Carbon Nanotubes as Electrical Interfaces with Neurons

Chapter · February 2010

DOI: 10.1007/978-90-481-8553-5_11

CITATIONS
6

READS
218

2 authors:

William Lee

18 PUBLICATIONS **426** CITATIONS

SEE PROFILE

Vladimir Parpura

University of Alabama at Birmingham

263 PUBLICATIONS **17,363** CITATIONS

SEE PROFILE

Chapter 11
Carbon Nanotubes as Electrical Interfaces with Neurons

William Lee and Vladimir Parpura

Abstract Carbon nanotubes (CNTs) are emerging as promising nanomaterials for biomedical applications. Due to their unique structural, mechanical and electronic properties, CNTs can be used as electrical interfaces with the brain in particular with neurons. CNT-based neural interfaces/electrodes have been employed in cell culture and in vivo; they offer advantages over standard metal-based electrodes in terms of monitoring and stimulation of neuronal activity. One of the challenges for interfacing brain and machine is the biocompatibility of the materials used for electrode construction. While CNTs appear biocompatible, the exposure limits have not been set thus far. An appropriate (inter)national standards/rules for the use of CNTs need to be established before CNT-based electrodes/devices can be used in human subjects.

Abbreviations

BBB	blood-brain barrier
CNTs	carbon nanotubes
DRG	dorsal root ganglion
EEG	electroencephalogram
ERP	event-related potentials
MEA	microelectrode array
MWNTs	multi-walled CNTs
PPy	polypyrole
RGCs	retinal ganglion cells
SWNTs	single-walled CNTs
TiN	titanium nitride

V. Parpura (✉)
Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, AL, USA
e-mail: vlad@uab.edu

M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*, DOI 10.1007/978-90-481-8553-5_11, © Springer Science+Business Media B.V. 2010

VACNFs vertically aligned carbon nanofibers
3D three-dimensional

11.1 Introduction

Carbon nanotubes (CNTs) are emerging as one of the most promising nanomaterials for applications in electronics, aerospace and biomedicine. In this chapter we discuss the use of CNTs as electrical interfaces with the brain in particular with its electrically excitable cellular components, neurons. We begin with a primer on CNTs unique structural, mechanical and electronic properties, which have captured the attention of physicists, chemists and material scientists and prompt the use of CNTs in biomedical applications (Section 11.2). This is followed by a discussion of a subset of experimental approaches using CNT-based neural interfaces in cell culture and in vivo to illustrate the advantages that CNTs can offer over standard metal-based electrodes in terms of monitoring and stimulation of neuronal activity (Section 11.3). Finally, we briefly discuss biosafety of CNTs and raise the concern as to the lack of exposure limit guidance to date (Section 11.4).

11.2 Primer on Characteristics of CNTs

Detailed description of the structure, properties and modification/functionalization of CNTs is available elsewhere [1, 2]. Briefly, CNTs are composed of graphene sheets rolled into cylinders, which have a hollow core. The cylindrical ends can be capped with a fullerene dome. Based on the number of concentric graphene cylinders within CNTs, they are classified into single-walled CNTs (SWNTs), double-walled CNTs, or multi-walled CNTs (MWNT). SWNTs commonly have their diameters between 0.7 and 2 nm, although their diameter down to 0.4 nm have been reported [3, 4]. MWNTs have an outer diameter that typically ranges from 2 to 100 nm, while the inner diameter varies between 1 and 3 nm. The length of synthesized CNTs is typically in the μm range, although SWNTs up to 4 cm have been reported [5]. CNTs have an exceptional mechanical strength with a Young's modulus of ~1 TPa. They are chemically relatively inert and non-biodegradable. In addition, CNTs exhibit unique electrical properties. CNT conductivity is endowed by the conformation of their hexagonal graphene lattice, which can be arm-chair, zig-zag, or chiral. They can be metallic or semi-conductive. In metallic CNTs, the graphene hexagonal lattice can be arranged in any of the three configurations, with all arm-chair CNTs being metallic. In semi-conductive CNTs, the lattice can be arranged in a zig-zag or chiral configuration. The combined physical properties make CNTs a durable nanomaterial for bio-engineering, especially in applications where a sustained presence of the material is desirable, such as stimulation/recording electrodes for interface with neural elements as discussed below.

11.3 CNT-Based Neural Interfaces for Stimulation and Monitoring of Neuronal Activity

An in-depth review on neural stimulation/recording electrodes is available elsewhere [6]. One of the challenges in designing electrodes for neural interfaces is to maximize delivery of electrical stimulation to cells with high selectivity, while minimizing tissue damage. In recording mode, electrodes with high sensitivity, as evidenced by high signal to noise ratio, are desirable. Amongst the nanomaterials available to date, CNTs display desirable properties for use in stimulation/recording electrodes. (i) CNT-based electrodes have been successfully miniaturized while they do not seem to inflict tissue damage. (ii) CNTs have the ability to operate as ballistic conductors which aids in lowering impedance and increasing charge transfer. (iii) CNTs display exceptional flexibility and they can be twisted and bent to a large degree, although they are five times mechanically stronger than steel [7]. These traits are advantageous for materials to be used for microelectrodes that would penetrate through the tissue. Such properties of CNTs allowed for their use in stimulating and monitoring of neuronal activity at various levels of spatial domains [see definition of levels in Ref. [8]], which includes (i) stimulation of action potentials/ Ca^{2+} excitability in a small group of neurons in culture using CNT films of multi-electrode arrays, (ii) stimulating and recording from neurons in hippocampal organotypic slice cultures as well and in the whole mount mouse retina, (iii) stimulation of and recording from rat and monkey cortices, and (iv) recording human electroencephalogram (EEG) through a CNT-based attachment to the superficial skin layer. We describe below a subset of experimental approaches demonstrating such usage of CNTs.

