that is, synaptically mediated, excitatory, and inhibitory stimulation (Popovych and Tass 2012). Based on these computational results, CR neuromodulation has the potential to provide a platform technology. For different diseases, CR might be applicable with different, appropriate stimulation modalities, for example, electrical stimulation via implanted or epicortical electrodes or sensory (e.g., acoustic) stimulation.

Long-lasting CR-induced desynchronization – animal experiments

The acute desynchronizing effect of electrical CR stimulation was experimentally verified in a hybrid neuroelectronic

system of coupled paddlefish electroreceptors (Neiman *et al.* 2007). To study CR aftereffects and, in particular, long-lasting desynchronization, electrical CR stimulation, consisting of sequentially delivered brief electrical bursts, was applied to the low-magnesium model of epileptiform activity in rat hippocampal slice (Tass *et al.* 2009). The low-magnesium model of seizure-like activity is characterized by robust neuronal synchronization (Haas and Jefferys 1984). CR stimulation caused a long-lasting desynchronization between hippocampal neuronal populations together with a long-lasting widespread decrease in the amplitude of the epileptiform activity (Tass *et al.* 2009). In contrast, periodic control stimulation induced a long-lasting increase in both synchronization and local field potential amplitude (Tass *et al.* 2009).

The best characterized model of experimental parkinsonism is the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaque monkey. Pre-clinical studies in that model played an important role in the development of stereotactic therapies of PD. For instance, in MPTP monkeys, it was convincingly demonstrated that an STN lesion (Bergman *et al.* 1990) as well as classical HF STN-DBS (Benazzouz *et al.* 1993) can reverse PD motor symptoms. HF STN-DBS decreases abnormal network activity in MPTP monkeys, but pathological synchrony reappears within seconds after cessation of DBS (Meissner *et al.* 2005). The latter observation corresponds to the fact that in PD patients standard HF DBS has neither long-lasting electrophysiological aftereffects (Kuhn *et al.* 2008) nor long-lasting clinical aftereffects (Temperli *et al.* 2003). Abnormal neuronal synchronization is found in MPTP monkeys (Nini *et al.* 1995; Hammond *et al.* 2007). Accordingly, long-lasting aftereffects of CR neuromodulation of the STN on motor symptoms were studied in MPTP-treated macaque monkeys (Tass *et al.* 2012b). It was shown that CR neuromodulation of the STN has sustained long-lasting aftereffects on motor function in MPTP monkeys. In contrast, long-lasting aftereffects were not observed with classical HF DBS.

Acoustic CR neuromodulation for the treatment of tinnitus

Subjective tinnitus is an acoustic phantom phenomenon, that is, a perception of sound without any physical sound sources (Lockwood *et al.* 2002; Eggermont 2003; Moller 2003; Weisz *et al.* 2005). It is typically initiated by a damage to the peripheral hearing system (Irvine *et al.* 2001; Lockwood *et al.* 2002; Norena and Eggermont 2003; Weisz *et al.* 2006) and characterized by abnormal neuronal synchronization (Llinas *et al.* 1999; Norena and Eggermont 2003; Weisz *et al.* 2005, 2007). Pathologically increased δ wave activity is observed in cortical regions lacking afferent input (Llinas and Steriade 2006; Steriade 2006). Accordingly, magnetoencephalography studies (Llinas *et al.* 1999; Weisz *et al.* 2005, 2007) as well as epidural recordings from the secondary auditory cortex (De Ridder *et al.* 2011) in patients with chronic subjective tinnitus revealed an increase of power in lower frequency bands (δ and θ) and higher frequency bands, especially γ, combined with a decrease of the power in the α band. The close link between increased δ and γ power is a prominent feature of a larger class of brain disorders featuring thalamocortical dysrhythmia (Llinas *et al.* 1999).

