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A method for the intracranial delivery of reagents to voltammetric recording sites

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Abstract

Carbon fiber microelectrodes are widely used for electrochemical monitoring in the intact brain. The local delivery of reagents to the recording site is often desirable. The approach of coimplanting a micropipette near the microelectrode presents some limitations that are overcome by the use of double-barreled devices. One barrel supports the carbon fiber and the other barrel serves as a pipet for local reagent delivery. Some studies have used iontophoretic delivery but here we consider the alternative approach of pressure ejection. However, placing the pipet so close to the electrode raises the risk that reagent can leak into the recording site. This problem is easily solved. We filled the tip of the pipet with vehicle solution, the barrel with a reagent solution, and separated the two solutions with an air gap to prevent their mixing. With this approach, reagent is delivered only after ‘priming’ pressure pulses: we show in two examples that unintended reagent delivery (leakage) prior to the priming pulses is non-detectable.

Keywords

Dopamine; in vivo voltammetry; double barreled electrode; electrical stimulation; striatum; segmented flow

1.0 INTRODUCTION

Voltammetry in conjunction with carbon fiber microelectrodes (Michael et al., 2007) provides unique insights into the neurochemistry of the intact brain. For example, voltammetry has elucidated significant details of the activity of dopamine (DA) in the rat striatum and nucleus accumbens (Kita et al., 2007; May and Wightman, 1989a, b; Moquin and Michael, 2009; Rodriguez et al., 2006; Zachek et al., 2010). Combining voltammetry with intracranial, rather than systemic, delivery of reagents to the recording site is a powerful enhancement of the technique. For example, the broadly distributed dopamine D2 receptor (D2R) plays a central role in regulating DA (Benoit-Marand et al., 2001; Cass and Gerhardt, 1994; Herr et al., 2010; Moquin and Michael, 2011). Local delivery of D2R agonists and antagonists to the recording site allows the role of D2Rs in DA terminals fields to be distinguished from that of D2Rs in the midbrain. Local reagent delivery also aids the in vivo calibration of microelectrodes (Garguilo and Michael, 1993; Herr et al., 2008) and the ultrastructural analysis of the recording sites (Peters et al., 2004).

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The co-implantation of glass (Cass et al., 1993; Cragg et al., 2001; Daws and Toney, 2007; Makos et al., 2009; Wang et al., 2010) and fused silica (Kulagina et al., 1999) pipets has been used to deliver reagents near recording sites. Co-implantation is somewhat cumbersome, involves two penetrations of the brain, and limits the precision of the delivery site relative to the recording site. Sufficient reagent to assure delivery across the distance of separation (typically 100–200 μm) is necessary (Sabeti et al., 2002). It is difficult to assess the geometrical symmetry of the delivery, which further limits precision of the method. And, in some cases the reagent is delivered into the pipet track rather than into the tissue itself (Garguilo and Michael, 1996).

A double-barreled electrode (DBE) solves these limitations (Herr et al., 2008; Moquin and Michael, 2011). One barrel contains the carbon fiber while the other barrel remains open and serves as the delivery pipet. This eliminates the need for double penetration of the brain and assures that reagent is always delivered to the recording site: this is especially valuable when the recording site is changed during an experiment, for example, to optimize the site (Garris et al., 1993). However, the close proximity of the pipet tip to the electrode raises the risk (see Results) that the reagent can affect the electrochemical recording by leaking from the pipet tip: here, we show that this is easily solved.

The leak problem is easily solved by introducing an air gap into the pipet to separate the fluid in the pipet tip from that in the pipet barrel. This does not prevent the leak per se, but it does prevent the leak from carrying reagent into the recording site.

