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Improved techniques for examining rapid dopamine signaling with iontophoresis

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1. ABSTRACT

Dopamine is a neurotransmitter that is utilized in brain circuits associated with reward processing and motor activity. Advances in microelectrode techniques and cyclic voltammetry have enabled its extracellular concentration fluctuations to be examined on a subsecond time scale in the brain of anesthetized and freely moving animals. The microelectrodes can be attached to micropipettes that allow local drug delivery at the site of measurement. Drugs that inhibit dopamine uptake or its autoreceptors can be evaluated while only affecting the brain region directly adjacent to the electrode. The drugs are ejected by iontophoresis in which an electrical current forces the movement of molecules by a combination of electrical migration and electroosmosis. Using electroactive tracer molecules, the amount ejected can be measured with cyclic voltammetry. In this review we will give an introduction to the basic principles of iontophoresis, including a historical account on the development of iontophoresis. It will also include an overview of the use of iontophoresis to study neurotransmission of dopamine in the rat brain. It will close by summarizing the advantages of iontophoresis and how the development of quantitative iontophoresis will facilitate future studies.

2. INTRODUCTION

Dopamine is the most abundant catecholamine in the brain and is involved in many key functions such as locomotion, learning, cognition, and the processing of rewarding stimuli. It has been extensively implicated in drug addiction due to its involvement in motivation and reward. A common model for studying reward-seeking, and by extension drug addiction, is intracranial self-stimulation (ICSS). In ICSS, animals are taught to press a lever to deliver an electrical stimulation to the brain which they find rewarding. These types of experiments established a clear role for dopamine in reward-seeking behavior, as animals learn to press quicker and press more frequently for stimulations to certain brain regions containing mainly dopamine neurons (1). However, despite decades of dopamine research on reward, the exact mechanisms by which dopamine influences reward-seeking behavior remain unclear. Technological advances continue to further our understanding of dopamine neurotransmission, and the work reviewed here will focus on the use of the carbon-fiber microelectrodes coupled to iontophoresis barrels for localized drug delivery.

2.1. Tools for studying dopamine neurotransmission in vivo

Dopamine neurotransmission can be studied either presynaptically at terminals that release dopamine, or post-synaptically at the cells to which dopamine terminals synapse to. Presynaptic release of dopamine can be studied with a variety of techniques, of which microdialysis and voltammetry are the most common. Microdialysis is a sampling method that relies on the diffusion of small molecules into a dialysis membrane implanted in the brain region of interest. The dialysate, which is the solution removed from the brain, is externally analyzed using techniques such as liquid chromatography with electrochemical detection or other detection schemes and capillary electrophoresis. This method of detection offers excellent chemical selectivity, and allows multiple analytes to be examined at once. However, despite recent advance, the timescale of detection (10's of s) remains much slower than the timescale at which neurotransmitter release occurs (ms) (2, 3). Due to their catechol moiety, catecholamines are electroactive and can be detected at modest potentials. In vivo voltammetric methods offer increased temporal resolution over microdialysis, but less chemical selectivity. In a recent review, Robinson et al discuss the available tools for studying neurotransmission, including the advantages and disadvantages of both electrochemical and non-electrochemical approaches (2).

Fast-scan cyclic voltammetry at carbon-fiber microelectrodes has emerged as the preferred electrochemical tool for in vivo monitoring of dopamine. Carbon-fiber microelectrodes are fabricated from a glass-encased carbon fiber and are used with a Ag/AgCl reference electrode. In our lab we use fibers that are ~5 µm in diameter and cut to a length of 50-100 µm. The small dimensions of this probe limit the tissue damage caused by implantation, and allow for mapping of microenvironments within brain regions (4). In these experiments, a triangular waveform is used that spans the range for oxidation of catecholamines and the current is measured. Due to the presence of a large background current at the high voltage scan rates (<100 V/s), electrochemical measurements are background subtracted so oxidative and reductive processes of the analytes can be better visualized. For instance, dopamine is oxidized at +0.6 V and reduced at 0.2 V. The current measured due to the oxidation and reduction of dopamine is directly proportional to the concentration of dopamine found at the site of detection. Typically, scans are repeated at 100 ms intervals. Thus, fast-scan cyclic voltammetry provides a method for the detection of dopamine release that is quantitative and has high temporal and spatial resolution.

Post-synaptic effects of dopamine neurotransmission in vivo are studied using electrophysiology, namely, extracellular single-unit recordings. Fine metal wires (few microns in diameter) encased in glass or saline filled micropipettes are routinely used as recording electrodes in these experiments (5). However, carbon-fiber microelectrodes may also be used for single-unit recordings. This approach offers the significant advantage of being able to monitor pre-synaptic and post-synaptic events in the same experiments. Additionally, carbon-fiber microelectrodes can also be coupled to iontophoretic drug delivery, making this approach even more advantageous.