Liopo et al. [9] demonstrated the ability to electrically stimulate neural cells directly through a CNT substrate. In this study, the neuroblastoma x glioma NG108 cell line or rat dorsal root ganglion (DRG) cells were cultured on planar and transparent SWNT films deposited onto overhead transparencies, i.e. polyethylene terephthalate sheets (Fig. 11.1a). NG108 cells and DRG neurons were subjected to electrophysiological recordings using a whole-cell patch clamp configuration to monitor the electrical activity of individual cells. These cells were then electrically stimulated either via a patch pipette or through a conductive SWNT film (Fig. 11.1b and c). Recorded currents due to two different stimulation methods appear qualitatively similar, indicating that an SWNT film can be used as a stimulation platform. Subsequent studies have demonstrated that various planar CNT films can be used for cellular growth and direct electrical stimulation of cultured primary neurons [10, 11], NG108 cells [12] and differentiated neural stem cells [13]. The ability of CNTs to deliver electrical stimulation to neurons can be attributed to their conductivity and their intimate contacts with neurons as revealed by electron microscopy [10–12, 14]. It should be noted, however, that while whole-cell patch clamp allows stimulating the same cell that is recorded from, CNT film stimulation excites the entire population of cells that are residing on the film which also serves as a planar growth scaffold/substrate. Since CNTs films/deposition is amenable to miniaturization, one possible solution for achieving the use of CNTs for

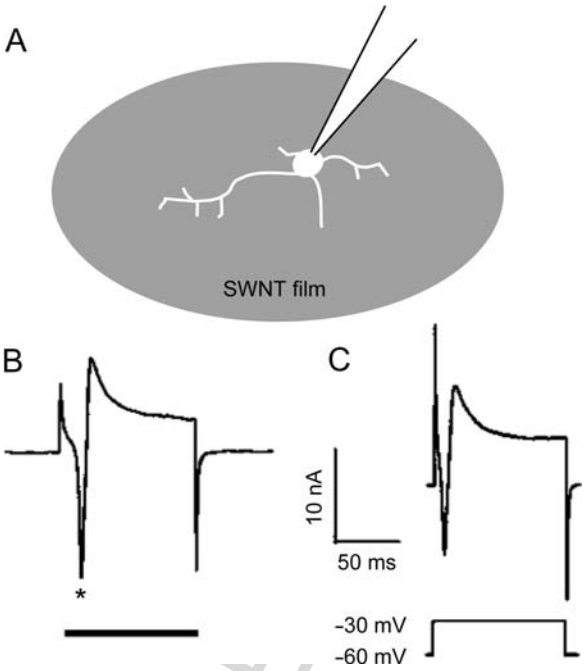

Fig. 11.1 Electrical stimulation of dorsal root ganglion (DRG) neurons via a planar SWNT film. (**a**) Schematic representation of an experimental setup. DGR neurons grown on SWNT films (*grey*) were subjected to electrophysiological recordings using a whole-cell patch clamp configuration. (**b**) Stimulation (direct current, *horizontal bar*) of a DRG neuron through the SWNT film causes an inward current (*asterisk*), similar to that seen when voltage steps were applied via a patch pipette (**c**). (**b–c**) Modified from [9], with permission

recording/stimulation of individual neurons, or a small group of these cells, within the network is to generate a so-called microelectrode array (MEA).

Wang et al. [15] developed a CNT-based MEA comprised of pillars made of vertically aligned conductive MWNTs (Fig. 11.2). The size, the geometry and location of the CNT pillars can be precisely controlled by lithography. CNT pillars electrodes have rectangular (30 × 30 μm, 50 × 50 μm, or 100 × 100 μm) or circular (50 μm in diameter) geometry with a height of 40 μm (Fig. 11.2a–c). Pillars were integrated onto a pre-patterned microcircuit and they were individually addressable. These electrodes have high charge injection capacity and operate without faradic/electrochemical reactions, that otherwise can lead to irreversible damage of the electrodes and surrounding tissue. Thus, they represent a prototype for efficient and biocompatible interfacing for neural prosthesis. Indeed, the authors demonstrated a potential of the MEA approach for neuronal stimulation by using cultured neurons. Dissociated hippocampal neurons were plated onto MEAs. Cells displayed viability and neurite outgrowth consistent with the previously shown biocompatibility of MWNTs [16, 17]. Rather than directly recording electrical activity of neurons, the authors assessed neuronal intracellular Ca^{2+} excitability due to electrical stimulation via CNT pillars. For dynamic Ca^{2+} imaging a fluorescent Ca^{2+} indicator was used. Since an inverted microscope was used while CNTs were nontransparent, the fluorescence emission from cells directly on the CNT pads could not be visualized. However, neurons that were in near proximity of stimulating electrodes