2.0 MATERIALS AND METHODS

2.1 Double barreled electrodes (DBE)

A carbon fiber (7- μm diameter, T650, Cytac Carbon Fibers LLC., Piedmont, SC) was inserted into one barrel of a double-barreled borosilicate glass capillary (dimensions prior to pulling 0.68 mm ID, 1.2 mm OD, A-M systems Inc., Sequim, WA). The double-barreled capillary was pulled to a fine tip with a vertical micropipette puller (Narishige, Los Angeles, CA). One barrel pulled snug around the carbon fiber and the other, which remains open, forms the pipet. Puller settings can be adjusted to alter the diameter of the open tip. To complete the electrode construction, the electrode barrel was backfilled with low-viscosity epoxy (Spurr Epoxy, Polysciences Inc., Warrington, PA) and the exposed fiber was trimmed to a length of 400 μm . Electrical contact between the fiber and a nichrome contact wire (Goodfellow, Huntingdon, Cambridgeshire, UK) was by means of a mercury droplet. For comparison purposes, single barrel electrodes were made by the same procedure.

The tips of some DBEs were examined by scanning electron microscopy (Fig 1). The EM images show that the glass forms a seal around the carbon fiber but leaves the pipet tip open. The dimensions of the tip depend on the pulling conditions. Two of the DBEs in the images of Fig 1 appear to be cracked (middle and right panel). The cracks visible in these images were caused by the EM procedures, most likely the vacuum. The DBEs used in the experiments reported below were not cracked.

2.2 Fast scan cyclic voltammetry

Fast scan cyclic voltammetry (FSCV) was performed with a high-speed potentiostat (EI-400, Ensmann Instruments, Bloomington, IN) and the program “CV Tar Heels v4.3” (courtesy of Dr. Michael Heien, Department of Chemistry, University of Arizona). The rest potential was 0 V vs Ag/AgCl and the voltammetric waveform consisted of three linear potential sweeps to +1 V, -0.5 V, and back to 0 V at a sweep rate of 400 V/s and with a repetition rate of 10 Hz. The DA oxidation current was recorded between 0.5 and 0.7 V of the first sweep. DA voltammograms were obtained by background subtraction.

2.3 Reagent delivery

The reagents delivered to the recording sites in this work were DA and raclopride, a D2 antagonist (both used as-received from Sigma Aldrich, St. Louis, MO). Dopamine hydrochloride (25 mM) and raclopride tartrate (2 mM) were dissolved separately in artificial cerebral spinal fluid solution (aCSF: 1.2mM Ca^{2+} , 152mM Cl^- , 2.7mM K^+ , 1.0mM Mg^{2+} , 145mM Na^+ , pH 7.4). In initial trials, we filled the pipet with one or other of the reagent solutions: however, this approach revealed the leak problem (see Results). Subsequently, we filled the pipet tip with aCSF vehicle, the pipet barrel with reagent dissolved in aCSF, and separated the two solutions with an air gap (1-1.5 mm in length) to prevent their mixing. To eject fluid from the pipet, we used a Picospritzer III (Parker Hannifin, Fairfield, NJ) to apply pressure pulses (20 psi N_2 , 0.2 s to 3 s) to the pipet barrel.

2.4 Beaker experiments

We characterized the DBEs by pressure ejecting DA into a beaker containing phosphate buffered saline (PBS: 155 mM Na^+ , 155 mM Cl^- , 100 mM phosphate, pH 7.4) and monitoring the ejected DA by FSCV.

2.5 In vivo experiments

Male Sprague-Dawley rats (250–350 g) (Hilltop, Scottsdale, PA) were anesthetized with isoflurane (2% by vol.) and placed in a stereotax (David Kopf Instruments, Tujunga, CA) with the incisor bar raised 5 mm above the interaural line (Pellegrino et al., 1979). A heating blanket (Harvard Apparatus, Holliston, MA) maintained body temperature at 37°C. A twisted, bipolar, stainless steel stimulating electrode was placed over the medial forebrain bundle (MFB) at the following coordinates from bregma: 1.6 mm lateral, 2.2 mm posterior, 8.0 mm below dura. The recording electrode was placed in the ipsilateral striatum (from bregma: 2.5 mm lateral, 2.5 mm anterior, initially 4.5 mm below dura). The stimulating electrode was lowered in small steps until evoked DA release was observed in the striatum: this is a well-established protocol for stimulating ascending DAergic fibers (Ewing et al., 1983; Heien et al., 2005; Kuhr et al., 1984). Baseline signals were recorded 20 minutes later.