Iontophoresis, also known as ionophoresis and microelectrophoresis, is the movement of ions and molecules under the influence of an applied current. The capillaries used for this technique are pulled to a fine tip making them ideal for localized ejection of drugs in biological systems. Iontophoresis has found a niche in neuroscience for the local application of neurotransmitters and drugs to discrete brain regions. It offers significant advantages over other drug delivery methods such as systemic injections, microinjection, and pressure ejection. For example, when a drug is administered systemically it non-selectively targets all regions of the brain possibly activating neuronal circuitry not under study, and thus confounding interpretation of the measured results. Additionally, metabolism of drugs in the periphery can reduce the drug's effects in the brain, and some drugs are unable to pass the blood-brain barrier (5). Microinjection and pressure ejection are two localized drug delivery tools which overcome the above disadvantages of systemic injections, but suffer from their own limitations. When compared to iontophoresis, microinjection and pressure ejection offer much less control over drug delivery, show problems with diffusive leakage, and the volume associated with delivery often causes damage to the tissue (6). Iontophoresis offers fine control of drug delivery; diffusive leakage can be controlled with retaining currents (or by using high resistance pipettes) and tissue damage is minimal because there is little volume associated with the ejection.

In this review we will give an introduction to the basic principles of iontophoresis, including a historical account on the development of iontophoresis. It will also include an overview of the use of iontophoresis to study neurotransmission of dopamine in the rat brain. It will close by summarizing the advantages of iontophoresis and how the development of quantitative iontophoresis will facilitate future studies.

3. BASIC PRINCIPLES OF IONTOPHORESIS

Iontophoretic drug delivery relies on the movement of ions under the influence of an applied current. Single or multi-barrel glass pipettes are pulled to a sharp tip, where each tip can be as small as 1 µm in diameter. Individual barrels are loaded with a drug solution prepared in NaCl to ensure adequate ion flow and an outward current is applied to a glass capillary. A potential difference is established between the ejecting solution and the outside buffer such that ions move in the direction opposite their charge. If a positive current is applied to the capillary, cations will be ejected out of the pipette; similarly, if a negative current is applied, anions will be ejected out of the pipette (5, 6). Figure 1 shows a schematic diagram of an iontophoresis probe containing ions X+ and Y- and neutrals Z^0. In the Figure 1A, a positive current is applied to the barrel, causing X+ to be ejected into solution, and Y- to be

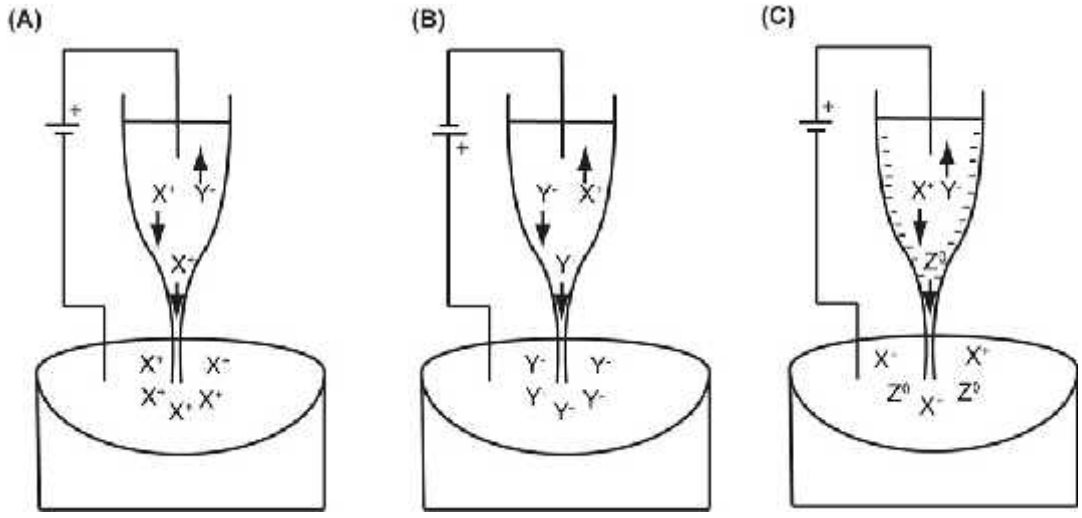

Figure 1. Schematic diagram of an iontophoretic barrel containing a salt solution of X+Y-. (A) Positive current is applied to the solution to initiate migration of cations (X+) out of the barrel. (B) A negative current is applied to retain cations (X+), and thus control diffusive leakage. A negative current may also be used to eject a drug which is an anion (Y-) in solution. (C) Compounds are also transported by electroosmotic flow (EOF) which is the bulk movement of flow due an electrical double-layer on the surface of the glass capillaries.

retained. A negative current is applied to the barrel in Figure 1B, and the ions move in opposite directions to that shown in the right panel.

In addition to the movement of ions due to the application of a current, there is also a significant contribution from electroosmosis (5, 7). Transport by electroosmosis, termed electroosmotic flow (EOF) is due to the presence of an electrical double-layer on the surface of the glass capillaries. The glass surface has negative charges on it which attract positive charges. When a potential is applied to the glass capillary, the cations on the surface are attracted to the anode. Since these cations are hydrated, this results in a bulk flow of the solution within the pipette. When a positive current is applied to the capillary, the bulk of the solution will move out of the capillary, carrying with it cations, anions, and neutrals alike (Figure 1C). Since this a bulk flow of solution, charged molecules as well as neutral molecules are transported with equal efficiency, unlike when transport is due to the migration of ions. To accurately describe drug delivery by iontophoresis, a distinction must be made between transport that is due to the migration of ions and that due to EOF. The migration of ions refers to electrophoretic movement and the movement due to bulk flow is electroosmotic. Thus, observed iontophoresis ejections are due the combination of these two processes.

