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Diamond electrodes for neurodynamic studies in *Aplysia californica*

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Abstract

As part of an ongoing effort to demonstrate that diamond is a versatile electrode material for biological applications, we present our progress in the development of diamond electrodes for the study of neurodynamics in an animal model, *Aplysia californica*. Diamond provides a unique opportunity to integrate neural stimulation and sensing in the same implantable device. Data from several parallel studies are presented: in vitro measurement of serotonin concentration, measurement of electrical activity, and neural stimulation. Using diamond microelectrodes, changes of the *Aplysia californica*'s feeding patterns are being studied as a function of concentration of serotonin, which can act as a neuromodulator of feeding behavior.

Keywords: Hot filament CVD; Diamond film; Sensors; Biomedical applications

1. Introduction

Conductive diamond is a chemically and mechanically robust sensor material that enables lower analyte concentrations and new chemistries to be investigated because of its low baseline current and wide potential water window of water stability, respectively. [1–4] The first report [5] of diamond for electrochemical applications was in 1983, with several reviews of the field. [3,4,6,7] Diamond electrodes show, by far, the *most stable* response of *any* electrode, and do not require extensive pretreatment to regenerate the electroactive surface. [3,8] Diamond provides advantage in chemical functionalization [9–11] to enhance sensor specificity. Also, a diamond electrode's response is relatively insensitive to oxygen, the ubiquitous biochemical interferent. [12] Preliminary testing of diamond electrodes as detectors of various bioanalytes, e.g., dopamine [8,13,14], serotonin [15], and sulfa drugs [16], have shown the potential of diamond biosensors. Diamond electrodes should also expand neural stimulation capabilities by avoiding water hydrolysis that leads to tissue damage and by providing long-term stability [2].

We are exploring the advantages of diamond electrodes using a specific, relatively well-defined neural circuit that provides known controls for comparison and minimizes surgical challenges. At the same time, data from this newly developed diamond device may provide insights into behavioral functions of this neural circuit. *Aplysia californica*, a marine mollusk, is a commonly used animal model for neurodynamic studies. Our focus is on the buccal mass, i.e., the muscular structure for feeding, and the associated neural circuitry: a collection of cells that control the feeding motor patterns (the buccal ganglion), and a specific large cell (the metacerebral cell) in the cerebral ganglion that has serotonin processes throughout the buccal mass. The somata (cell bodies) of the buccal motor neurons are large (~50–150 μm),

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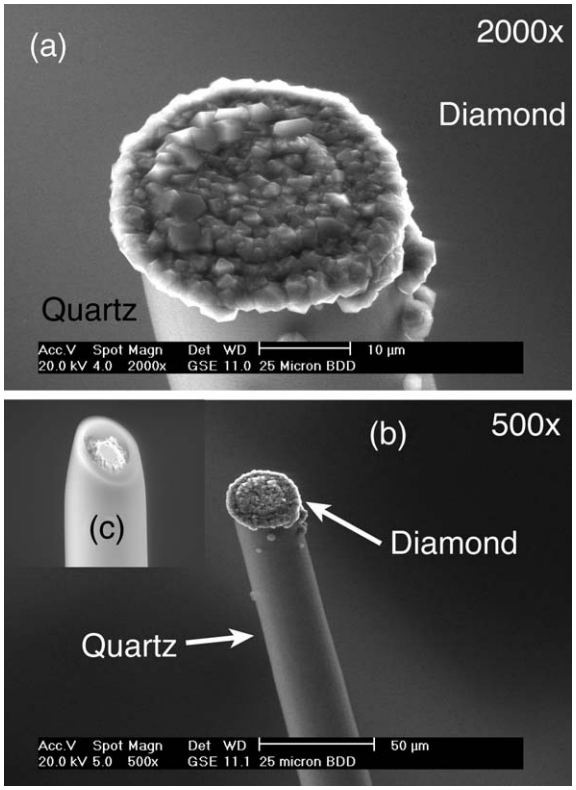

Fig. 1. SEM images of (a,b) selective diamond deposition onto a (c) tungsten microelectrode (quartz insulator); final tip diameter, 35 µm. [22].

identifiable, and readily accessible; thus, it is possible to obtain electrophysiology recordings and monitor chemical release from a single cell. In addition, neural activity in the buccal ganglion motor neurons can often be directly correlated with muscular response; e.g., patterns of buccal nerve recordings in vivo (corresponding to recordings of multiple neurons firing) can distinguish between the three fundamental feeding behaviors: biting, rejecting, and swallowing [17]. All three behaviors are initiated by the activation of the I2 muscle that pushes the central grasper forward [18].