2.6 Electrical stimulation

The stimulus was an optically isolated, constant-current, biphasic waveform (frequency 60 Hz, pulse height 270 μA , pulse width 2 ms, duration 3 s).

2.7 Electrode calibration

Voltammetric responses recorded in vivo were converted to units of DA concentration by post-calibrating the electrodes in DA standards dissolved in aCSF.

2.8 Data analysis

The maximum amplitude of evoked or exogenous DA was measured using the DA oxidation potential (~650 mV) at the end of each event. Statistical analysis was by repeated measures ANOVA with a Tukey post-hoc test.

3.0 RESULTS

3.1 The pipet leaks

Conventional single barrel electrodes typically equilibrate and reach a stable baseline within 20 min of insertion into brain tissue (Borland and Michael, 2004; Heien et al., 2005; Phillips et al., 2003; Robinson et al., 2003; Wightman et al., 2007). The baseline noise from DBEs with solution in the pipet is approximately twice that of conventional electrodes (Fig 2A).

The extra baseline noise provided a preliminary indication that leakage from the pipet can affect the electrochemical signals even without any purposeful ejection procedure. There was no visual evidence of solution flowing from the pipet tips prior to their insertion into the brain. So, the leakage process may be diffusional, as solutes exchange between the pipet and the surroundings due to concentration gradients.

3.2 Raclopride affects evoked DA release without purposeful ejection

DBEs preloaded with raclopride (2 mM in aCSF; a new DBE in each rat) were implanted into the striatum and used to record evoked DA release during electrical stimulation of the MFB. An initial evoked DA response was recorded immediately after positioning the DBE into a naïve recording site: to establish a naïve recording site, the DBE was lowered 0.5 mm deeper into the brain with the stereotaxic manipulator. The amplitude of a second evoked response, recorded 5 min later at the same site, was consistently and significantly larger (Fig 2B, see figure legend for ANOVA details). Because no purposeful pressure ejection was performed in these experiments, we conclude that the increased response amplitude is due to the effects of raclopride that leaked into the recording site from the pipet tip. Raclopride's ability to increase the amplitude of evoked DA responses by blocking presynaptic D2 autoreceptors is well known (Benoit-Marand et al., 2007; Herr et al., 2010; May and Wightman, 1989a; Moquin and Michael, 2009).

3.3 The air gap: a simple yet effective solution

Several attempts to prevent the raclopride leak were unsuccessful (see Discussion). But, the introduction of an air gap into the delivery pipet (Fig 3) was both simple and effective. In this approach, the pipet tip is filled with aCSF and the barrel is filled with the solution containing the reagent of interest. The two solutions are separated by an air gap (~1 mm in length) that prevents their mixing. Pressure pulses (20 psi N₂, 0.2–3s, Picospritzer III) compressed the air gap (visible under a stereomicroscope) and caused the barrel solution to mix with the tip solution (Fig 3B). After several priming pulses, the reagent of interest is ejected from the tip (Fig 3C). The air gap always remains in-place inside the pipet.

3.4 Beaker characterization of the air gap's function

DBEs with aCSF in the tip and DA (25 mM in aCSF) in the barrel were tested in beakers containing PBS. No DA response was observed during an initial series of pressure pulses (20 psi, 0.5 s, see Fig 4a: the circles mark the start of each ejection). Clear DA responses were observed during subsequent pulses (Fig 4A), confirming that DA is ejected only after the initial priming pulses. The observed responses were consistent among several (n=3) DBEs (Fig 4B). Thus, DA ejection did not occur until after the initial series of pressure pulses primed the pipet by mixing the DA solution in the barrel with the aCSF solution in the tip. There was no detectable DA signal prior to the priming pulses.