Iontophoresis probes are constructed from multi-barrel glass, allowing one of the barrels to serve as a recording electrode. In some cases the barrel is filled with 4-5 mM NaCl and is used to measure voltage changes in response to the application of a drug. For in vitro preparations, such as that of the neuromuscular junction, fluctuations in membrane potentials are measured. Measurements by the recording electrode in the central nervous system (CNS) are usually of the electrical properties of single neurons by extracellular single-unit recordings. The coupling of a recording electrode to multiple iontophoresis probes allows for quick comparison of the differences or similarities in the measured results due to the application different drugs.

3.1. Origin of iontophoresis in neurobiology

In the 1950s, the actions of acetylcholine at the frog neuromuscular junction, and how the release of acetylcholine affected the muscle's permeability to different ions were of great interest to neurobiologists. In 1953, William Nastuk published what is widely accepted to be the first account of iontophoresis to deliver acetylcholine to the junctions locally and quickly (8). The paper describes the use of pulled glass capillaries filled with acetylcholine that were electrically controlled to deliver acetylcholine. A recording electrode measured changes in membrane potential and showed that as the iontophoretic probe approached the end-plate region of the neuromuscular junction, depolarization slowly occurred. It also showed that application of a positive current to the barrels caused the muscle to depolarize and application of a negative current repolarized the muscle (9). Studies by del Castillo and Katz furthered the development of iontophoresis, and their papers gave valuable insight to the mechanisms of acetylcholine at neuromuscular junctions. Equally important, however, was that they introduced other researchers to the advantages of iontophoresis and its usefulness for mapping end-plate potentials (10-19). The body of this work opened up a whole new area of research using iontophoresis, and it was soon expanded to the CNS and other families of neurotransmitters.

3.2. Quantification of iontophoretic drug delivery

Early on, from del Castillo and Katz's very first papers on iontophoresis, a critical analysis of the practical considerations necessary to use the technique effectively

had taken place. The advantages of the technique were immediately apparent: it allows for rapid and local application of acetylcholine, easily simulating the actions of synaptic acetylcholine release to map receptor distributions. They also, however, noted the drawbacks, chiefly that an incomplete understanding of how drug delivery occurred made it difficult to interpret some of the results. They noted variability in the electrical properties of the iontophoresis barrels (most likely changes in tip resistance) which led to changes in the magnitude of current and the distance between the tip and tissue necessary to elicit a response with acetylcholine application (15). These same findings have been continuously referred to in the literature (6, 8, 20, 21).

Due to the variations observed in neuronal response, a need to understand how iontophoretic drug delivery occurred grew. The first efforts were put toward developing a theory that could predict drug ejection, both as it traveled down an iontophoretic barrel and once out of the barrel. To describe transport within the barrel, a modified form of Faraday's Law was used where the iontophoretic flux (moles), M, is defined as:

$$M = n\frac{it}{zF} \quad (1)$$

where n is the transport number, i is the current applied (A), t is the ejection time (s), z is valence charge, and F is the Faraday constant (96,485 C/mol) (22). The transport number, n, is determined empirically and refers to the percentage of the total current that the ion carries during ejection. From this simple relationship, it is expected that the amount of delivered material can be predicted by knowing the transport number and the applied current and ejection time, which are controlled by the experimenter. Unfortunately, in practice, this is not the case, as great variability in ejection exists from barrel to barrel. This is likely due to additional contributions in delivery from electroosmosis which is not included in equation 1. Our work described in section 4.2 of this review provides confirmation that this is the case.

3.3. Determination of transport numbers

Despite the incomplete description of delivery given by equation 1, much research was focused on determining the transport number for all the neurotransmitters and drugs most commonly used. This was done by measuring the amount of material delivered in vitro using a variety of detection schemes, including bioassays, electrochemistry, and most commonly, radiolabeling assays (21, 23-28). The quantities of material detected were highly variable from barrel to barrel leading to high variability in reported transport numbers. To account for this, researchers took the average of many electrodes to determine the transport number, although different researchers still obtained different transport numbers. For example, the transport number determined for norepinephrine ranges from 0.09 to 0.35 over 6 different published reports (21, 29-33). Interestingly, different transport numbers for 5-hydroxytryptamine were found for small versus large tip iontophoresis probes (21). This seems indicative of the effect electroosmosis and spontaneous diffusion has on the measured transport numbers, and highlights the ineffectiveness of equation 1. Electroosmosis contributes significantly to observed iontophoretic ejections and increases in tip diameter positively correlate with increases in the amount of electroosmosis observed (7).

3.4. Effects of retaining currents

In an effort to more completely describe iontophoretic delivery, an expression for iontophoretic flux (q) which included a factor termed "diffusional efflux, qD" was derived and is shown below (22).

$$q = \frac{q_F}{\exp\left(\frac{q_F}{q_D}\right) - 1} \quad (2)$$

Here, qF is equal to M from equation 1. Although this modified equation still does not accurately predict iontophoretic delivery, development of this equation led to a critical analysis of retention currents applied in between ejections, which affect diffusional efflux. These studies noted that the observed ejection is a function of the magnitude of the retention current applied as well as the duration and frequency for which it was applied. A lag in rise-time to reach steady state will be increased with increased retention time and magnitude, highlighting the importance of knowing the "history of the pipette" (22, 25, 34). Indeed, since application of current opposite in polarity to the ejecting current will cause ions to migrate up the barrel, the solution of ions for ejection at the tip of the barrel will become depleted. Because of this, low retention currents are typically applied (5 nA or less of opposite polarity to the ejecting current), and often a "warm-up" period is necessary to replenish the solution at the tip of ions. A warm-up period consists of at least 2 or 3 cycles of ejection and retention to achieve fast rise and decay time courses during experiments. The need to reach equilibrium between ejection and retention is crucial considering the nature of the experiments typically performed with iontophoresis. For example, one use of iontophoresis is to study receptor-drug interactions, which would be greatly affected by a variable time course in drug delivery.

