Advancing neurochemical monitoring

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Two new approaches to neurochemical monitoring vivo—an improved real-time microsensor and genetically engineered cells that sense neurotransmitter levels—address the critical issue of brain reactivity to implanted devices.

Identifying the neural basis of behavior is a core focus of neuroscience. One prominent methodology in this pursuit is monitoring the neurotransmitters that underlie communication between neurons. Although technical improvements have advanced neurochemical measurements to the real-time domain, one critical limitation of present methods is the highly invasive nature of implanting a recording device and the subsequent reaction of brain tissue. Neuroinflammation not only alters the sampled microenvironment, but also results in a diffusion barrier that encapsulates the probe and therefore restricts access to released neurotransmitters. Taking radically different strategies, two new approaches address this key hurdle for achieving the long-standing goal of chronic, real-time neurochemical monitoring. In this issue of Nature Methods, Clark et al.[1] describe a microelectrode that retains the capability for subsecond dopamine measurements in

vivo for months. In Nature Neuroscience, Nguyen et al.[2] report implantable genetically engineered cells for electrode-free acetylcholine sensing.

Microdialysis[3] and voltammetry[4] have dominated the modern era of neurochemical monitoring invivo. With exquisite sensitivity and selectivity by virtue of removing brain analytes for ex vivo determination, microdialysis is better suited for measuring basal neurotransmitter levels. By using electrochemistry at the probe tip for in situ detection, the superior temporal resolution of voltammetry is more appropriate for capturing faster chemical signals.

Recent advances in voltammetry have overcome the historical criticisms of poor sensitivity and chemical specificity. Indeed, by providing nanomolar and subsecond measurements and a chemical signature in the form of a voltammogram, fast-scan cyclic voltammetry (FSCV) has met the demanding analytical criteria for monitoring phasic dopamine

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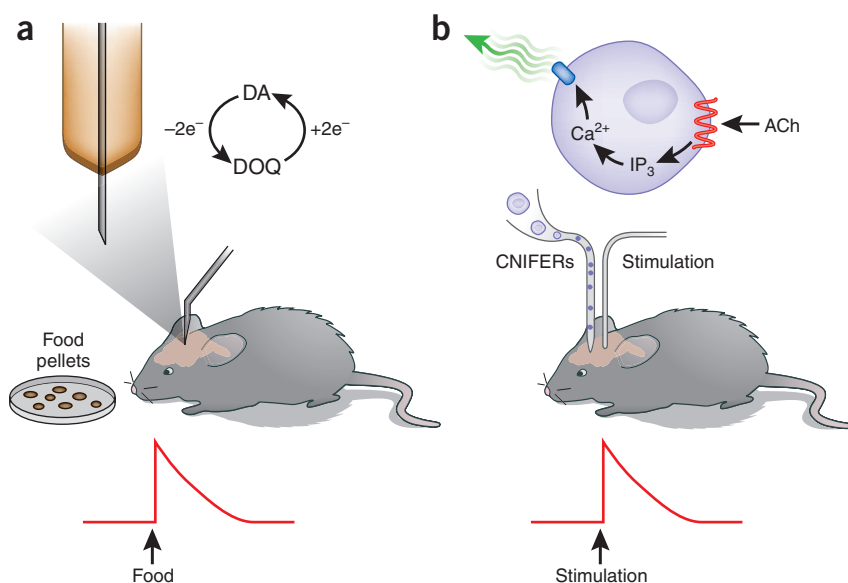
Figure 1 | New strategies for neurochemical monitoring *in vivo*. (**a**) The sensing portion of the carbon-fiber microelectrode is the exposed length of a single carbon fiber (7 μm diameter) sealed in the insulating fused-silica capillary. In fast-scan cyclic voltammetry, dopamine (DA) is oxidized to dopamine-ortho-quinone (DOQ) and then reduced to DA again. Unexpected food delivery transiently elevates brain dopamine. (**b**) CNiFERs respond to neuronally released acetylcholine (ACh) *in vivo* by increasing intracellular Ca^{2+} levels via a G protein–coupled mechanism; Ca^{2+} is then detected via altered fluorescence of expressed protein Ca^{2+} sensors. Electrical stimulation elicits rapid changes in cortical acetylcholine.

release in freely behaving rats[5]. Important for goal-directed behavior, these transient signals are generated by short bursts of rapid neuronal firing in response to primary rewards and to cues predictive of these rewards[6]. Excessive drug-induced phasic dopamine release, thought to modulate synaptic plasticity, may underlie a 'pathological usurpation' of reward-related learning mechanisms in addiction[7]. However, to avoid signal loss with chronic implantation, monitoring dopamine release with FSCV relies on an acutely lowered probe, which obviates longitudinal studies comparing dopamine responses in the same animal across many days.