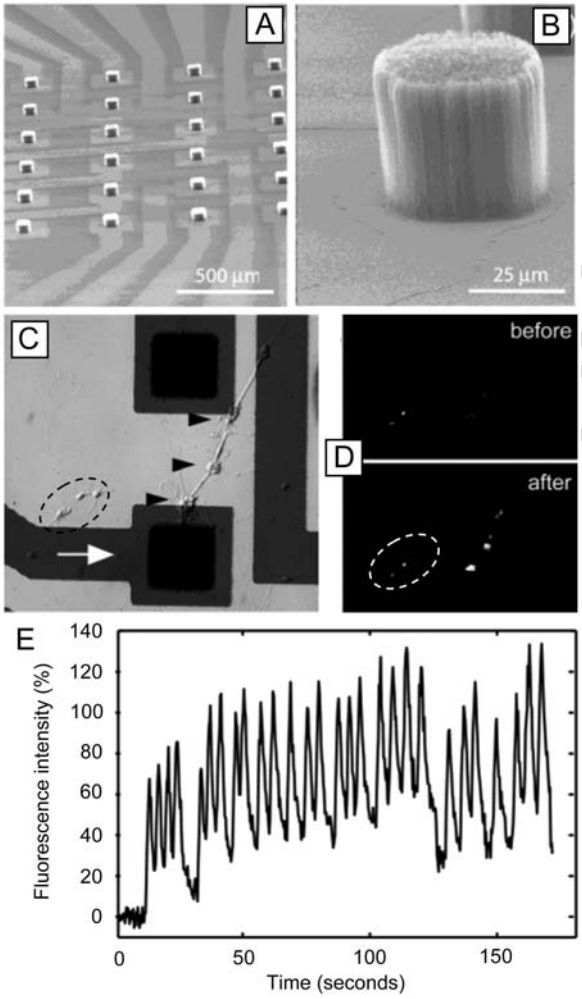

Fig. 11.2 Electrical stimulation of hippocampal neurons grown on an MWNT-based microelectrode array (MEA). (**a**) CNT-based MEA comprised of pillars made of vertically aligned conductive MWNTs and having either rectangular (30 × 30 μm) or (**b**) circular (50 μm in diameter) geometry with a height of 40 μm. (**c**) Pillars are integrated onto a pre-patterned microcircuit and they are individually addressable. Hippocampal neurons grown on MEA (100 × 100 μm pillars) were electrically stimulated (*white arrow*). (**d**) Neurons loaded with a fluorescent Ca^{2+} indicator before and after electrical stimulation, which causes the increase in intracellular Ca^{2+} levels in neurons in contact with each other and a CNT pillar (*black arrowheads* in **c**). Note that neurons that were not in contact with CNT pillars show no changes in intracellular Ca^{2+} excitability (*left, dashed oval*; also see **c**). (**e**) Time course of intracellular Ca^{2+} dynamics due to repetitive cell stimulation. Modified from [15], with permission

showed increases in intracellular Ca^{2+} levels seen as the increase in the fluorescence intensity (Fig. 11.2d). Repeated stimulation paradigm caused transient increases in intracellular Ca^{2+} levels (Fig. 11.2e). In contrast, neurons that were not in contact with CNT pillars show no changes in intracellular Ca^{2+} excitability. Taken together, these experiments show that MEA made out of CNTs have potential for use in tissue and show promise as bio-compatible and efficient electrodes.

Yu et al. [18] used vertically aligned carbon nanofibers (VACNFs), a form of carbon material closely related to MWNTs, to generate MEAs which they utilized to stimulate neurons and record from these cells in cultured organotypic hippocampal slices. In this study, 40 individually addressable VACNF electrodes, 10 μm in height and spaced 15 μm apart, were arranged in a linear array with a total length of 600 μm (Fig. 11.3a). Individual VACNF electrodes assumed a cone-like geometry, which aids their penetration of the tissue leading to improved electrical interface with neurons (Fig. 11.3b). VACNF electrodes had effective radius of up to ~17 μm [see Fig. 2 in Ref. [18]]. Although such size of VACNF electrodes is smaller than traditional metal surface electrodes, the electrical noise level recorded for VACNFs electrodes was comparable to that of various metal-based MEAs reported elsewhere

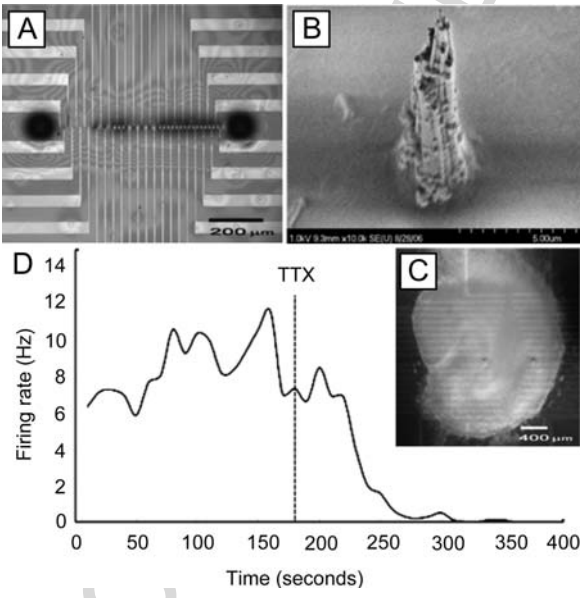

Fig. 11.3 Vertically aligned carbon nanofibers (VACNFs) forming MEA record spontaneous electrical activity within the slice. (**a**) Individually addressable VACNF electrodes were arranged in a linear array (light micrograph). (**b**) An individual VACNF electrode assumes cone-like geometry (electron micrograph). (**c**) A hippocampal slice on a VACNF MEA (light micrograph). (**d**) Spontaneous action potential discharges/firing from a slice recorded by MEA is sensitive to tetrodotoxin (TTX; the time point of application indicated by the *vertical dashed line*), a blocker of voltage-gated Na^+ channels, confirming a neuronal source for spontaneous activity. Modified from [18], with permission