3.5. Other factors affecting iontophoretic delivery

In addition to transport numbers and diffusional efflux, the literature is laced with other factors not included in equations 1 or 2 that are thought to contribute to iontophoretic delivery (5). Factors such as the concentration of the drug solution and the dimensions of the barrels have obvious implications on the observed ejection. The acidity of the drug solution will affect the solubility of the drug, and will also affect EOF since there will be more anions on the glass surface at lower pHs. The concentration of drug in solution and the dimensions of the barrel are not expected to affect delivery due to ion migration, but will have a significant effect on diffusional efflux and electroosmosis since both contribute to bulk flow of solution. Thus, using higher concentrated drug

solutions as well as barrels with larger tips, will lead to larger observed ejections.

Some other factors include the method used for filling the barrels and the age of the constructed electrode. One can see how these factors may influence drug delivery, but not many reports exist which specifically document their effects. Additionally, the medium into which compounds are iontophoresed will also affect delivery, but this more likely a consequence of transport away from the tip, than the ejection itself. Transport away from the tip has been modeled as diffusion from a point source, and factors such as volume fraction and tortuosity of the brain have been included (35). The extracellular volume fraction of the brain is given the symbol α, and is defined as the fraction of the total brain volume that is extracellular space. In the brain, tortuosity, given the symbol λ, is a measure of how much the movement of substances is hindered by cellular components such as cell bodies or processes.

4. IONTOPHORESIS IN THE CENTRAL NERVOUS SYSTEM

The technique of iontophoresis as it is used today is essentially the same as that described in a study by David Curtis and Rosamund Eccles, which is the first account of using iontophoresis in the CNS (36). Using five-barrel pipettes pulled to a fine tip, they loaded four of the barrels with drugs, and the central barrel, filled with 4M NaCl, served as the recording electrode. Each barrel was individually controlled, with positive currents applied to cause ejection and retaining currents applied in between ejections to minimize leakage effects. Importantly, they determined that Renshaw cells can be locally modulated by iontophoresis, and that there is a differential response between some drugs when administered systemically versus iontophoretically. This led them to the conclusion that the blood-brain barrier may have been causing some drugs to seem unresponsive, and that by using iontophoresis this problem was circumvented since application occurs directly onto the receptor sites (36).

From 1960s into the 1980s iontophoresis was increasingly used in the CNS to study the pharmacological activity of various drugs at cells in specific brain regions. Research quickly moved beyond studying acetylcholine and into studying all known neurotransmitters and the receptors that they activate. There was particular interest in sensitivity variations from cell to cell, pharmacology of new antagonists, and the mechanism of action of these agents. Some of the most commonly studied neurotransmitters with iontophoresis include glutamate, GABA, acetylcholine, norepinephrine, dopamine, and serotonin.

4.1. Studying the role of dopamine in the striatum with iontophoresis

Dopamine neuron cell bodies located in the substantia nigra and ventral tegmental area project to discrete areas of the striatum. Complex signaling of dopamine in the striatum has led many researchers to ask what the exact role of dopamine in the striatum is. Iontophoresis should be uniquely suited for tackling this question because it allows for dopamine to be directly applied to medium spiny neurons (MSNs), the principal output neurons of the striatum. However, the results are contradictory. Extracellular single-unit recording in anesthetized animals has been coupled to iontophoresis of exogenous dopamine, and it was found to excite MSN firing at low ejection currents and inhibit it at high ejection currents (37-40). A caveat in these measurements is that the anesthesia suppressed spontaneous firing of MSNs. Therefore, the effects of iontophoretically applied dopamine were examined on cells activated by iontophoretically applied glutamate or by stimulation of the cortical inputs. More recently, however, these conclusions have been questioned. It has been shown that MSNs that project to the dopamine cell bodies contain D1 receptors whereas those that project to the globus pallidus contain D2 receptors. In general D1 activation promotes unit activity whereas activation of D2 receptors inhibits it (41). Further complicating interpretation of these data are the fact that D1 and D2 receptors exhibit low and high activity states and that they modulate synaptic plasticity. Thus future research with iontophoresis is required to distinguish these seemingly contradictory findings.

Work done in awake rats has generally confirmed results obtained from anesthetized rats and suggests that the role of dopamine in the striatum is to gate incoming glutamatergic signaling and control the level of spontaneous MSN firing or "noise" (42-44). Hence, dopamine is thought to act as a low-pass filter, effectively increasing the "signal-to-noise" of striatal signaling. However, recent evidence demonstrates that glutamate is co-released with dopamine from dopamine terminals, further complicating interpretation of the role of dopamine in the striatum (45-47). Indeed, to tease apart the roles of dopamine and glutamate in the striatum selective and localized pharmacology is needed, and iontophoresis will be particularly useful for these experiments.