The metacerebral cell (MCC) in the cerebral ganglion is connected to the buccal ganglion through the cerebral–buccal connective (CBC); it has multiple roles in feeding behavior that, in part, involve the release of serotonin, a neuromodulator, throughout the buccal muscular structure [19]. The MCC contains approximately 0.94 ± 0.61 mM serotonin in the soma and 4% is released with the generation of an action potential [20,21].

Through real-time detection with diamond electrodes, we seek to determine the release site and concentration of *serotonin* from the MCC during a normal feeding pattern. In addition, we will explore diamond's capabilities as a sensor for electrical activity and a neural stimulating electrode. Our long-term goal is to integrate these functions into a single diamond device that can both manipulate and monitor aspects of neural activity. Our animal model, *Aplysia*, provides unique advantages for development of an integrated device since all three aspects – stimulation, electrical activity and neurotransmitter sensing – can be probed at the neuron cell body.

2. Methods

Microelectrodes (~30 µm diameter) were fabricated (Fig. 1) using hot-filament-assisted CVD to selectively deposit boron-doped diamond (Fig. 1a and b) onto a tungsten microelectrode

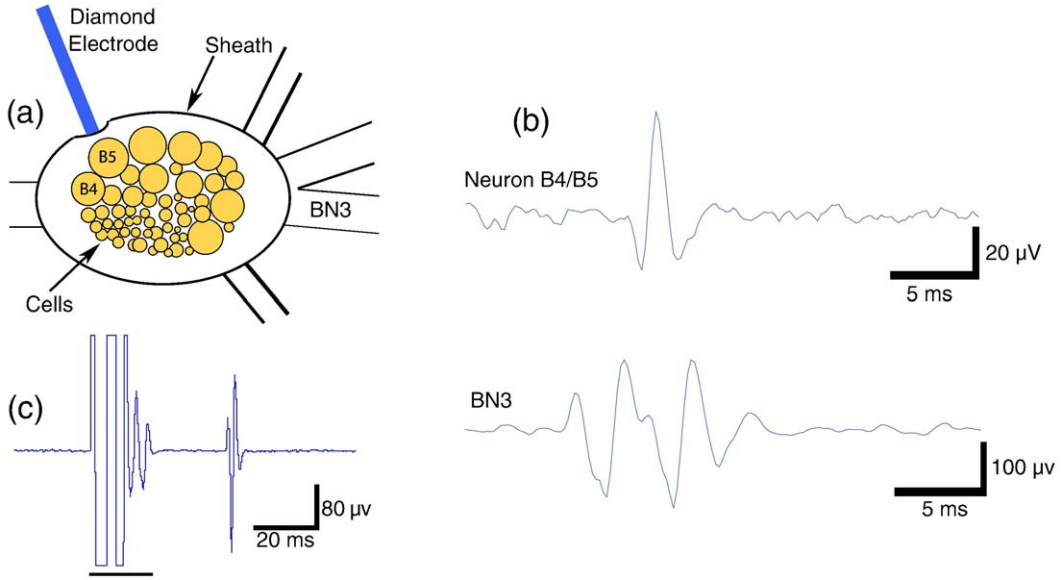

Fig. 2. Extracellular recording and stimulation using a diamond microelectrode. (a) Schematic of the in vitro, extracellular recording/stimulation apparatus. The diamond electrode is pressed into the insulating sheath over a neural cell of interest to specifically record or stimulate that cell. (b) The diamond electrode (top) recorded single-cell activity above the B4 or B5 cell, while a suction electrode on the nerve (bottom) recorded activity from both cells. (c) Extracellular stimulation at B4 or B5 produced a clear, single action potential after stimulation.