[see detailed comparison on p. 2190 of Ref. [18]]; the average noise level was inversely proportional to the electrode dimensions. The recording noise level of VACNF MEAs assessed in solution was favorable to enable extracellular recordings from cultured organotypic hippocampal slices. Prior to use of VACNF MEA for recordings from slices, the chips were treated with a mixture of cell adhesion permissive substrates poly-l-lysine and laminin to aide the adherence of slices to the chip. Slices cultured separately were then applied onto chips (Fig. 11.3c) and were held in place using a nylon mesh. Spontaneous electrical activity was simultaneously recorded in CA3 pyramidal and dentate gyrus granule cell layers; this activity could be blocked by tetrodotoxin, a blocker of voltage-gated Na^+ channels underlying action potentials producing spikes in the recordings (Fig. 11.3d). Conversely, the removal of the inhibitory inputs by the addition of bicuculline, a blocker of gamma-amino butyric acid receptors type A, resulted in epileptiform activity. In addition to recording of spontaneous neuronal electrical activity, the application of stimuli between two VACNF electrodes resulted in evoked field potentials. Taken together, this study demonstrated that VACNF-based MEAs can deliver stimuli to the tissue and record from it with improved spatial control compared to CNT films. The three-dimensional (3D) cone-like protrusions offer recordings from single units with amplitudes doubled from those seen in metal-based MEAs [19]. A lingering issue with the use of VACNF-based MEA, shared with conventional metal-based electrodes, is the rigidity of their surfaces. Namely, neural cells display sensitivity to the mechanical stiffness of the scaffold [20, 21]. Consequently, to improve the use of VACNF MEAs, Nguyen-Vu et al. [22] implemented VACNF brush-like electrodes that have been additionally coated with the conductive polymer polypyrole (PPy). Such co-deposition approach found applications when using CNTs for recordings of neuronal activity in vivo [see below Ref. [23]].

Shoval et al. [24] implemented the use of MWNT MEAs, which were produced and packaged as previously reported [25], to record from whole-mount retinas. Here, the deposition of CNTs onto titanium nitride (TiN) patterned substrate results in highly conductive and porous/rough CNT islands/electrodes with low impedance [25], which represent a good cell-adhesive surface as neurons entangle into a 3D CNT matrix [26]. Bare TiN electrodes were designed to be porous achieving high surface area and low impedance as well. Freshly isolated whole mount retinas where placed onto MEAs with the retinal ganglion cells (RGCs) layer facing down. It should be noted that RGCs represent the output cells from the retina encoding the information transfer to the cortex by the frequency of their action potential discharges. In many of the retinal dystrophies, these cells may remain intact to transmit information, while photoreceptors degenerate. Consequently, there is an urge to develop retinal implants that would by pass photoreceptors and directly opto-electrically couple to RGC. Nonetheless, whole-mount retinas were restrained onto chips using a polyester membrane filter. For comparison two chips were used: bare TiN MEAs and MEAs containing additional coating with CNTs. In both cases, some of the CNT or TiN islands on the chip were not electrically accessible, thus do not represent any of the 60 active electrodes within the MEA (Fig. 11.4a). These “spare” islands appear to assist in stabilizing the whole tissue. All islands

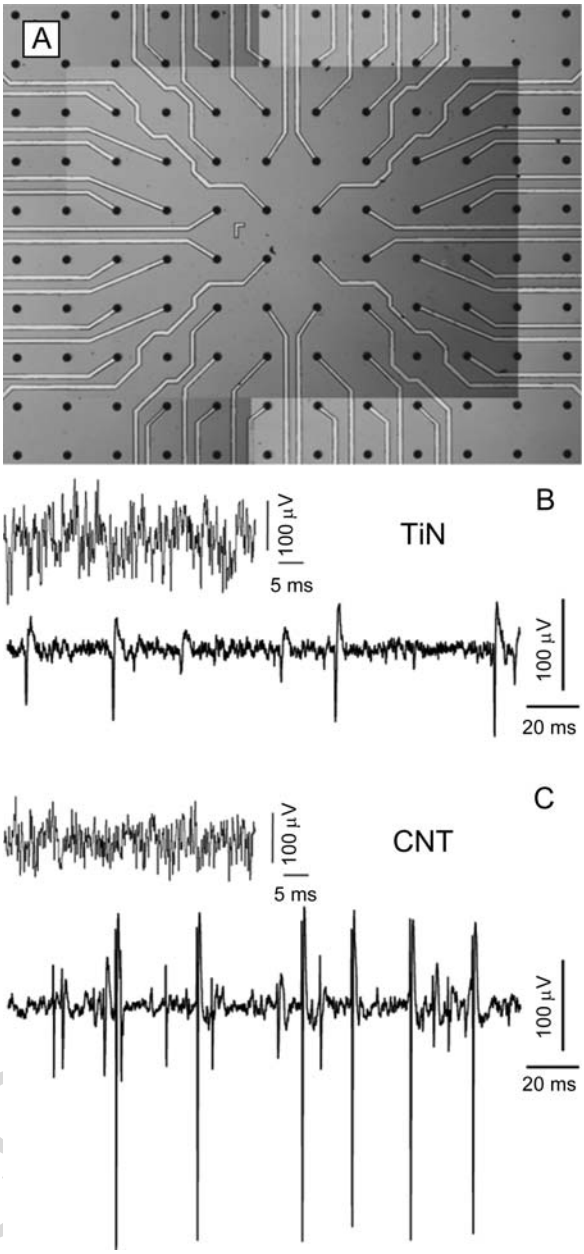

Fig. 11.4 Electrical recordings of neural activity from the whole-mount retina using an MWNT MEA. (**a**) CNTs islands (*black dots*) deposited onto titanium nitride (TiN) patterned substrate. Note that not all CNT island are electrically active, since they are not connected to individual leads. (b–**c**) Recordings obtained from a whole-mount retina using a bare TiN and an MWNT-coated hybrid electrode, respectively. Top traces in b and c represent baseline noise level, while lower traces disclose spontaneous discharges of action potentials from retinal ganglion cells. Modified from [24], with permission

were 30 μm in diameter and spaced 200 μm apart; CNT islands had heights of several μm. Electrical recordings from retinas indicate that both types of electrodes can record spontaneous activity with RGCs discharging burst of action potentials (Fig. 11.4b and c). Individual electrodes of both types of MEAs appeared to record from at least two RGCs simultaneously as two sets of signal amplitudes