4.2. Real-time monitoring of iontophoretic delivery

The pioneering design by Millar and co-workers couples iontophoretic barrels to carbon-fiber microelectrodes for real-time monitoring of electroactive compounds using fast-scan cyclic voltammetry (26). Our work with carbon-fiber iontophoresis probes has allowed us to gain a better understanding of iontophoretic drug delivery, with a particular emphasis on electroosmosis and iontophoretic variability. We have found that the inherent variability in the construction of iontophoresis barrels leads to variability in EOF, ultimately causing variability in the observed iontophoretic ejection (7). For example, the delayed onset of iontophoresis ejections can readily be quantitated with this approach (Figure 2). Furthermore, the relative rate of transport of anions or cations, as it compares to transport of a neutral EOF marker molecule remains the same across barrels. Thus, quantification of iontophoretic delivery for electroinactive drugs is possible by monitoring the rate of EOF and knowing the relative rate of transport for the drugs which can be obtained by capillary electrophoresis.

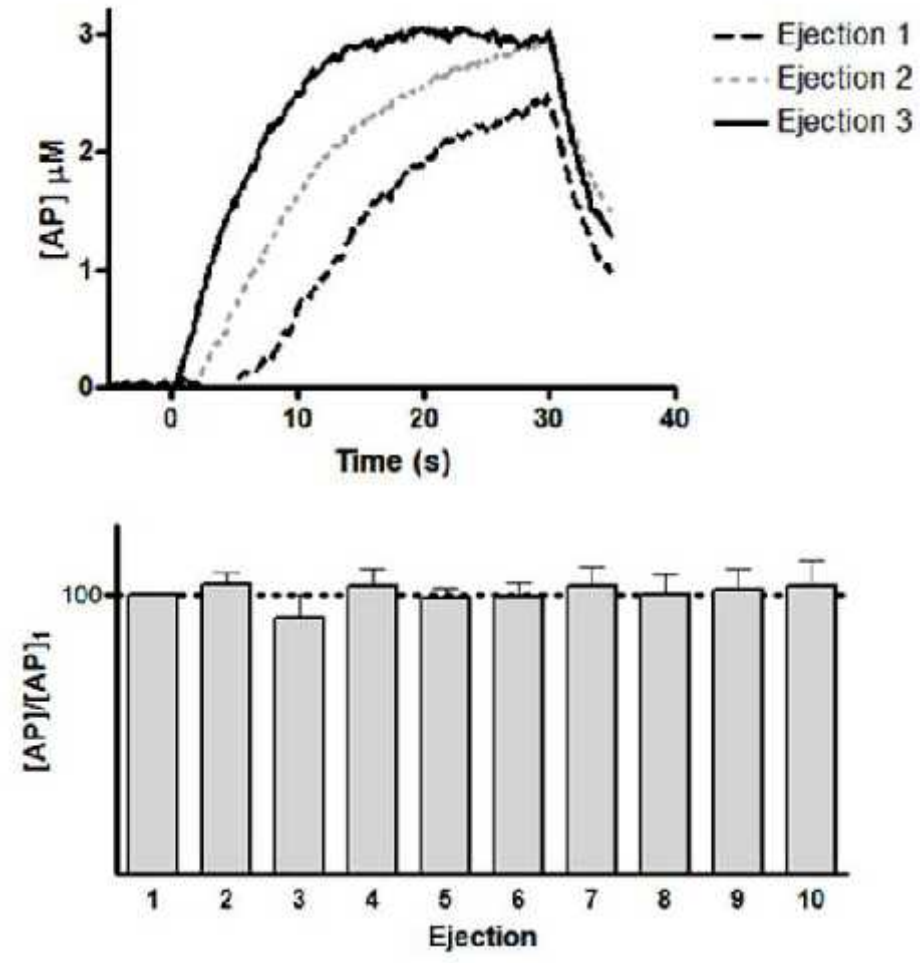

Figure 2. Temporal profile of consecutive iontophoretic ejections. A) Current vs. time trace for the first 3 ejections of AP in the rat brain. Ejection current was turned on at t = 0 s and off at t=30 s. Ejection 1 shows a characteristic delay in rise time, consistent with a "warm-up" period for ejections. Subsequent ejections, 2 and 3, have less of a delay, and ejection 3 reaches steady state. B) 10 consecutive ejections into PBS buffer after "warm-up" period. [AP]/[AP]1 represents the amount of AP measured at steady state compared to the amount measured from the first ejection after the "warm-up" period. Reprinted with permission from (54).

Using this approach, we have established a methodology for quantifying the delivery of electroinactive drugs which is compatible with in vivo experiments already carried out in our lab. We validated the methodology by examining the regulation of dopamine neurotransmission in the striatum of urethane anesthetized rats, a well understood biological system (48-53). Our data showed that acetaminophen can be used as a marker of EOF without affecting dopamine release or clearance (Figure 3). Thus, we can quantitatively deliver drugs such as raclopride, quinpirole, and nomifensine by co-ejecting acetaminophen and monitoring its delivery (54). The drug concentration is then estimated from the amount of acetaminophen measured and the amount of time elapsed from the end of delivery. Recently we have also expanded its use to include monitoring of norepinephrine in discrete subregions of the bed nucleus of the stria terminalis (BNST, unpublished work). In that study we also use acetaminophen to monitor iontophoretic delivery of electroinactive drugs such as idazoxan and desipramine. Interestingly, because of the high spatial resolution of carbon-fiber microelectrodes, we were able to observe differences in release and clearance between two relatively small subregions of the BNST. In both studies, iontophoresis allows for rapid drug manipulations directly at the site of measurement. In addition, multiple sites within a single animal can be assessed given the highly localized nature of iontophoretic drug delivery.

