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The dopamine patchwork of the rat nucleus accumbens core

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Abstract

The dopamine (DA) terminal field in rat dorsal striatum is organized as a patchwork of domains that exhibit distinct DA kinetics. The rate and short-term plasticity of evoked DA release, the rate of DA clearance, and the actions of several dopaminergic drugs are all domain-dependent. The patchwork arises in part from local variations in DA's basal extracellular concentration, which establishes an autoinhibitory tone in slow but not fast domains. The present study addressed the hypothesis that a domain patchwork might also exist in the nucleus accumbens core (NAcc), a DA terminal field involved deeply in reward processing and the mechanisms underlying substance abuse. DA recordings in the NAcc by fast-scan voltammetry during electrical stimulation of the medial forebrain bundle confirm that the NAcc contains a patchwork of fast and slow domains exhibiting significantly different rates of evoked DA release and DA clearance. Moreover, the NAcc domains are substantially different from those in the dorsal striatum. There are no signs in the NAcc of short-term plasticity of DA release during multiple consecutive stimuli and no signs of a domain-dependent autoinhibitory tone. Thus, the NAcc domains are distinct from each other and from the domains of the dorsal striatum.

Keywords

dopamine; nucleus accumbens; short-term plasticity; evoked release; voltammetry

INTRODUCTION

Dopamine (DA) is critically important in numerous aspects of normal CNS function (O'Donnell, 2003; Schultz, 2007) and the etiology of several CNS disorders, including Parkinson's disease (Schultz, 1982; Lotharius & Brundin, 2002), schizophrenia (Abi-Dargham *et al.*, 2000), substance abuse (Phillips *et al.*, 2003), and attention deficit hyperactivity disorder (Sagvolden *et al.*, 2005). DA's diversity of function and dysfunction imparts deep significance to understanding the mechanisms that control extracellular DA concentrations and their spatiotemporal dynamics. Such mechanisms have been extensively studied in the subregions of the striatum (i.e. dorsal striatum and nucleus accumbens) that play central roles in motor control (Kish *et al.*, 1988; Graybiel *et al.*, 1994; Doya, 2000; Durieux *et al.*, 2012) and the mediation of reward-related behaviors (Schultz, 1998; Ikemoto & Panksepp, 1999; Wise, 2004; Ariansen *et al.*, 2012).

The kinetics of evoked DA release and DA clearance in the striatum are heterogeneous (May & Wightman, 1989; Garris *et al.*, 1994b; Zachek *et al.*, 2010). Consequently, it has become common practice during voltammetric recording of extracellular DA to seek out "hot spots", i.e. recording sites that produce maximally robust DA responses. The concept of

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hot spots, however, is inconsistent with the ultrastructure of the striatum, which contains a regular array of DA terminals arranged in a lattice-like formation (Moss & Bolam, 2008). Thus, a truly comprehensive understanding of dopaminergic mechanisms requires knowledge also of the properties and functions of sites that do not qualify as hot spots.

Voltammetric DA recordings in the dorsal portion of the rat striatum have revealed the presence of a patchwork of domains exhibiting significant differences in the kinetics and plasticity of evoked DA release, the kinetics of DA clearance, and the actions of several dopaminergic drugs (Moquin & Michael, 2009; Wang *et al.*, 2010; Moquin & Michael, 2011, Taylor *et al.*, 2012 & 2013). These differences derive, in part, from local variations in DA's basal concentration, which establishes an autoinhibitory tone in the slow, but not the fast, domains. The objective of the present study was to address the hypothesis that a domain patchwork might exist also in the core of the nucleus accumbens (NAcc). Our findings confirm the existence of a domain patchwork in the NAcc but also show that the NAcc domains are substantially different from those in the dorsal striatum.