were clearly designated. The quality of recordings, however, were much better with CNT-coated electrodes, as evidenced by decreased levels of baseline noise to half of that seen in TiN electrodes and by more than doubled amplitudes of recorded action potential amplitudes, resulting in a exceptionally high signal-to-noise ratio of the CNT-coated electrode and clear single unit recordings (Fig. 11.4c, high amplitude signals). Over time (minutes to hours) the number of electrodes that could record RGCs activity increased on both type of chips. The amplitude of action potentials recorded from TiN electrodes were stable, while recordings from CNT-coated electrodes kept improving over time, showing enhanced amplitudes of recorded action potentials with a 2% per minute increase; recordings lasted up to several hours. This time-dependent improvement in recordings made by CNT-coated electrodes could be attributed to improved coupling between electrodes and the tissue. Moreover, the stimulation proof-of-concept experiments were executed. Stimulation via an individual CNT-coated electrode (80 μm in diameter) can be used to record evoked action potentials generally on a single neighboring electrode (200 μm spacing). Taken together, the results of this study indicate that CNT-based MEAs have promise for use in vivo.

Keefer et al. [23] established a procedure to coat planar and 3D electrodes with MWNTs and compared their performances with the uncoated electrodes using cultured neurons, the motor cortex of anaesthetized rats, and the V4 region of the visual cortex of a conscious trained monkey. The initial experiments were done on planar MEAs in the absence of any brain cells. Deposition of CNTs on indium-tin oxide based MEAs reduced the impedance of electrodes by ~20 fold and increased the charge transfer by ~45 fold. Follow-up experiments used dissociated cultures of frontal cortical neurons plated onto MEAs with bare gold surfaces or MEAs containing an additional coating with CNTs. Both gold and CNT surfaces were permissive substrates for neuronal growth. Spontaneous activity of the established neuronal networks could be recorded for up to 3 months in culture with similar success with either of MEA. However, the stimulation delivered via CNT-coated MEAs was more effective than that of the bare gold-based MEAs, a finding consistent with the CNTs' ability to lower impedance and increase charge transfer. One consequence of such an effect by the CNTs was a decrease in noise levels by ~65%, which led to the increased sensitivity of CNT-coated MEAs, without a change in their selectivity. These proof-of-principle experiments using planar MEAs were followed by 3D electrodes and work in vivo. Commercially available 3D tungsten and stainless steel sharp electrodes were over-coated with CNTs. Once again, when tested in solution, these electrodes outperformed the bare metal electrodes by offering lower impedance and higher charge transfer. This performance could be further enhanced if CNTs were co-deposited with conductive polymers such as PPy which itself has been previously proven successful in experiments in vivo [27]. Two different animal models were used to test CNT-coated sharp electrodes: motor cortex (controlling limb movement) of anesthetized rats and V4 cortex (involved in perception of form-with-color) of an awake trained monkey. In experiments with rats, two parallel electrodes, referred to as stereotrodes, one coated with CNTs and the other bare tungsten, spaced apart by 125 μm, were used. For experiments with the monkey cortex, the animal's task was to look at a flashing color square

(form-with-color) on a screen, while recordings were performed using "stereotrodes" spaced 1 mm apart and containing one uncoated stainless steel (control) and another CNT-coated electrode (Fig. 11.4). In both in vivo experimental models, CNT-coated electrodes outperformed their paired control electrodes in terms of reduced noise (~17 dB) and increased sensitivity of detection (on average 7.4 dB more power) of spontaneous electrical neuronal activity throughout various ranges of acquisition frequencies (1–1000 Hz) relevant to brain (patho)physiology (Fig. 11.5a). As one would predict from their mechanical strength, CNTs endured the advancement of electrodes through the dura mater and remained intact even after recordings were completed, as assessed by electron microscopic investigation of the used electrodes (Fig. 11.5b). Taken together the coating of planar and 3D electrodes with CNTs allowed for their enhanced performance while recording neuronal electrical events in culture and in vivo. Thus, these hybrid electrodes could now be readily tested in exciting ongoing projects in the field of brain-machine interfaces, such as, the restoration of movement in tertraplegia [28]. Of course this should not occur with adequate testing for biosafety of CNT-based devices.

Thus far, we outlined the development of CNT-based electrodes used in direct contact with neural cells/brain tissue. However, it is commonly accepted that the safest way to record neural electrical activity is by using the EEG, since it is non-invasive procedure where electrodes are placed on a scalp. The location of such electrodes in humans is about 2–3 cm away from the surface of the cortex so that the electrical signal has low amplitude. Consequently, EEG recordings are generated by a rather large population of neural cells that are synchronously active. It

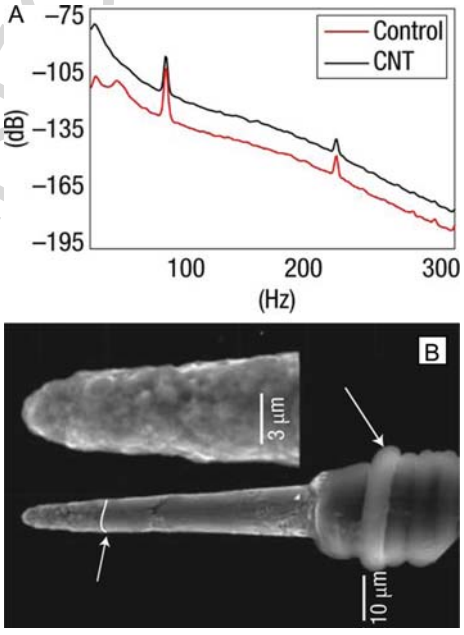

Fig. 11.5 CNT-coated electrodes record electrical activity in the primate visual cortex. (**a**) Power spectra obtained from CNT-coated and bare (control) metal electrodes indicate enhanced performance by CNT-coated electrodes. (**b**) CNT-coated electrode after recording from the monkey cortex. While insulating thin layer is peeled back (*right arrow*) from its original near the tip position (*white line, left arrow*) during penetration through the dura mater, the covalently attached CNTs at the electrode tip remain intact (inset). Modified from [23], with permission