5. CONCLUSIONS

Fast-scan cyclic voltammetry and iontophoresis have proven to be powerful tools for understanding the dynamics of dopamine release in discrete brain regions.

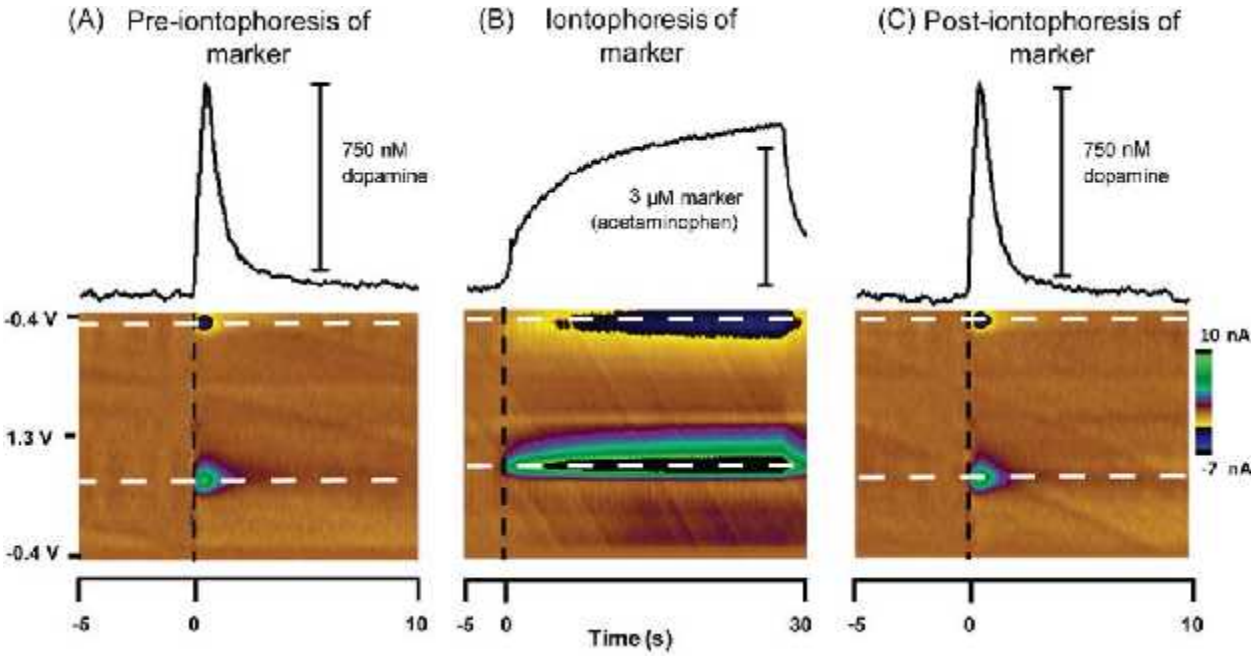

Figure 3. Effect of the EOF marker acetaminophen on stimulated dopamine release. The top panels show current as a function of time while the lower panels are two dimensional color plots where current is shown in false color on the potential vs. time axes. The white dashed lines on the color plots indicate the voltages at which oxidation (lower lines) and reduction (top lines) is occurring. (A) A representative baseline current trace and color plot for the stimulated release of dopamine. The black dashed line (t=0) indicates time of stimulation. (B) Representation of iontophoretic ejection of 3 µM acetaminophen. The black dashed line (t=0) indicates the application of a positive current to the barrel. (C) Current trace and color plot for stimulated release after ejection seen in B. The black dashed line (t=0) indicates time of stimulation. There is no change in the extracellular concentrations of dopamine seen in A and C elicited with a stimulation indicated by the black dashed line and t=0 for each trace. Reprinted with permission from (54).

Pharmacological manipulation by iontophoresis offers unique and complimentary information to that obtained by systemic drug delivery. Specifically, with iontophoresis local effects at the site of measurement can be examined, independent of drug effects in other regions. In addition, because drugs are localized to the area immediately adjacent the probe, multiple sites within a single region can be assayed and compared. Quantitative iontophoresis has facilitated these types of studies and will be useful in evaluating differences between discrete brain regions in neurotransmitter release and regulation. Future efforts will focus on expanding the dynamic range of drug delivery to enable localized, in-animal dose-response curves. This is being accomplished by loading multiple barrels with drug concentrations spanning 1-2 orders of magnitude. Additionally, we wish to transition this methodology to awake rats performing ICSS and natural reward seeking behaviors.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. R. A. Wise: Dopamine, learning and motivation. *Nat Rev Neurosci*, 5(6), 483-94 (2004)

2. D. L. Robinson, A. Hermans, A. T. Seipel and R. M. Wightman: Monitoring rapid chemical communication in the brain. *Chem Rev*, 108(7), 2554-84 (2008)