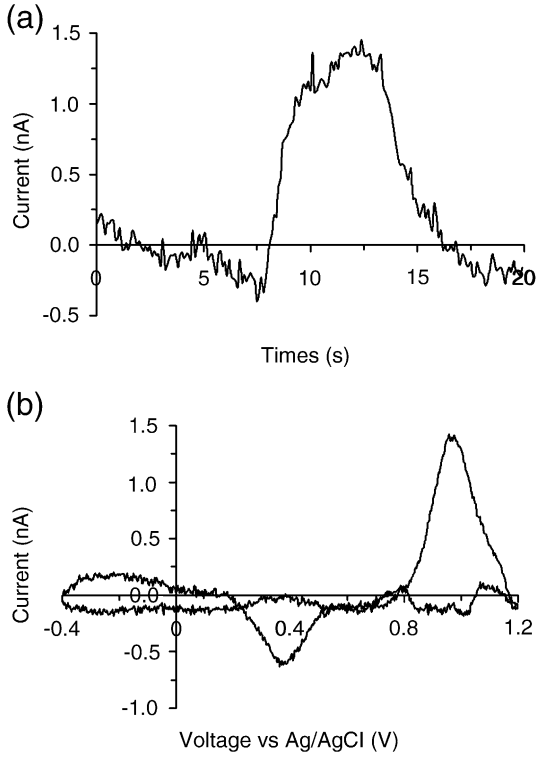

Fig. 3. Serotonin detection (1 nM in *Aplysia* saline) using fast scan cyclic voltammetry (300 V/s) combined with flow injection analysis. The signal to noise ratio was 17.2; all scans were background subtracted from the first 5 s of saline injected. (a) The representative peak current vs. time profile obtained at +0.958 V vs. SCE, for likely the first electron transfer step in serotonin oxidation. (b) The cyclic voltammogram of serotonin with an oxidation peak at +0.958 V and a reduction peak at +0.362 V.

(Fig. 1c); for details, see Ref. [22]. Flow cell calibrations were used to estimate detection limits; background-subtracted, fast scan cyclic voltammetry (FSCV) was the serotonin detection method. A linear voltage sweep, from −0.4 V to +1.2 V vs. Ag/AgCl, at a rate of 300 V/s was applied at 10 Hz. Typically, 10 buffer scans were averaged for the background subtraction. Standard electrophysiology techniques were employed for measurement of electrical activity.

Aplysia were anesthetized (333 mM $MgCl_2$ solution injection) and the cerebral and buccal ganglia, with the buccal mass, removed; these studies were limited to in vitro preparations, with intention to eventually conduct in vivo studies. The lipid sheath (insulating layer surrounding the cells) was removed as necessary for intracellular or electrochemical measurements. As controls, standard Ag/AgCl pipette and suction electrodes were used for intracellular (i.e., within the cell), and extracellular (i.e., outside the cell with, typically, the sheath intact) measurements, respectively. For intracellular recordings, a Ag/AgCl electrode (housed in a sharply pulled glass pipette, <1 μm diameter, potassium acetate electrolyte) was inserted into the soma and the time varying, membrane potential recorded; this technique provides a direct measurement of electrical activity from a specific cell. Additionally, intracellular electrodes can inject small amounts of current within the cell, enabling simultaneous stimulation and recording.

Extracellular techniques are less invasive and thus more desirable for in vivo studies, but penetration of the sheath will eventually be necessary for in vivo detection of serotonin. In our initial in vitro studies, diamond micro-