Clark *et al.*[1] modify the conventional carbon-fiber microelectrode (CFM) to develop a chronically implantable probe for FSCV (**Fig. 1a**). This alternative design, which replaces the borosilicate shaft with a polyimide-coated fused-silica capillary, was viable when implanted in the rat brain for monitoring phasic dopamine release associated with unexpected food reward for up to 4 months. Rather amazingly, the researchers found no glial encapsulation around the electrode shaft or carbon-fiber tip. The authors did not determine what exactly about this new probe minimizes tissue reactivity, but fused silica is less

rigid than borosilicate and polyimide is highly biocompatible.

Regardless, and attesting to the utility of the new CFM design for extended learning paradigms, Clark *et al.*[1] monitored dopamine for 25 consecutive days in the same rat during Pavlovian conditioning. In the first phase of this experiment, dopamine release evoked by food delivery was stably transferred to a cue predicting this reward. In the second phase, increasing reward value (that is, providing more food) altered dopamine dynamics, and omission of food delivery extinguished the responses.

Biosensors, which use a 'biological recognition element' to interact with an analyte, have been developed for monitoring non-electroactive neurotransmitters that go undetected with voltammetry[8]. A common design for such biosensors incorporates an oxidase enzyme generating the electroactive hydrogen peroxide, but others have been developed. One of the earliest biosensors was the 'sniffer pipette', constructed from an excised membrane patch containing ionotropic cholinergic receptors[9]. Binding of acetylcholine, a neurotransmitter implicated in cognitive processes and schizophrenia[10], opens ion channels that are interrogated

electrophysiologically. Optical imaging techniques have also been recently used for biosensing, using implantable cells containing organic dyes for imaging Ca^{2+} *in vivo*[11] and using neurotransmitter-sensitive fluorescent proteins expressed in cultured neurons[12].

Nguyen *et al.*[2] make use of these recent advances in biosensing to develop electrode-free, noninvasive methodology for monitoring acetylcholine. In their cell-based reporters, called CNiFERs—a clever twist of both the moniker and the approach of the original 'sniffer pipette'—detection is also based on cholinergic receptors, in this case a metabotropic receptor. CNiFERs are an immortal cell line genetically engineered to express a fluorescent Ca^{2+} protein sensor and the M1 muscarinic receptor (**Fig. 1b**). Binding of acetylcholine initiates a biochemical cascade involving a G protein, phospholipase C and the second messenger inositol trisphosphate, ultimately leading to increased intracellular Ca^{2+} levels and altered fluorescence.

When chronically implanted in the rat cortex and monitored by two-photon laser-scanning microscopy, CNiFERs robustly respond to basal and electrically evoked levels of acetylcholine for up to six days[2]. Suggestive of a physiological role, measured signals temporally coincided with the electrophysiological response recorded locally. Most importantly, there was minimal tissue damage at the implantation site, as indicated by few reactive astrocytes, no cell proliferation and an intact vasculature.

What does the future hold for these new neurotransmitter sensing technologies? As it is already coupled to an established monitoring technique, the new CFM developed by Clark *et al.*[1] appears poised for immediate applications. The small shaft size compared to the conventional CFM (80 μm versus ~1 mm) permits implantation of several probes in a single animal for concurrent assessment of regional differences in dopamine dynamics. Incorporating telemetry into the probe design would also remove the restriction of the cable tether linking animal and recording equipment. CNiFERs developed by Nguyen *et al.*[2] are at a more nascent stage. Because the monitoring scheme based on G protein–coupled receptors should generalize to most neurotransmitters, this methodology has great

promise for broad applications in neuro-chemical monitoring in vivo. Extending these cell-based measurements to deep brain structures and to awake, especially freely behaving animals would be very attractive as well.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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