METHODS AND MATERIALS

Solutions, drugs, and reagents

All reagents were used as-received from their suppliers and all aqueous solutions were prepared in ultrapure water (NANOPure, Barnstead, Dubuque, IA, USA). Dopamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) standard solutions were prepared fresh just before use in N₂-purged artificial cerebrospinal fluid (aCSF, 144 mM Na⁺, 1.2 mM Ca²⁺, 2.7 mM K⁺, 149.1 mM Cl⁻, 1.0 mM Mg²⁺, 2.0 mM phosphate, pH 7.4). Raclopride tartrate (Sigma-Aldrich) was dissolved in phosphate buffered saline (PBS, 155 mM NaCl, 100 mM sodium phosphate, pH 7.4). Paraformaldehyde (PFA, 4% in PBS) was prepared from resin (Sigma-Aldrich) and stored at 4°C.

Carbon fiber electrodes

Single carbon fibers (7-μm diameter T650, Cytec Carbon Fibers, LLC, Piedmont, SC, USA) were placed inside fire polished borosilicate glass capillaries (0.58 mm I.D., 1.0 mm O.D.; Sutter Instruments, Co., Novato, CA, USA). Each capillary was pulled to a fine tip (Narishige puller, Tokyo, Japan) and sealed with low-viscosity epoxy (Spurr Embedding Kit, Polysciences, Inc., Warrington, PA, USA). The exposed fiber was trimmed to 200 μm with a surgical blade and an electrical connection between the fiber and a Ag-coated copper wire (Squires Electronics, Inc., Cornelius, OR, USA) was formed with a droplet of mercury. The electrode tip was immersed in purified reagent-grade isopropyl alcohol (Sigma-Aldrich) for at least 20 min (Bath *et al.*, 2000).

Fast-scan cyclic voltammetry (FSCV)

FSCV was performed with a custom-built potentiostat (electronics shop, Department of Chemistry, University of Pittsburgh) and a current amplifier (Keithley 428, Keithley Instruments, Inc., Cleveland, OH, USA) under software control (CVTarHeels 4.3, courtesy of Prof. Michael Heien, Department of Chemistry, University of Arizona (Heien *et al.*, 2003)) running on a PC equipped with analog interface boards (PCI-6052E and PCI-6711, National Instruments, Austin, TX, USA). The potential between scans was 0 V vs. Ag/AgCl. The triangular potential waveform (three linear sweeps to 1.0 V, then to -0.5 V, and back to 0 V at 400 V/s) was repeated at 10 Hz.

Electrode calibration

Electrodes were pre- and post-calibrated in DA standard solutions using a custom-built flow cell (University of Pittsburgh, Department of Chemistry Mechanics Shop) with gravity-fed aCSF. In vivo DA concentrations were determined by post-calibration.

Subjects

Protocols were in accordance with NIH guidelines (publication 86–23). The Institutional Animal Care and Use Committee of the University of Pittsburgh approved all procedures involving animals (male Male Sprague-Dawley rats, $n = 38$, 250–275 g, Hilltop Laboratories, Scottsdale, PA, USA).

Surgery

Rats were anesthetized with isoflurane (5% by vol. initially and 2.5% for maintenance) delivered via a ventilator (Harvard Apparatus, Holliston, MA, USA) and wrapped in a 37°C homoeothermic blanket (Harvard Apparatus). A carbon fiber electrode was placed into the dorsal striatum (AP +1.2 mm, ML +1.4 mm, DV 5.0 mm below dura, (Paxinos & Watson, 1998) and a Ag/AgCl reference electrode (Bioanalytical Systems Inc., West Lafayette, IN, USA) was placed in contact with the contralateral cortical surface via a salt bridge. A bipolar, stainless-steel stimulating electrode (MS303/B, Plastics One, Inc., Roanoke, VA, USA) was placed just above the ipsilateral medial forebrain bundle (MFB) (AP –4.3 mm, ML +1.2 mm, DV 7.5 below dura) and then slowly lowered until evoked DA release was detected in the striatum (Ewing *et al.*, 1983; Kuhr *et al.*, 1984; Michael *et al.*, 1987). Thereafter, without moving the stimulating electrode the carbon fiber was slowly lowered into the NAcc (DV 6.6–7.6 mm below dura). Consistent with prior reports (Kuhr *et al.*, 1986; Stamford *et al.*, 1988; Garris *et al.*, 1994b), electrical stimulation of the MFB evoked DA release in the dorsal striatum and NAcc.