should be noted that in addition to neuronal signals, the current flow created by glial cells contributes to the EEG. For example, glial Müller cell potentials have a time course similar to a component in the electoretinogram, referred to as the b wave [29]. Additional consequence of low amplitude measurements presents itself in the skin preparation for EEG recordings, which requires the use of electrolytic gels and takes several minutes per electrode. Owing to the diffusion of such gels into the skin, there is an additional requirement for stabilization of recordings. To overcome some of these limitations, Ruffini et al. [30] developed a dry electrophysiology sensor based on MWNTs to record the EEG from humans. Arrays of MWNTs were grown on silicon disks to form brush like structures (Fig. 11.6a); individual MWNTs were 50 nm in diameter and 10–15 μm length/height. Disks containing an array of MWNTs were then diced into squares (1 × 1 cm) and mounted onto commercial active electrodes with on-site amplification (Fig. 11.6b). As a standard for comparison, conventional electrodes were used. Both types of electrodes were connected to a commercial research electrophysiology recording system. Prior to the use on human, a series of tests were run on a pig skin to record test signals applied beneath the skin. Here, commercial electrodes were applied using an electrolytic gel, while CNT-based electrodes were applied "dry" without skin preparation. The

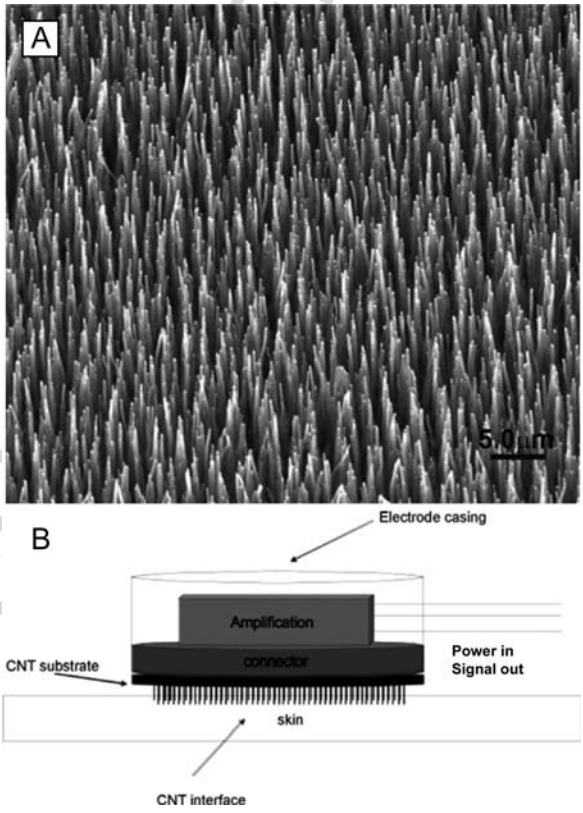

Fig. 11.6 MWNT-based electrode for EEG. (**a**) Scanning electron microscope image of an array of MWNTs grown on a silicon disk, which can be mounted onto a commercial active electrode with on-site amplification (**b**). (**b**) Schematics of CNT-based EEG electrode. MWNT array penetrates a superficial layer of the skin owing to the height/length of CNTs. Modified from [30], with permission

results showed similar performance of two types of electrodes. It should be noted, however, that CNT-based electrodes only penetrated the superficial layer of skin owing to the MWNT length/height. They caused neither pain nor any skin reaction as reported by a human subject who was subjected to EEG recordings when dry CNT and wet commercial electrodes were placed next to each other on the scalp for comparison of their performances. The recordings were done simultaneous using both types of electrodes using the protocol that encompassed spontaneous EEG and event-related potentials (ERP). Spontaneous EEG consisted of two periods, one with eyes open and the other with eyes shut, where the subject did not perform a task, allowing recording of β and α waves, respectively. ERP were tested in response to an auditory stimulus. In all conditions, dry CNT electrodes performed similarly to the commercially available state of the art research-oriented wet electrodes.

11.4 Biosafety of CNTs

The promising and exciting possibilities for the use of CNTs in biomedical applications also raise concerns about their safety and exposure limits [reviewed in Refs. [31, 32]]. Besides the above described use of CNTs in electrodes for neural interfaces, they are also emerging as a tool for targeted drug delivery [reviewed in Ref. [33]] and, in this context, understanding of their biodistribution and biostability is necessary. This knowledge could also be relevant to the applications of CNTs in neural interfaces, since there is a possibility that CNTs could leach out from electrodes and get distributed systemically if they can pass the blood-brain barrier (BBB). Biodistribution of a variety of functionalized SWNTs indicate that, following their delivery to the circulation, their blood retention can vary between 1 hour and 1 day, depending on the animal model used and modifications of SWNTs; the excretion/clearance of SWNTs via biliary and renal pathways is evident [34–37]. Interestingly, the biodistribution of CNTs in the brain 24 hours after their intravenous injection is much lower than that in the spleen and lungs, although these organs are all highly vascularized, suggesting that the intact BBB can effectively shield the entrance of CNTs into the brain [35]. Conversely, whether the BBB prevents CNTs that leach out from implanted electrodes in the brain to exit into the circulation has not yet been determined. However, even if this proves to be a clearance pathway, CNTs available in the circulation would be at very low quantities, so that such a scenario highly likely represents a trivial issue. Consequently, a pressing concern presents itself in the possible direct effects that CNTs may have on neural cells, which they contact as being an integral part of CNT-based electrodes.