The background-subtracted cyclic voltammogram collected after the 2nd pressure pulse exhibits a small-amplitude, non-DA peak (Fig 4C). This 'aCSF peak' is likely due to differences in the composition of aCSF and the PBS. The background-subtracted voltammogram collected after the 5th pressure pulse exhibits both the aCSF peak and the expected DA peaks (Fig 4D). Subtraction of the aCSF peak (Fig 4C) from the aCSF+DA peaks (Fig 4D) yields a "clean" DA voltammogram that matches the DA voltammogram obtained during calibration (Fig 4E). This confirms that DA ejection is responsible for the signal observed after the priming pulses.

3.5 Characterizing the air gap in vivo

DBEs prepared with aCSF in the tip and DA (25 mM in aCSF) in the barrel were implanted into the rat striatum. The air gap did not reduce the baseline noise in vivo (data not shown: the results were similar to those in Fig 2A), suggesting that solution continues to leak from the tip. In this case, however, the tip contains only aCSF and no other reagent of interest. No DA signal was observed during a series of priming pressure pulses (Fig 5A): voltammograms recorded during the priming pulses exhibited no DA peaks (Fig 5A, inset). DA responses were clearly observed during subsequent pressure pulses (voltammograms omitted: they resemble those in Fig 4). Thus, the air gap prevented DA delivery prior to the purposeful priming pulses.

DBEs with aCSF in the tip and raclopride in the barrel (2 mM in aCSF) were implanted into the striatum. A naïve recording site was established by lowering the DBE 0.5 mm deeper into the brain and two evoked responses were recorded 5 min apart: no purposeful raclopride ejection was performed during this phase of the experiment. The two initial evoked responses were essentially identical (Fig 5B), indicating that the airgap prevented delivery of raclopride into the recording site in the absence of pressure pulses (compare to Fig 2B). Next, pressure pulses were applied to the pipet until a non-specific voltammetric response signaled the ejection of raclopride (raclopride is not electroactive and did not cause consistent voltammetric responses but the change in composition of the solution near the electrode was sufficient to indicate that raclopride delivery had occurred). A third stimulus response recorded immediately after the last pressure pulse exhibited clearly the expected effects of autoreceptor blockade (Fig 5B). Thus, Fig 5 shows clearly that effective concentrations of raclopride were only delivered to the recording site after the purposeful application of pressure pulses to the pipet.

4.0 DISCUSSION

The DBE (Fig 1) enables the local delivery of reagents of interest to voltammetric recording sites in the brain. Preliminary studies (Fig 2) showed clearly that solution leaks from the pipet. This was not due, in any obvious way, to bulk flow from the pipet and is likely a diffusional process. If so, there is little that can be done to prevent solutes exchanging between the pipet tip and the surroundings. If the pipet is remote from the recording site, this phenomenon is not noticeable. However, in the case of the DBE the leakage affects the electrochemical signal. The air gap did not stop solute exchange per se but was highly effective at preventing the barrel solution from mixing with the tip solution until purposeful pressure pulses were applied to the pipet.

4.1 Tip leakage

Solute exchange between the tip and the surroundings (leakage) was readily apparent during preliminary DBE studies. The leakage increased the baseline voltammetric noise even if the pipet contained only aCSF (Fig 2A). The impact of raclopride on evoked DA responses, without any purposeful ejection procedure, was also clear (Fig 2B). Even under a microscope, there was no evidence of bulk flow from the pipets: no observable drops formed when the tips were suspended in air. However, it is possible droplet formation in air was prevented by surface tension at the tip, which would not exist once the tip was immersed in solution. Thus, it seems likely that the leakage is diffusional and due to concentration gradients between the tip solution and the surroundings. This might especially affect non-endogenous substances, such as raclopride, since the concentration of raclopride in surrounding brain tissue is zero.