In a separate group of rats, once the carbon fiber was positioned in the NAcc, stimulation (60 Hz, 250 μ A, 3 s) was repeated at 5-min intervals. Between each stimulus the stimulating electrode was lowered slowly through the MFB in 100 μ m vertical steps (results in Supplementary Fig S6).

Electrical stimulation

The electrical stimulus was a constant-current, biphasic square wave (duration 0.2–3 s, frequency 60 Hz, pulse intensity 150–550 μ A, pulse width 2 ms) supplied by a pair of optical stimulus isolators (NL800A, Digitimer, Ltd., Letchworth Garden City, UK). When multiple consecutive trains were used, the four trains were 1-s long and separated by a 2-s interval.

Drug administration

Each rat received a series of pre-raclopride stimuli, a single dose of raclopride (2 mg/kg i.p.), and a final post-raclopride stimulus 30 min after drug administration.

Histology

A small lesion (12 V AC, 3–5 s) was used to identify the recording sites because carbon fiber tracks are otherwise not visible by light microscopy (Peters *et al.*, 2004). Because the lesion procedure destroys the electrode, it was performed after electrode post-calibration: electrodes were retracted from the tissue, post-calibrated, and returned to their final stereotaxic coordinates for the lesion procedure. Every possible effort was made not to disturb the way the electrode was mounted on the carrier arm during post-calibration. After the lesion, rats were perfused via the heart with 160 mL PBS followed by 160 mL PFA.

Brain tissue was removed from the skull, stored in cold PFA, cut into 30 μm coronal sections (Vibratome, St. Louis, MO, USA), stained with cresyl violet, cover-slipped, and examined with a light microscope (Leica Microsystems). All results reported herein are from recording sites in the NAcc (Supplementary Fig S1).

Data Analysis

The initial rate of evoked dopamine overflow was determined from the slope of the evoked responses during the first 300 ms of each electrical stimulus. The linear dopamine clearance rate was measured from the slope of the descending phase of the responses after the stimulus: linear segments were defined by at least three data points with an $r^2 > 0.96$. Overshoot duration was defined by the time needed after the end of the stimulus for the evoked response to reach its maximum amplitude. The overshoot concentration was the amount by which the dopamine concentration continued to increase after the end of the stimulus. Statistical analyses were by t-test and two-way analysis of variance (ANOVA) with a repeated measures design (IBM SPSS Statistics 20).

RESULTS

DA exhibits two distinct kinetic profiles in the NAcc

Electrically evoked DA release in the NAcc exhibits two distinct kinetic profiles, depending on the recording location, within individual animals (Fig 1). During a brief stimulus (200 ms, 60 Hz, 250 μA) the fast kinetic profile (Fig 1, solid line) exhibits evoked DA release without delay when the stimulus begins and short-term facilitation of release as the stimulus proceeds: short-term facilitation is evident in the greater increase in DA caused by the 2nd 100 ms of the stimulus compared to the first 100 ms. When the stimulus ends, the DA signal continues to increase (overshoot) for an additional 200 ms before starting to return towards the baseline. In contrast, a slow recording location in the same animal exhibits no response (Fig 1, dashed line). However, if the stimulus is extended to 1 s, the slow profile (Fig 1, dash-dot line) exhibits robust DA release after a ~200-ms initial delay. The slow profile also exhibits short-term facilitation and overshoot. The presence or absence of the initial ~200 ms delay in evoked release provides an objective basis for classifying NAcc responses as fast or slow, respectively.