3. M. Perry, Q. Li and R. T. Kennedy: Review of recent advances in analytical techniques for the determination of neurotransmitters. *Anal Chim Acta*, 653(1), 1-22 (2009)

4. R. M. Wightman, M. L. Heien, K. M. Wassum, L. A. Sombers, B. J. Aragona, A. S. Khan, J. L. Ariansen, J. F. Cheer, P. E. Phillips and R. M. Carelli: Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. *Eur J Neurosci*, 26(7), 2046-54 (2007)

5. T. W. Stone: Microiontophoresis and Pressure Ejection. John Wiley & Sons, London (1985)

6. D. R. Curtis and W. L. Nastuk: Micro-electrophoresis. In: *Physical Techniques in Biological Research*. Academic Press, New York (1964)

7. N. R. Herr, B. M. Kile, R. M. Carelli and R. M. Wightman: Electroosmotic flow and its contribution to iontophoretic delivery. *Anal Chem*, 80(22), 8635-41 (2008)

8. T. P. Hicks: The history and development of microiontophoresis in experimental neurobiology. *Prog Neurobiol*, 22(3), 185-240 (1984)

9. W. L. Nastuk: Membrane potential changes at a single muscle endplate produced by transitory application of acetylcholine with an electrically controlled microjet. *Fed. Proc.*, 102(12) (1953)

10. J. Axelsson and S. Thesleff: A study of supersensitivity in denervated mammalian skeletal muscle. *J Physiol*, 147(1), 178-93 (1959)

11. B. Katz and R. Miledi: Further Observations on the Distribution of Actylcholine-Reactive Sites in Skeletal Muscle. *J Physiol*, 170, 379-88 (1964)

12. R. Miledi: The acetylcholine sensitivity of frog muscle fibres after complete or partial devervation. *J Physiol*, 151, 1-23 (1960)

13. K. Peper and U. J. McMahan: Distribution of Acetylcholine Receptors in the Vicinity of Nerve Terminals on Skeletal Muscle of the Frog. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 181(1065), 431-440 (1972)

14. J. Del Castillo and B. Katz: On the localization of acetylcholine receptors. *J Physiol*, 128(1), 157-81 (1955)

15. L. Del Castillo and B. Katz: A study of curare action with an electrical micromethod. *Proc R Soc Lond B Biol Sci*, 146(924), 339-56 (1957)

16. J. Del Castillo and B. Katz: Interaction at end-plate receptors between different choline derivatives. *Proc R Soc Lond B Biol Sci*, 146(924), 369-81 (1957)

17. J. Del Castillo and B. Katz: A comparison of acetylcholine and stable depolarizing agents. *Proc R Soc Lond B Biol Sci*, 146(924), 362-8 (1957)

18. J. Del Castillo and B. Katz: The identity of intrinsic and extrinsic acetylcholine receptors in the motor end-plate. *Proc R Soc Lond B Biol Sci*, 146(924), 357-61 (1957)

19. J. Del Castillo and B. Katz: Localization of active spots within the neuromuscular junction of the frog. *J Physiol*, 132(3), 630-49 (1956)

20. F. E. Bloom: To spritz or not to spritz: The doubtful value of aimless iontophoresis. *Life Sciences*, 14, 1819-1834 (1974)

21. P. B. Bradley and J. M. Candy: Iontophoretic release of acetylcholine, noradrenaline, 5-hydroxytryptamine and D-lysergic acid diethylamide from micropipettes. *Br J Pharmacol*, 40(2), 194-201 (1970)

22. R. D. Purves: The physics of iontophoretic pipettes. *J Neurosci Methods*, 1(2), 165-78 (1979)

23. K. Krnjevic and J. W. Phillis: Iontophoretic studies of neurones in the mammalian cerebral cortex. *J Physiol*, 165, 274-304 (1963)

24. K. Krnjevic and J. W. Phillis: Acetylcholine-sensitive cells in the cerebral cortex. *J Physiol*, 166, 296-327 (1963)

25. R. D. Purves: The release of drugs from iontophoretic pipettes. *J Theor Biol*, 66(4), 789-98 (1977)

26. M. Armstrong-James, K. Fox, Z. L. Kruk and J. Millar: Quantitative ionophoresis of catecholamines using multibarrel carbon fibre microelectrodes. *J Neurosci Methods*, 4(4), 385-406 (1981)

27. M. Armstrong-James, J. Millar and Z. L. Kruk: Quantification of noradrenaline iontophoresis. *Nature*, 288(5787), 181-3 (1980)

28. Z. L. Kruk, M. Armstrong-James and J. Millar: Measurement of the concentration of 5-hydroxytryptamine ejected during iontophoresis using multibarrel carbon fibre microelectrodes. *Life Sci*, 27(22), 2093-8 (1980)

29. K. Krnjevic, R. Laverty and D. F. Sharman: Iontophoretic release of adrenaline, noradrenaline and 5-hydroxytryptamine from micropipettes. *Br J Pharmacol Chemother*, 20, 491-6 (1963)

30. S. Barasi and M. T. Roberts: Responses of motoneurones to electrophoretically applied dopamine. *Br J Pharmacol*, 60(1), 29-34 (1977)

31. M. Sasa, S. Igarashi, H. Fujiwara and C. Inagaki: Comparison between fluorimetric and radiochemical assays of iontophoretically released noradrenaline from a seven-barreled micropipette. *Jpn J Pharmacol*, 28(4), 639-42 (1978)