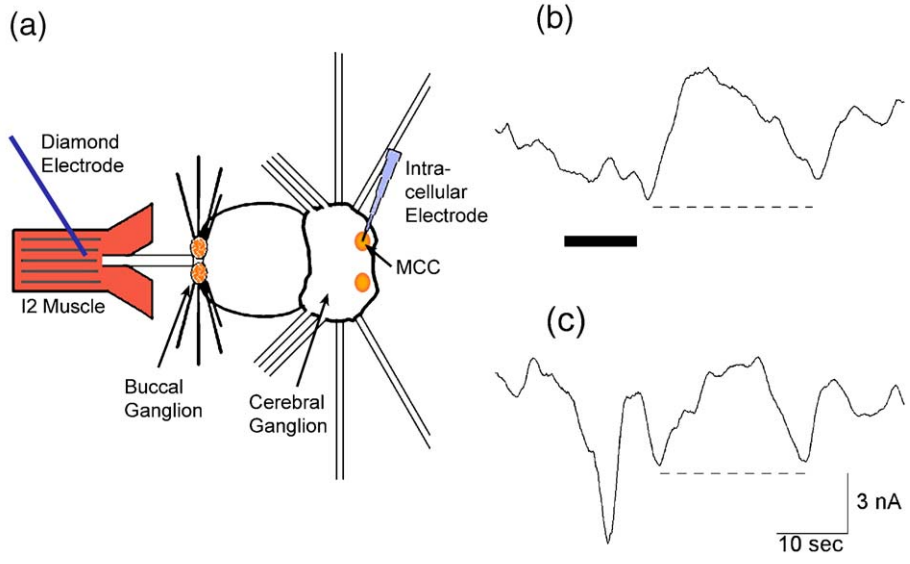

Fig. 4. In vitro detection of serotonin at the I2 muscle of the *Aplysia*. (a) Experimental Setup – The cerebral ganglion is desheathed in a Petri dish with inlet and outlet flow of high divalent cationic solution. An intracellular electrode is placed inside the MCC and a diamond electrode is placed within the muscle fibers of the I2 muscle. (b) MCC stimulation – The muscle tissue is washed for 10 min keeping the MCC at rest before experiment. A background current is taken for the first 10 s, then a stimulus is added causing 36 action potentials (black bar), and a current vs. time is recorded at 0.667 V vs. Ag/AgCl. (c) Control – After wash, 10 mL of 10 nM serotonin was added to the bath and a similar current vs. time change was recorded at 0.667 V vs. Ag/AgCl.

electrodes were tested to either (a) detect extracellular serotonin from a *desheathed* ganglia, or (b) extracellularly record electrical activity or stimulate cells from outside the intact sheath. For recording or stimulating through the sheath, the extracellular diamond electrode was pressed into the sheath above the desired cell, resulting in the sheath surrounding the electrode, insulating it from the water (Fig. 2a). The diamond electrode can stimulate or record. Standard, extracellular suction electrodes measured nerve activity (i.e., the combined signal representing activity in axons from multiple neurons). Each neuron was distinguished by visual inspection and by its signal amplitude on the nerve. *Aplysia* saline (450 mM NaCl, 33 mM $MgSO_4$•$7H_2O$, 22 mM $MgCl_2$•$6H_2O$, 10 mM KCl, 10 mM $CaCl_2$•$2H_2O$, 10 mM MOPS, and 10 mM glucose) was used for the electrophysiological measurements; high divalent cationic solution (334 mM NaCl, 40 mM $MgCl_2$•$6H_2O$, 10 mM KCl, 40 mM $CaCl_2$•$2H_2O$, 10 mM MOPS, and 10 mM glucose) was used for chemical measurements to eliminate spontaneous neuron activity.

3. Results

Using the diamond microelectrode, preliminary, extracellular recording and stimulation were conducted from a single neuron, B4 or B5 (two electrically coupled neighboring neurons) (Fig. 2). Although our ultimate interest is in the MCC, the neurons B4/B5 were used for preliminary studies because they are the most surgically accessible, and our group has extensive experience in studying their function. An action potential produced by neurons B4/B5 is the largest signal on the nerve designated buccal nerve 3 (BN3). For extracellular recording from B4/B5, a one-to-one relationship was observed on the diamond electrode, indicating that it was selectively recording from one neuron, whereas the suction electrode on BN3 recorded two signals, reflecting activity in both neurons (Fig. 2b). For extracellular stimulation by the diamond electrode, a one-to-one relationship was also observed, indicating that one neuron was stimulated without activating neighboring cells (Fig. 2c).

In flow cell calibration, nominally 1 nM serotonin was detected (Fig. 3). Prior to study of in vitro serotonin release from the MCC, connections between the MCC and the buccal mass were established (data not shown); the MCC has processes to the I2 muscle, the primary muscle for protraction [23]. Therefore, it is expected that serotonin will be released from the processes of the MCC that are within the I2 muscle. In parallel, we established that exogenously injected serotonin enhances the I2 muscle's contractile strength, thus indicating that serotonin plays a direct role in the behavioral function of I2 [23]. To elicit serotonin release from the MCC, an intracellular (Ag/AgCl) electrode was placed in the MCC, and a diamond electrode was placed at the muscle fibers of an intact I2 muscle (Fig. 4a). Upon a generation of 36 action potentials within the MCC, a measurable level of serotonin was detected, with the peak current at +0.667 V vs. Ag/AgCl (Fig. 4b). As a control, 10 mL of 10 nM serotonin was added to the bath and a similar peak current at +0.667 V vs. Ag/AgCl observed (Fig. 4c).

4. Conclusions

We have shown that diamond electrodes can record activity and stimulate specific cells in vitro. Diamond's specificity was verified through the controls. The same diamond electrode was able to qualitatively record increased levels of serotonin at the I2 muscle upon specific stimulation of the MCC; additional calibration is necessary for further quantification of the level of release. Overall, we have demonstrated that diamond electrodes can record, stimulate, and detect neurochemicals in the same animal model. In the future, these functions will be integrated at the same diamond electrode, and incorporated into microelectrode arrays, for mapping the chemical and electrical signals in vivo during a complete feeding behavior.

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