The fast and slow NAcc profiles are consistent across multiple animals (Fig 2: stimulus = 1 s, 60 Hz, 250 μA : solid lines are the average of multiple fast ($n = 8$) and slow ($n = 8$) profiles recorded in separate rats: the dotted lines are confidence intervals based on the SEMs: the same presentation format is used in subsequent figures). The responses are significantly different (see ANOVA results in the figure legend). These two profiles completely define evoked DA responses in the NAcc, i.e. no other profiles were detected. The maximum amplitude (Fig 2b), initial rate of overflow (Fig 2c), and rate of DA clearance after the stimulus (Fig 2d) are significantly profile-dependent. However, the two profiles exhibit similar overshoot duration (Fig 2e). Whether a fast or slow profile is observed at any given recording location is not determined by the location of the stimulating electrode (Supplementary Fig S6).

Effects of current intensity

Distinct fast and slow NAcc profiles are consistent over a broad range of stimulus current intensities (Fig 3, stimulus = 3 s, 60 Hz, 150–550 μA : SEM ranges omitted for clarity). At intensities 300 μA and 450 μA the fast and slow profiles, respectively, exhibit a constant initial rate of DA clearance (see also Supplementary Fig S2a): this indicates saturation of DA clearance and justifies the use of the observed clearance rates as the *apparent* V_{max} of

DA uptake in each domain. The apparent $V_{\max}$ of the fast profile, $3.90 \pm 0.57 \mu\text{M/s}$ ($n = 4$), is significantly ($p < 0.05$) higher than that of the slow profile, $2.24 \pm 0.22 \mu\text{M/s}$ ($n = 6$).

Short-term facilitation of NAcc fast DA release

The NAcc fast profile exhibits short-term facilitation of DA release, whereas the dorsal-striatum fast profile exhibits short-term depression (Moquin & Michael, 2009). Since this is a unique aspect of the NAcc response, we examine it in more detail here (Fig 4). During brief stimuli over a range of current intensities (200 ms, 60 Hz, 150–550 μA), the slow profiles exhibit either no or minimal evoked DA release but the fast profiles exhibit a steady increase in maximum response amplitude. However, after the first 100 ms of the stimulus, the amplitude of DA release in the fast profile is independent of the stimulus intensity (Supplementary Fig S3). The amplitude increases steadily with stimulus intensity during the second 100 ms of the stimulus (short-term facilitation) and during the 100 ms after the stimulus ends (overshoot).

NAcc responses to multiple consecutive stimuli

The NAcc yields distinct fast and slow profiles during consecutive stimuli (stimuli = 1-s pulse trains with 2-s intervals, 60 Hz, 250 μA , Fig 5a). The amplitudes of the fast and slow responses are significantly different (Fig 5b: two-way ANOVA with repeated measures $F_{(1,14)} = 17.058$, $n = 16$, $**p < 0.001$) but there are no significant train-to-train differences in amplitude in either domain. Each consecutive train returned the DA concentration to the same absolute level (Fig 5b). When the response amplitudes were measured with respect to the signal at the beginning of each train, there was a slight but not significant decrease in the amplitudes normalized with respect to the first train (Fig 5c). Both the fast and slow profiles exhibit a ~200-ms overshoot at the end of each consecutive train.

Effects of raclopride

Both the fast (Fig 6) and slow (Fig 7) NAcc profiles are sensitive to the D2 receptor antagonist, raclopride (2 mg/kg i.p.). In both domains, raclopride increased the overall amplitude of the responses but had no significant effect on the first 100 ms of the fast profile or the first 200 ms of the slow profile and had no significant effect on the rate of DA clearance after the end of the stimulus. Raclopride only significantly increased the overshoot duration of slow profiles (Fig 7e, paired-samples t-test, $p < 0.0005$).

DISCUSSION

The findings presented herein confirm that the NAcc exhibits two distinct DA kinetic profiles. The nature, fast or slow, of evoked DA responses depends solely on the location of the recording electrode, so it appears that the DA terminal field of the NAcc is organized as a patchwork of fast and slow spatial domains, in similar fashion to the patchwork we have identified in the dorsal striatum (Moquin & Michael, 2009; Wang *et al.*, 2010; Moquin & Michael, 2011, Taylor *et al.*, 2012 & 2013). Nevertheless, the NAcc domains are substantially different from those in the dorsal striatum in terms of short-term plasticity and the role of autoinhibition.