The goal of CR neuromodulation is to desynchronize a tinnitus-related synchronized focus in the tonotopically organized central auditory system. To this end, the concept of electrical CR neuromodulation was extended to acoustic CR neuromodulation (Tass and Popovych 2012; Tass *et al.* 2012a). Using the tonotopic organization of the central auditory system, electrical stimulation bursts sequentially applied via different stimulation contacts were replaced by acoustically delivered sequences of tones of different pitch in the vicinity of the dominant tinnitus frequency (Tass and Popovych 2012; Tass *et al.* 2012a). Safety and efficacy of different doses of acoustic CR neuromodulation were studied in the RESET study, a prospective, randomized, single blind, placebo-controlled trial in 63 patients with chronic tonal tinnitus and up to 50-dB hearing loss (Tass *et al.* 2012a). Clinical scores, visual analog scale, and tinnitus questionnaire (TQ), as well as spontaneous electroencephalography (EEG), were recorded. CR treatment turned out to be safe and well-tolerated. Acoustic CR caused a significant decrease of tinnitus loudness and symptoms. According to evaluation studies of visual analog scale (Adamchic *et al.* 2012b) and TQ scores (Adamchic *et al.* 2012c), the CR-induced improvements in visual analog scale and TQ scores obtained in the RESET study were not only statistically significant but also clinically significant. Therapeutic effects achieved in 12 weeks of CR treatment persisted through a preplanned 4-week therapy pause. Moreover, sustained long-term CR effects were observed after 10 months of therapy. Seventy-five percent of patients were responders (with a reduction of at least six TQ points), with a mean TQ reduction of 50% among responders. In the course of the CR therapy, the tinnitus frequency significantly decreased. In addition, CR therapy counteracted the tinnitus-related EEG abnormalities in a network of auditory and non-auditory brain areas (Fig. 4). CR induced a decrease of δ and γ power combined with an increase of α power. Both decrease of the tinnitus frequency and reversal of tinnitus-related EEG power are indicative of CR-induced neuroplastic changes. Hence, acoustic CR neuromodulation causes a significant clinical improvement as well as a significant decrease of pathological neuronal synchronization. In addition, an EEG subgroup analysis showed that the CR-induced change of tinnitus frequency is associated with characteristic EEG changes in the α and, in particular, γ band, indicative of a CR-induced reduction of tinnitus-related auditory binding in a pitch processing network (Adamchic *et al.* 2012a).