4.2 Some pros and cons of the air gap approach

The most obvious drawback of this approach is the increased baseline noise (Fig 2) that raises the detection limit of the electrochemical recording: we have not attempted to quantify the detection limit of the DBEs.

The air gap (Fig 3) proved to be a simple yet effective means of preventing pressure ejection of reagents of interest (i.e. DA and raclopride) from the pipet barrel prior to a series of priming pulses both in a beaker (Fig 4) and in vivo (Fig 5). The number of priming pulses needed varied among DBEs because it was difficult to precisely control the volume of the tip solution or the air gap. But, during this work the voltammetric responses identified the onset of reagent delivery, even though raclopride is not electroactive (i.e. it does not oxidize or reduce at the carbon fiber electrode). The non-specific voltammetric response to raclopride was very effective at predicting the subsequent raclopride-enhanced evoked DA response (Fig 5B).

The air gap renders the DBE a “one shot device,” i.e. a new DBE must be prepared for each experiment. However, (see Moquin and Michael, 2011) this is quite feasible as the DBEs are not substantially more difficult to make than conventional carbon fiber electrodes. In practice, a new conventional electrode is used for each experiment too.

The reagent of interest is diluted during the priming procedure, so the concentration of the ejected reagent is uncertain. We should mention, however, that pipet infusions likely always involve concentration uncertainty due to the complexities of diffusion, clearance, and binding, etc., of reagents in the extracellular space of the brain. In our hands (see Moquin and Michael 2009 and 2011), the DBEs were useful despite this issue. For example, we used intrastriatal delivery of raclopride to confirm the role of striatal, as opposed to midbrain, DA receptors in regulating evoked DA release. Herr et al (2008, 2010) demonstrated the use of an electroactive “tracer” to quantify iontophoretic reagent delivery: pressure ejection does not exclude a similar approach.

The air gap approach permitted the in vivo quantification of DA clearance kinetics (Moquin and Michael 2011). The literature describes two approaches to quantifying DA clearance kinetics in vivo. In one, voltammetry is used to observe clearance after electrically evoked release of endogenous DA (Wu et al., 2001). In the other, voltammetry is used to observe DA clearance after the pressure ejection of exogenous DA into the extracellular space (Cass et al., 1993; Sabeti et al., 2002). These approaches have resulted in some controversy because clearance of exogenous DA usually appears to be slower than clearance of endogenous DA. However, by means of the DBE we demonstrated that the clearance of endogenous and exogenous DA is identical (see Fig 3e of Moquin and Michael, 2011). Thus, we attribute the prior discrepancy between clearance rates to the usual distance (100–200 μm) between the DA pipet and the recording site: due to this distance, the observed DA kinetics are affected by DA diffusion. The diffusional contributions are minimized by positioning the infusion site at the recording site.

4.3 Failed attempts

Several other attempts to stop reagent leak were unsuccessful. We tried an oil gap instead of air, in case there might be an advantage to the oil's incompressibility. In fact, the oil prevented the barrel and the tip solutions from mixing even when pressure was applied. Second, we tried smaller tips. If the tip is too small (Fig 1), the ejection requires high pressure (>30 psi) and causes fluid percussion injury to the recording site (Frey et al., 2009; Thompson et al., 2005): ejections at high pressure caused a loss of the evoked DA responses. Third, we tried pressure ejections without a tip solution, i.e. just air in the pipet tip. This failed because the pressure pulses ejected the air and injured the recording site.

5.0 CONCLUSIONS

The air gap idea, based on segmented flow (Gunther et al., 2004; Lada and Kennedy, 1996; Rogers et al., 2011), proved a simple yet effective means to prevent reagent delivery prior to the priming pulses. The approach has some drawbacks, as explained above, but also several benefits. A key advantage is that the DBE can be implemented in a manner that avoids percussion injury to the recording site.