The current literature on safety exposure limits and toxcitiy of CNTs on neural cells is limited. Most of the CNT toxicity studies were done using non-neural cells or cell-lines; these in vitro studies reported that the exposure to CNTs increased oxidative stress and cell death in a dose-dependent manner [38–43]. However, some of these studies were performed in such a manner that they do not directly address the toxicity effect solely attributed to CNTs. For instance, the synthesis of CNTs

often involves the use of a metal catalyst, in which the residual content in CNTs could be responsible for some of the observed toxic effects. Hence, non-purified CNTs, containing higher content of a metal catalyst, have been shown to be more potent in inducing oxidative stress in macrophages than the purified CNTs [44, 45]. Nonetheless, the length of CNTs can contribute to differential cellular response. The degree of inflammatory response to subcutaneously applied CNTs in vivo was greater for 825 nm in length CNTs than for shorter 220 nm in length CNTs [46]. It should be noted, however, that no severe inflammatory response, such as necrosis, was observed around both CNTs examined. Therefore, an evaluation of CNTs' effect on cells/tissue must take into consideration the type of CNTs and the presence of impurities.

The development of CNT-based materials for biomedical applications is still at its infancy, and it is timely to investigate effects of CNTs on neural cells/tissue before CNT-based devices become wide-spread in use. A recent in vitro study showed that CNTs are biocompatible with neural cells [47]. However, a more systematic approach, using a variety of CNTs, is needed to address acute and long-term effects that CNTs may have on the brain and the whole living organism in order to establish safety guidance for the use of this promising nanomaterial in biomedical applications in the near future.

11.5 Concluding Remarks and Future Directions

The intent of this chapter was to review the use of CNT-based neural interfaces for stimulation and monitoring of neuronal activity. Owing to unique physical properties of this nanomaterial, CNT-based electrodes have potential to replace bare metal electrodes for many of the medical applications, most notably brain-machine interfaces. Further improvement in CNT-based electrodes may include their chemical modifications, so they can detect various transmitters [discussed in Ref. [48]]. However, the use of CNT-based electrodes in human subjects must not occur without adequate testing. It is encouraging that systemic administration of CNTs [36] indicates that this form of carbon does not have any deleterious effect on mammalian health. This pilot study could be used as a springboard to determine safe exposure limits, both general and brain-specific, in humans. From the stance of implantation in the brain, the chronic response of the tissue to the resident CNT-based electrodes will have to be compared to the "classical" electrodes that have been in use for decades [49]. Although the retention of CNTs on planar electrodes over the period of ~3 months has been demonstrated [23], similar studies will need to be performed with the CNT-coated 3D electrode arrays using even longer implantation times, before we can make a full assessment of CNTs biocompatibility.

Acknowledgements We thank J. Robert Grammer for his comments on previous versions of this manuscript. The authors' work is supported by a grant from the National Institute of Mental Health (MH 069791) and National Science Foundation (CBET 0943343). We dedicate this chapter to the late Glenn I. Hatton, whose work inspired new views of astrocyte-neuronal interactions.

References

1. Bekyarova E, Haddon RC, Parpura V. Biofunctionalization of Carbon Nanotubes, In: Kumar CSSR (ed) Biofunctionalization of Nanomaterials (Nanotechnologies for the Life Sciences). Wiley-VCH, Wienhein-Berlin, Germany, 2005; pp. 41–71
2. Bekyarova E, Ni Y, Malarkey EB, et al. Applications of Carbon Nanotubes in Biotechnology and Biomedicine. J Biomed Nanotechnol 2005; 1:3–17
3. Qin L-C, Zhao X, Hirahara K, et al. The smallest carbon nanotube. Nature 2000; 408:50
4. Wang N, Tang ZK, Li GD, et al. Single-walled 4 A carbon nanotube arrays. Nature 2000; 408:50–51
5. Zheng LX, O'Connell MJ, Doorn SK, et al. Ultralong single-wall carbon nanotubes. Nat Mater 2004; 3(10):673–676
6. Cogan SR. Neural stimulation and recording electrodes. Annu Rev Biomed Eng 2008; 10:275–309
7. Krishnan A, Dujardin E, Ebbesen TW, et al. Young's modulus of single-walled nanotubes. Phys Rev B 1998; 58(20):14013–14019
8. Schwartz AB, Cui XT, Weber DJ, et al. Brain-controlled interfaces: movement restoration with neural prosthetics. Neuron 2006; 52(1):205–220
9. Liopo AV, Stewart MP, Hudson J, et al. Biocompatibility of native and functionalized single-walled carbon nanotubes for neuronal interface. J Nanosci Nanotechnol 2006; 6(5): 1365–1374
10. Mazzatenta A, Giugliano M, Campidelli S, et al. Interfacing neurons with carbon nanotubes: electrical signal transfer and synaptic stimulation in cultured brain circuits. J Neurosci 2007; 27(26):6931–6936
11. Cellot G, Cilia E, Cipollone S, et al. Carbon nanotubes might improve neuronal performance by favouring electrical shortcuts. Nat Nanotechnol 2009; 4(2):126–133
12. Gheith MK, Pappas TC, Liopo AV, et al. Stimulation of neural cells by lateral layer-by-layer films of single-walled currents in conductive carbon nanotubes. Adv Mater 2006; 18(22):2975–2979
13. Kam NWS, Jan E, Kotov NA. Electrical stimulation of neural stem cells mediated by humanized carbon nanotube composite made with extracellular matrix protein. Nano Lett 2009; 9(1):273–278
14. Lovat V, Pantarotto D, Lagostena L, et al. Carbon nanotube substrates boost neuronal electrical signaling. Nano Lett 2005; 5(6):1107–1110
15. Wang K, Fishman HA, Dai HJ, et al. Neural stimulation with a carbon nanotube microelectrode array. Nano Lett 2006; 6(9):2043–2048
16. Mattson MP, Haddon RC, Rao AM. Molecular functionalization of carbon nanotubes and use as substrates for neuronal growth. J Mol Neurosci 2000; 14(3):175–182
17. Hu H, Ni YC, Montana V, et al. Chemically functionalized carbon nanotubes as substrates for neuronal growth. Nano Lett 2004; 4(3):507–511
18. Yu Z, McKnight TE, Ericson MN, et al. Vertically aligned carbon nanofiber arrays record electrophysiological signals from hippocampal slices. Nano Lett 2007; 7(8): 2188–2195
19. Heuschkel MO, Fejtl M, Raggenbass M, et al. A three-dimensional multi-electrode array for multi-site stimulation and recording in acute brain slices. J Neurosci Meth 2002; 114(2): 135–148
20. Balgude AP, Yu X, Szymanski A, et al. Agarose gel stiffness determines rate of DRG neurite extension in 3D cultures. Biomaterials 2001; 22(10):1077–1084
21. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005; 310(5751):1139–1143
22. Nguyen-Vu TD, Chen H, Cassell AM, et al. Vertically aligned carbon nanofiber architecture as a multifunctional 3-D neural electrical interface. IEEE Trans Biomed Eng 2007; 54(6 Pt 1): 1121–1128