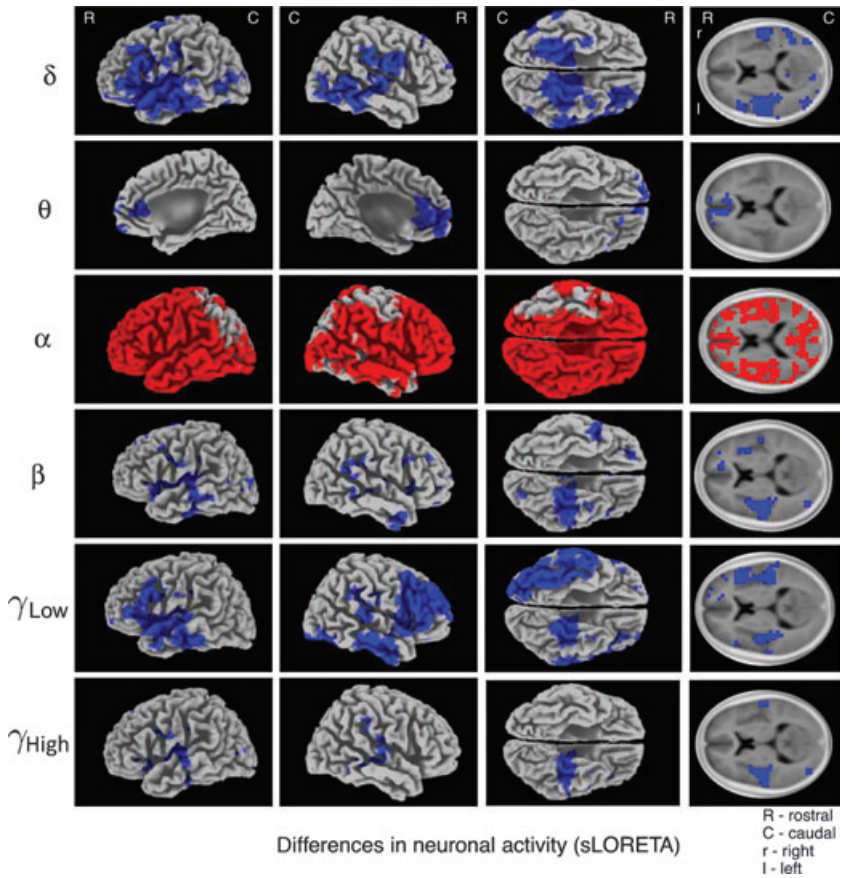

Fig. 4 Effects of 12 weeks of acoustic coordinated reset (CR) neuromodulation on brain oscillations in patients with chronic bilateral subjective tinnitus as revealed by the RESET study (Tass *et al.* 2012a). Spontaneous electroencephalograph was recorded with eyes closed before and after 12 weeks of CR therapy in the off-stimulation state, that is, with the acoustic CR stimulator being turned off for at least 2.5 h prior to electroencephalography recording. Statistical non-parametric maps from standardized low-resolution brain electromagnetic tomography (sLORETA) (Pascual-Marqui 2002) provide the localization of significant CR-induced changes of the current source density power in different frequency bands: δ (1–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–30 Hz), γ-low (30–48 Hz), and γ-high (52–90 Hz). To increase the signal-to-noise ratio, 12 patients with bilateral tinnitus (from all treatment groups, including those with suboptimal daily dose) were selected by means of a tinnitus questionnaire based reliable-change-index (Jacobson and Truax 1991). 3D maps are superimposed onto a horizontal brain section (right column) and onto a three-dimensional brain (first three columns). Statistical significance of sLORETA changes was non-parametrically tested on a voxel-by-voxel basis with a randomization test (Nichols and Holmes 2002). Significantly decreased oscillatory power after CR therapy compared to baseline is labeled blue, while increased oscillatory power is labeled red (corrected, $p < 0.05$). Figure from Tass *et al.* (2012a) reprinted with permission from the authors, copyright by Forschungszentrum Jülich.

In all computational studies, CR neuromodulation turned out to be a stimulation technique which causes an effective desynchronization, not only during stimulation (Tass 2003a, b). In fact, the CR approach aims at an unlearning of pathological connectivity and, hence, at the achievement of long-lasting desynchronization (Tass and Majtanik 2006; Hauptmann and Tass 2007, 2009; Tass and Hauptmann 2007, 2009; Popovych and Tass 2012). Long-lasting desynchronization caused by electrical CR stimulation was verified in animal experiments, in epileptic rat hippocampal slice (Tass *et al.* 2009) and in MPTP monkeys (Tass *et al.* 2012b). Furthermore, long-lasting desynchronization of acoustic CR neuromodulation provides a therapy for patients with chronic subjective tinnitus (Tass *et al.* 2012b).

According to a computational analysis, CR-induced desynchronization and anti-kindling can be achieved by direct stimulation of the neuronal soma or indirect, that is, synaptically mediated, excitatory or inhibitory stimulation (Popovych and Tass 2012). Based on these computational results as well as on the experimental and clinical findings reviewed above, CR neuromodulation may finally prove to be a platform technology. With appropriately chosen stimulation modalities, CR might provide therapies for different brain diseases characterized by abnormal neuronal

synchrony. Indeed, apart from PD and tinnitus, there are several such diseases, for example, dystonia (Chen *et al.* 2006), schizophrenic spectrum disorder, obsessive–compulsive disorder, and depressive disorder (Schulman *et al.* 2011).

Advancing deep brain stimulation technology by human electrochemical detection

Over the past 20 years, significant advances in stereotactic neurosurgical techniques have permitted surgical alternatives for neuropsychiatric disorders, such as ablation surgery and neuromodulation (Nandhagopal *et al.* 2008; Remple *et al.* 2008; Poewe 2009). These technological improvements, combined with increased understanding of neuropsychiatric pathophysiology, have generated marked increase in the application of restorative neurosurgical techniques, such as electrical stimulation of specific brain nuclei, known as DBS. DBS has become increasingly utilized and is now Food and Drug Administration approved for a variety of neurological disorders and targets, such as the STN or globus pallidus internus for PD (Limousin *et al.* 1998; Favre *et al.* 1999; Kumar *et al.* 2000; Rodriguez-Oroz *et al.* 2005), ventral-intermediate nucleus of the thalamus for essential tremor (Benabid *et al.* 1993), globus pallidus internus for dystonia (Greene, 2005), and nucleus accumbens for obsessive-compulsive disorder (Greenberg *et al.* 2006; Lipsman *et al.* 2007).