Acknowledgments

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Abbreviations

aCSF	artificial cerebral spinal fluid solution
DA	dopamine
D2R	dopamine D2 receptor
DAT	dopamine transporter
DBE	double barrel electrode
PBS	phosphate buffer solution

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Highlights

- Double barrel electrodes combine detection and reagent delivery in a single device.
- This design enables electrode optimization and highly localized reagent delivery.
- However, capillary tips leak reagents at physiologically relevant concentrations.
- Segmenting the reagent solution from the tip solution prevents premature delivery.
- Pressure ejections mix reagent into the tip solution, enabling reagent delivery.

Figure 1. Scanning electron microscope images of the tip of double barrel electrodes

One barrel pulls around the carbon fiber while the other barrel remains open to form a pipet. The fiber protrudes beyond the tip. The cracks in the glass evident in the middle and right hand panels were caused by EM procedures: the DBE used during the experiments described in the text were not cracked.

Figure 2. The double barrel electrode capillary tip leaks

(A) Baseline recordings (average of $n=3$, error bars are SEMs) in the rat striatum with single (conventional) carbon fiber electrodes and DBEs containing raclopride ($25 \mu\text{M}$ aCSF, no air gap). The baseline noise of the DBE is approximately twice that of the single barreled electrode. (B) The DBE containing raclopride was lowered to a new recording location and a 3 s stimulus response was recorded immediately ($t = 0$). A second stimulus response was recorded 5 min later ($t = 5$). The second response was enhanced by raclopride even though no pressure ejection was performed. This confirms that raclopride leaked from the capillary tip in sufficient quantity to affect D2 autoreceptors near the recording electrode. (E)

Raclopride leaking from the capillary tip significantly affected the response amplitude ($n=3$, one-way ANOVA, $F_{(1,4)}=26.0$, $\dagger p < 0.005$).

Figure 3. Schematic of a double barrel electrode utilizing segmented flow

(A) The capillary tip is filled with vehicle solution (grey) while the barrel is filled with vehicle containing the reagent of interest (black): the vehicle and reagent solutions are separated by an air gap (~ 1 mm in length). (B) Pressure pulses compress the air gap and cause the reagent solution to mix with the tip solution. (C) Subsequent pressure pulses eject the reagent.

Figure 4. Ejections of DA into a PBS solution using the air gap technique

(A) A representative trace of the DA signal obtained during repeated pressure pulses of 20psi. The initial pulses produce no response because there is no DA in the tip. Subsequent pressure pulses produce responses as DA mixes with the tip solution as is ejected. (B) The subsequent DA responses are reproducible ($n=3$, repeated measures ANOVA, Tukey post-hoc, $F_{(5,12)}=128.4$, *, †, ‡, $p < 0.0001$). (C-D) Cyclic voltammograms recorded during the 2nd and 5th pressure pulse. (C) During the 2nd pressure pulse the voltammogram has peak at 250 mV caused by differences in the ion concentration of PBS and aCSF. The DA peaks are absent. (D) During the 5th pressure pulse the voltammogram has the features associated with PBS and with DA. (E) Subtracting the voltammogram in C from the voltammogram in D produces a voltammogram (solid line) that agrees well with a DA voltammogram obtained during electrode calibration (dashed line).

Figure 5. In vivo ejections using the air gap technique

(A) Ejection of DA into the striatum using the air gap technique. The initial pulses produce only small non-DA responses (the inset voltammogram, from the first pressure pulse, shows no DA peaks). DA responses eventually appear after the barrel solution containing DA mixes. (B) The air gap technique prevents the delivery of raclopride by leaking. The DBE contained aCSF in the tip and raclopride (25 μ M in aCSF) in the barrel: the solutions were separated by a 1 mm air gap. A 3-s stimulus response was recorded immediately upon placing the DBE in a new recording site ($t=0$). The response recorded at the same site 5 min later, with no pressure pulses, was identical ($t=5$), confirming that the air gap prevented raclopride from leaking into the recording site. A final stimulus response recorded 5 min

later, after raclopride ejection ($t=10$), showed the amplitude enhancement associated with the blockade of presynaptic D2 autoreceptors.