32. B. J. Hoffer, N. H. Neff and G. R. Siggins: Microiontophoretic release of norepinephrine from micropipettes. *Neuropharmacology*, 10(21), 175-80 (1971)

33. C. M. Bradshaw, R. Y. Pun, N. T. Slater and E. Szabadi: A procedure for comparing the mobilities of unlabeled drugs used in microelectrophoresis experiments. *J Pharmacol Methods*, 5(1), 67-73 (1981)

34. R. D. Purves: Effect of Drug Concentration on Release from Ionophoretic Pipettes. *Journal of Physiology-London*, 300(MAR), P72-P73 (1980)

35. M. E. Rice and C. Nicholson: Diffusion and Ion Shifts in the Brain Extracellular Microenvironment and Their Relevance for Voltammetric Measurements. In: *Voltammetric methods in brain systems Voltammetric methods in brain systems*. Humana Press, Totowa, N.J. (1995)

36. D. R. Curtis and R. M. Eccles: The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J Physiol*, 141(3), 435-45 (1958)

37. X. T. Hu and F. J. White: Dopamine enhances glutamate-induced excitation of rat striatal neurons by

cooperative activation of D1 and D2 class receptors. *Neurosci Lett*, 224(1), 61-5 (1997)

38. S. R. White, G. C. Harris, K. M. Imel and M. J. Wheaton: Inhibitory effects of dopamine and methylenedioxymethamphetamine (MDMA) on glutamate-evoked firing of nucleus accumbens and caudate/putamen cells are enhanced following cocaine self-administration. *Brain research*, 681(1-2), 167-176 (1995)

39. G. V. Williams and J. Millar: Differential Actions of Endogenous and Iontophoretic Dopamine in Rat Striatum. *The European journal of neuroscience*, 2(7), 658-661 (1990)

40. X. T. Hu and R. Y. Wang: Comparison of effects of D-1 and D-2 dopamine receptor agonists on neurons in the rat caudate putamen: an electrophysiological study. *J Neurosci*, 8(11), 4340-8 (1988)

41. Surmeier DJ, Carrillo-Reid L, and Bargas J: Dopaminergic modulation of striatal neurons, circuits, and assemblies. *Neuroscience*, 198, 3-18 (2011)

42. E. A. Kiyatkin and G. V. Rebec: Striatal neuronal activity and responsiveness to dopamine and glutamate after selective blockade of D1 and d2 dopamine receptors in freely moving rats. *Journal of Neuroscience*, 19(9), 3594-3609 (1999)

43. E. A. Kiyatkin and G. V. Rebec: Modulation of striatal neuronal activity by glutamate and GABA: iontophoresis in awake, unrestrained rats. *Brain research*, 822(1-2), 88-106 (1999)

44. E. A. Kiyatkin and G. V. Rebec: Iontophoresis of amphetamine in the neostriatum and nucleus accumbens of awake, unrestrained rats. *Brain Res*, 771(1), 14-24 (1997)

45. N. Chuhma, W. Y. Choi, S. Mingote and S. Rayport: Dopamine neuron glutamate cotransmission: frequency-dependent modulation in the mesoventromedial projection. *Neuroscience*, 164(3), 1068-83 (2009)

46. G. D. Stuber, T. S. Hnasko, J. P. Britt, R. H. Edwards and A. Bonci: Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J Neurosci*, 30(24), 8229-33

47. Tecuapelta et al. Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. *J. Neurosci.* 30(20), 7105-7110 (2010)

48. P. A. Garris, E. L. Ciolkowski and R. M. Wightman: Heterogeneity of evoked dopamine overflow within the striatal and striatoamygdaloid regions. *Neuroscience*, 59(2), 417-27 (1994)

49. S. R. Jones, P. A. Garris and R. M. Wightman: Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *J Pharmacol Exp Ther*, 274(1), 396-403 (1995)

50. G. E. Mickelson, P. A. Garris, M. Bunin and R. M. Wightman: In vivo and in vitro assessment of dopamine uptake and release. *Adv Pharmacol*, 42, 144-7 (1998)

51. P. A. Garris, E. A. Budygin, P. E. Phillips, B. J. Venton, D. L. Robinson, B. P. Bergstrom, G. V. Rebec and R. M. Wightman: A role for presynaptic mechanisms in the actions of nomifensine and haloperidol. *Neuroscience*, 118(3), 819-29 (2003)

52. J. M. Kita, L. E. Parker, P. E. Phillips, P. A. Garris and R. M. Wightman: Paradoxical modulation of short-term facilitation of dopamine release by dopamine autoreceptors. *Journal of neurochemistry*, 102(4), 1115-1124 (2007)

53. P. A. Garris, E. L. Ciolkowski, P. Pastore and R. M. Wightman: Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *J Neurosci*, 14(10), 6084-93 (1994)

54. N. R. Herr, K. B. Daniel, A. M. Belle, R. M. Carelli and R. M. Wightman: Probing presynaptic regulation of extracellular dopamine with iontophoresis. *ACS Chem Neurosci*, 1(9), 627-638 (2010)

Abbreviations: Intracranial self-stimulation (ICSS), central nervous system (CNS), electroosmotic flow (EOF), medium spiny neuron (MSN)

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