Despite its well-established clinical efficacy, the mechanism of DBS is incompletely understood. Because ablative surgery is similarly effective for treating movement disorders such as tremor and PD, the stimulation-evoked silencing of pathologically hyperactive neurons was initially postulated as the primary mechanism (Benabid *et al.* 1987; Bergman *et al.* 1990; Patel *et al.* 2003). However, recent studies have reported activation (vs. silencing) of output nuclei (Garcia *et al.* 2005), thereby altering neurotransmission and generating downstream effects within a neural network. The implications of this hypothesis for DBS are that it should evoke changes in neural activity and transmission in interconnected structures within the neural network and that it is these changes that underlie clinical benefit. Nevertheless, our understanding of these distal DBS effects remains far from complete, in large part because of the technical difficulties of combining global assessment of neural activity and chemical-specific monitoring.

Given the strong electrophysiological and imaging evidence for DBS neuromodulation, it is not surprising that preclinical studies have shown neurochemical release in various efferent targets during DBS. *In vivo* microdialysis, which removes analyte from brain extracellular fluid for *ex vivo* analysis, has shown in rats that STN DBS significantly increases glutamate release in the globus pallidus (Windels *et al.* 2000, 2003; Savasta *et al.* 2002). However, the relatively large size of these probes disrupts tissue in the vicinity of the probe, which results in underestimation of extracellular dopamine levels as compared with measurement techniques that utilize *in vivo* electroanalysis in combination with chemical microsensors (Clapp-Lilly *et al.* 1999; Robinson *et al.* 2003; Borland *et al.* 2005). In addition, microdialysis requires relatively long periods of stimulation (e.g., 1 min or more). To solve this problem, a novel neurochemical monitoring system suitable and safe for human DBS surgery has been developed. The system components include (i) Wireless Instantaneous Neurotransmitter Concentration Sensing (WINCS) system, a wireless self-contained potentiostat and current sensor, (ii) WincsTrode, an in-house (Mayo Clinic) designed and fabricated neurochemical recording electrode, and (iii) WincsNanotrode, a National Aeronautics and Space Administration (NASA)-Mayo Clinic collaboratively developed multiplexed areal electrode. The integration of WINCS and the electrodes allows simultaneous detection and analysis of changes in neurotransmitter release during the application of DBS stimulation.

WINCS

The WINCS device was specifically developed to monitor neurochemical release during both experimental and clinical DBS surgical procedures (Fig. 5). For this reason, patient safety, signal fidelity, medical device safety, and integration with existing DBS surgical procedures, were key priorities in its development. WINCS consists of a relatively small, wireless, sterilizable, battery-powered unit that can interface with carbon-fiber microelectrodes (CFMs) or enzyme-based microsensors for real-time monitoring of neurochemical release in the brain (Agnesi *et al.* 2009, 2010; Bledsoe *et al.* 2009; Chang *et al.* 2009; Kimble *et al.* 2009; Griessenauer *et al.* 2010).

The system has been extensively tested using WINCS-based neurochemical recordings in a large animal model (pig) of DBS as a prelude to the studies in humans. It can measure *in vivo* dopamine and adenosine release with CFMs and glutamate release with an enzyme-linked biosensor during DBS (Agnesi *et al.* 2009; Bledsoe *et al.* 2009; Shon *et al.* 2010a,b).

WincsTrode

Fast-scan cyclic voltammetry, like other electroanalytical methods, has the advantage of allowing on-line correlation between neurochemical and behavioral changes. A major advantage of the fast-scan cyclic voltammetry procedure is that subsecond temporal resolution can be achieved, making it one of the fastest methods available for measuring changes in extracellular concentrations of electroactive molecules. Two other advantages are minimization of tissue damage and high spatial resolution. Conventional CFM electrodes consist of a glass-insulated carbon fiber (tip dimensions typically 50–250 μm length by 5–10 μm outer