23. Keefer EW, Botterman BR, Romero MI, et al. Carbon nanotube coating improves neuronal recordings. Nat Nanotechnol 2008; 3(7):434–439
24. Shoval A, Adams C, David-Pur M, et al. Carbon nanotube electrodes for effective interfacing with retinal tissue. Front Neuroengineering 2009; 2(4):8
25. Gabay T, Ben-David M, Kalifa I, et al. Electro-chemical and biological properties of carbon nanotube based multi-electrode arrays. Nanotechnology 2007; 18(3):035201 (6pp)
26. Sorkin R, Gabay T, Blinder P, et al. Compact self-wiring in cultured neural networks. J Neural Eng 2006; 3(2):95–101
27. Cui X, Wiler J, Dzaman M, et al. In vivo studies of polypyrrole/peptide coated neural probes. Biomaterials 2003; 24(5):777–787
28. Hochberg LR, Serruya MD, Friehs GM, et al. Neuronal ensemble control of prosthetic devices by a human with tetraplegia. Nature 2006; 442(7099):164–171
29. Miller RF, Dowling JE. Intracellular responses of the Muller (glial) cells of mudpuppy retina: their relation to b-wave of the electroretinogram. J Neurophysiol 1970; 33(3):323–341
30. Ruffini G, Dunne S, Fuentemilla L, et al. First human trials of a dry electrophysiology sensor using a carbon nanotube array interface. Sensors and Actuators a-Physical 2008; 144(2): 275–279
31. Helland A, Wick P, Koehler A, et al. Reviewing the environmental and human health knowledge base of carbon nanotubes. Environ Health Perspect 2007; 115(8):1125–1131
32. Stern ST, McNeil SE. Nanotechnology safety concerns revisited. Toxicol Sci 2008; 101(1): 4–21
33. Klumpp C, Kostarelos K, Prato M, et al. Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. Biochim Biophys Acta 2006; 1758(3):404–412
34. Cherukuri P, Gannon CJ, Leeuw TK, et al. Mammalian pharmacokinetics of carbon nanotubes using intrinsic near-infrared fluorescence. Proc Natl Acad Sci USA 2006; 103(50): 18882–18886
35. Liu Z, Cai W, He L, et al. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. Nat Nanotechnol 2007; 2(1):47–52
36. Liu Z, Davis C, Cai W, et al. Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy. Proc Natl Acad Sci USA 2008; 105(5):1410–1415
37. Singh R, Pantarotto D, Lacerda L, et al. Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. Proc Natl Acad Sci USA 2006; 103(9):3357–3362
38. Cui D, Tian F, Ozkan CS, et al. Effect of single wall carbon nanotubes on human HEK293 cells. Toxicol Lett 2005; 155(1):73–85
39. Manna SK, Sarkar S, Barr J, et al. Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-kappaB in human keratinocytes. Nano Lett 2005; 5(9):1676–1684
40. Monteiro-Riviere NA, Nemanich RJ, Inman AO, et al. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. Toxicol Lett 2005; 155(3):377–384
41. Jia G, Wang H, Yan L, et al. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Environ Sci Technol 2005; 39(5):1378–1383
42. Bottini M, Bruckner S, Nika K, et al. Multi-walled carbon nanotubes induce T lymphocyte apoptosis. Toxicol Lett 2006; 160(2):121–126
43. Magrez A, Kasas S, Salicio V, et al. Cellular toxicity of carbon-based nanomaterials. Nano Lett 2006; 6(6):1121–1125
44. Kagan VE, Tyurina YY, Tyurin VA, et al. Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron. Toxicol Lett 2006; 165(1):88–100
45. Pulskamp K, Diabate S, Krug HF. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol Lett 2007; 168(1):58–74

46. Sato Y, Yokoyama A, Shibata K, et al. Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell line THP-1 in vitro and subcutaneous tissue of rats in vivo. Mol Biosyst 2005; 1(2):176–182
47. Dubin RA, Callegari GC, Kohn J, et al. Carbon nanotube fibers are compatible with mammalian cells and neurons. IEEE Trans Nanobiosci 2008; 7(1):11–14
48. Parpura V. Instrumentation: carbon nanotubes on the brain. Nat Nanotechnol 2008; 3(7): 384–385
49. Hubel DH. Tungsten microelectrode for recording from single units. Science 1957; 125(3247):549–550