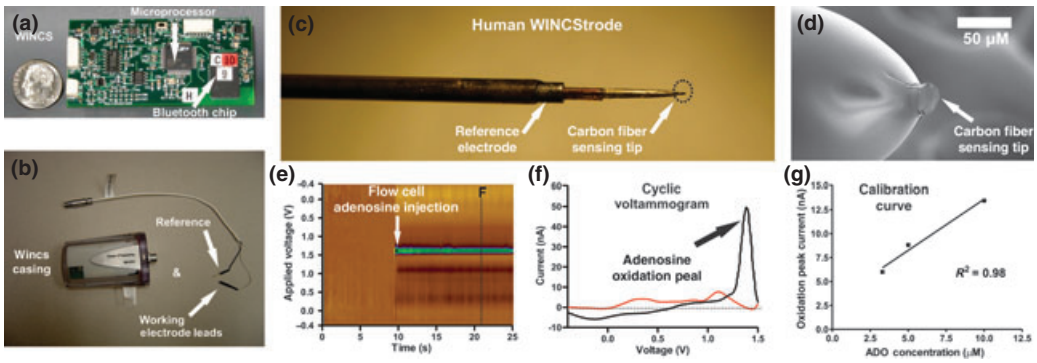

Fig. 5 Wireless Instantaneous Neurotransmitter Concentration Sensing (WINCS) system. (a) The WINCS device with Microprocessor and Bluetooth labeled. (b) WINCS encased in its sterilizable polycarbonate case. (c) WincsTrode, a neurochemical electrode designed for fast-scan cyclic voltammetry recordings in humans consisting of a carbon-fiber sensing tip. To achieve maximum safety, the carbon fiber is cut to provide limited exposure. The stainless-steel ring on the sheath was utilized as the reference electrode. (d) Scanning electron microscopy image of the sensing tip in (c) (dotted circle and arrow) showing the polyimide coating and seal around the exposed carbon-fiber tip (arrow). Modified from Chang *et al.* (2012). (e) Pseudo-color plot of adenosine detection in a flow cell using WINCS. (f) A single cyclic voltammogram showing oxidation peak of adenosine from (e). (g) Calibration curve of electrode demonstrating the linear response to adenosine.

diameter). However, conventional CFMs are fragile and pose a significant risk for human brain recordings. For this reason, a significantly safer and more durable electrode was developed. This electrode, called the WincsTrode, is insulated with polyimide and specifically designed for human brain recordings (Chang *et al.* 2012) (Fig. 5). Experimentally, the WincsTrode has been found to have a limit of detection of $\sim$100 nM for dopamine and adenosine. Because the physiological range of the adenosine concentration is generally thought to be
20–200 nM at basal extracellular levels (Latini and Pedata 2001), the WincsTrode clearly demonstrates sensitivity to adenosine that is physiologically relevant.

Adenosine is a neurochemical of interest to understand DBS mechanisms. Proposed as a chemical mediator of thalamic DBS for the treatment of essential tremor (Bekar *et al.* 2008), caudate adenosine release can be measured at CFMs during electrical stimulation of the nigrostriatal dopaminergic tract (Cechova and Venton 2008). Importantly, increases in adenosine appear to correlate with elevations in cerebral blood flow that result from an increase in neural activity (Brundege and Dunwiddie 1997; Phillis 2004). STN DBS elicits caudate adenosine release as measured by CFMs (Shon *et al.* 2010a). In addition, there is adenosine release in humans following insertion of the DBS electrode and during DBS in the ventral-intermediate thalamus of patients with essential tremor during DBS neurosurgery (Chang *et al.* 2012).

WincsNanotrode

Carbon nanoelectrodes have been shown to be an excellent substrate for electrochemical detection, demonstrating ultra high sensitivity, high signal-to-noise ratio, and rapid sampling, while at the same time providing an improved brain–electrode interface (Nguyen-Vu *et al.* 2007; Koehne *et al.* 2011). As the size of the exposed electrode is reduced, the sensitivity and temporal resolution can be dramatically improved. Nanoelectrodes can greatly improve the measurement of low-concentration neurotransmitters in real time in comparison with currently used microelectrodes. Carbon nanofibers (CNFs) grow well-separated and vertically aligned from catalytic metal coated substrates by plasma-enhanced chemical vapor deposition. The diameter can vary from 25 to 100 nm and the length can vary from hundreds of nanometers to many micrometers. The open ends of CNFs have a very fast electron transfer rate (similar to graphite edge planes), while the side wall has very slow charge transfer rate (similar to graphite basal planes). For sensing applications, electrochemical signals can be picked up at the open end and transported to the other end in contact with underlying circuits. CNF sensing arrays can be encapsulated with either SiO_2 or Parylene C so that only the reactive open end interfaces with the analyte solution (Koehne *et al.* 2011) (Fig. 6). These fabrication processes are compatible with semiconductor processing techniques, and thus can be mass-produced with low cost, which makes application studies possible.

A multiplexed device with 3 × 3 electrode pads, of 200 µm × 200 µm lateral dimension, is fabricated using standard photolithography methods. Nickel catalyst defines the location of CNF growth on the electrode pads, as shown in Fig. 6. Nickel catalyst can be deposited by UV-lithography, producing bulk CNF growth within a micron scale region, or by e-beam, generating individually separated CNFs. Each of these electrode pads can be selectively encapsulated with either SiO_2 or Parylene C for added stability and superior sensing capability. The microfabricated

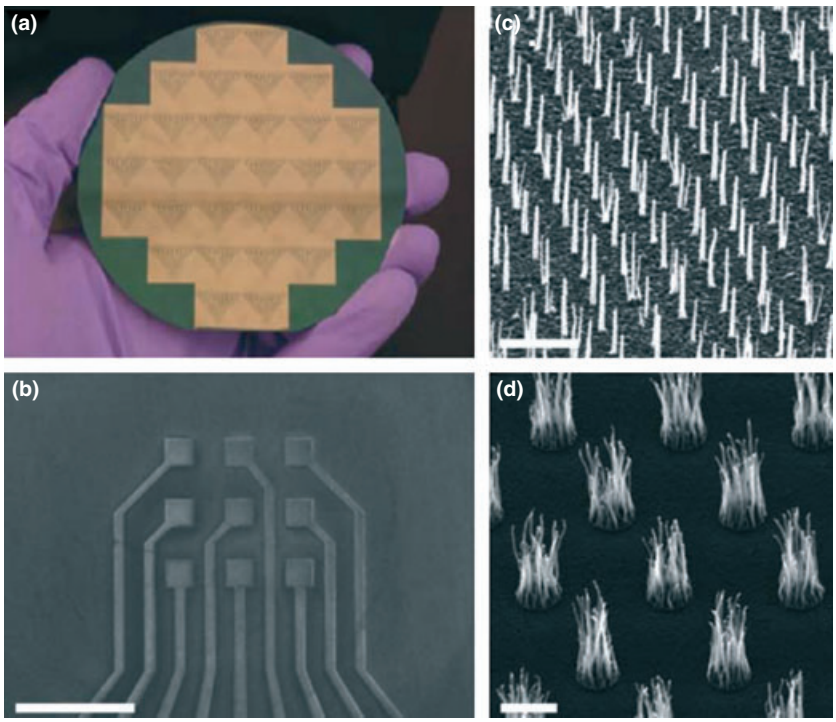

Fig. 6 WincsNanotrode. Field emission scanning electron microscope images of 3 × 3 electrode device. (a) Image of 4-inch silicon wafer with 30 patterned electrodes. (b) zoom in of the 3 × 3 array, each pad is 200 μm × 200 μm. (c) Individual carbon nanofibers made by e-beam patterned catalyst. (d) Clusters of carbon nanofibers made by UV-lithography patterned catalyst. Adopted from Koehne *et al.* (2011); reproduced by permission of The Royal Society of Chemistry; http://pubs.rsc.org/en/Content/ArticleLanding/2011/AN/c1an15025a.

array devices afford high spatial resolution of the thalamic slice by allowing recording with nanoelectrode ensembles of exactly 200 μm separation intervals.

We anticipate that the devices described here will provide novel insights into the mechanism of action of DBS and new techniques in the treatment of neuropsychiatric disorders. Indeed, WINCS was specifically designed for human use and has already been successfully implemented for electrochemical recordings in human patients undergoing DBS neurosurgery (Chang *et al.* 2012). Such neurochemical recordings in human patients during DBS is laying the foundation for an implantable closed-loop 'smart' device incorporating microsensor, feedback control, and neuromodulation to optimize neurotransmitter levels continuously for improved clinical efficacy.

Concluding remarks

One of the major advantages of pharmacological interventions (either oral or intravenous, but especially the former) is their minimally invasive nature. A pill is ingested, absorbed into the bloodstream, and transported to the nervous system. No incisions, no holes in the skull, and no electrodes skewering brain tissue. Not so with the present brain neuromodulation systems, be they depth electrodes (i.e. DBS), arrays of microelectrodes penetrating the brain cortex or cortical surface electrodes placed outside or inside the dura. All require a surgical procedure, and all are insultingly inelegant for interacting with such a marvelous structure as the nervous system. However, the lack of specificity of drugs is a serious drawback, one for which targeting techniques to latch onto tumor cells (such as tumor antigens) may solve in the realm of neurooncology, but for the broader field of neuromodulation to address functional disorders (from movement disorders to epilepsy to mood disorders to headache to obesity, etc.) such targeting techniques are likely to prove much more elusive.

Just as the bloodstream is the highway of sustenance and waste disposal for the nervous system (and the 'FedEx' of pharmacological interventions!), the bloodstream can serve as the distribution channel for precision cellular-level neuromodulation, both electrical and chemical. Our neurointerventional colleagues have made remarkable strides over the past two decades in placing catheters in progressively more minute blood vessels in the brain. Rodolfo Llinás and colleagues have shown that microcatheters can record and stimulate the nervous system through the capillary wall as effectively as an electrode placed in the nervous system parenchyma (Watanabe *et al.* 2009). Moreover, as presented above, nanomaterials are being developed to fabricate micron (and potentially submicron) size arrays for both electrical and chemical recording and stimulating of nervous system tissue. The era of cellular, and even intracellular, precision neuromodulation will soon be upon us.

But you ask, 'How can we pay for all this intricate interacting with the brain?' That is, how will the energy needs for these devices, potentially dozens or hundreds of

them constantly monitoring and tweaking the brain in different crucial locations (the locations depending on the disorder involved), be delivered to these micron size modulators? Not to worry! Our colleagues in materials science are developing nanogenerators that will tap into the nervous system's own energy sources. Either piezoelectric nanodevices (capitalizing on the movement of the brain and/or the pulsations of the intracranial blood vessels), heat-sensitive energy generators, or perhaps even energy sources based (like the brain itself) on the metabolism of glucose within the capillary blood (or other equally ingenious techniques), will tap into the energy present within the cranium itself (Lee *et al.* 2012; Xu *et al.* 2012). And with regard to the more literal meaning of 'pay' for such nanoneuromodulators, to the extent that dirt contains carbon, one might say they will be 'dirt cheap'. Consider how we would have reacted in 1960 to today's cell phone (costing perhaps $20 in 1960 dollars), voice and digital interaction with any place on earth, multimegapixel camera, global positioning system, apps galore, etc. Nothing proposed here is in the least bit fanciful.

Two towering early figures in the nanorealm were Richard Feynman and Richard Smalley. To quote Feynman in December, 1959, and Smalley in June, 1999, respectively: 'It is a staggeringly small world that is below. In the year 2000, when they look back at this age, they will wonder why it was not until the year 1960 that anyone began seriously to move in this direction.'; and '…20 years from now, nanoscale missiles will target cancer cells in the human body and leave everything else blissfully alone. I may not live to see it. But I am confident it will happen'.

We might paraphrase these two prescient scientists, with regard to neuromodulation, as follows: 'It is a staggeringly complex and marvelous world that is the nervous system. Twenty years from now we will be able to converse with the nervous system on its own level and on its own terms. Subtle diplomacy will have substituted for brute force in transforming the malfunctioning nervous system back to normalcy. In the year 2030, when they look back on this age, they will wonder why we saw such rapid progress in fields like personal computers in the late 20th century but such glacial inertia in the development of techniques for true neuromodulation'.

Acknowledgments

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Conflict of interest statement

Dr. Peter A. Tass has a contractual relationship with ANM Adaptive Neuromodulation GmbH. Dr. Russell J. Andrews is a consultant for Cyberonics, Inc.

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