Patchwork v. long-range diffusional distortion of evoked DA responses

Prior studies of evoked DA release have tended to focus on “hot spots” and so the literature contains no prior reports of a DA patchwork in the NAcc. Low-amplitude, delayed responses might be diffusional distortions of the fast responses (discussed by Taylor *et al.* 2012 & 2013) but several observations support the alternative view that the slow profiles reflect a distinct kinetic phenomenon. For example, in the NAcc the fast and slow profiles

exhibit no clear difference in the duration of the post-stimulus response overshoot (Fig 2e). The overshoot of the slow profiles would be longer if the slow profiles were derived from diffusion of DA from fast domains. Instead, we observe no domain difference in the time for the maximal DA concentration to reach the recording electrode. Also, we observe a narrow range of response amplitude in the slow domains: this also speaks against a diffusion process because, if diffusion were involved, intermediate response amplitudes would be observed based on the distance between the electrode and the fast domains serving as the diffusion source. Instead, our results show no evidence of a concentration gradient between NAcc fast and slow domains. Our findings are much more consistent with DA diffusing *within* rather *between* the respective domains, a similar conclusion we have reached in the dorsal striatum (Taylor *et al.*, 2012).

Patchwork v. short-range diffusional distortion

In past studies, optimizing the recording site did not necessarily eliminate all signs of distortion by diffusion or the electrode response. The response overshoot has been attributed to short-range diffusional distortion caused by a Nafion film on the electrode or possibility a distortion associated with DA absorption kinetics on the electrode surface (Engstrom *et al.*, 1988; Wightman *et al.*, 1988; Kawagoe *et al.*, 1992; Venton *et al.*, 2002). Several approaches have been developed to correct these distortions to gain access to the intrinsic kinetics of DA release and clearance (Garris *et al.*, 1994a; Garris & Wightman, 1995; Garris *et al.*, 1997; Wu *et al.*, 2001b; Addy *et al.*, 2010). However, our findings suggest that overshoot may also be a consequence of DA kinetics. For example, we observe overshoots when recording brief responses (stimulus = 200 ms, 60 Hz, 250 μ A) in the NAcc (Fig 2) but not in the dorsal striatum (Moquin & Michael, 2009; Taylor *et al.*, 2012). During the present study, we confirmed that the same electrodes and recording technique that produce overshoots in the NAcc did not do so in the dorsal striatum (Supplementary Fig S4). Thus, the overshoot appears to be a feature of the NAcc rather than an inherent feature of the FSCV recording technique.

The origin of overshoot in the NAcc

The blockade of DA uptake with nomifensine dramatically increases overshoot duration and amplitude in the dorsal striatum (Taylor *et al.*, 2012). This raises the possibility that overshoots in the NAcc might be due to DA uptake, which is inherently slower in the NAcc than the dorsal striatum (Cass *et al.*, 1992; Cass *et al.*, 1993; Cragg *et al.*, 2000; Wu *et al.*, 2001a). Although the apparent $V_{\max}$ values for DA clearance in the fast and slow domains of the NAcc were significantly different, they were not significantly different from the corresponding values reported by the same experiment conducted in the dorsal striatum (Taylor *et al.*, 2013 and Supplementary Fig S2b). However, in the NAcc, the domains did not exhibit any substantial difference in the time course of DA clearance (Supplementary Fig S5): this is a stark contrast with the dorsal striatum, where the time course of DA clearance is domain-dependent (Moquin & Michael, 2011; Taylor *et al.*, 2012). So, DA clearance in the NAcc appears to operate under near pseudo-first order conditions, especially at stimulus intensities <500 μ A. Thus, we attribute the consistent overshoots in the NAcc to a higher value of the apparent K_M of DA clearance.

Our findings agree with prior reports that DA clearance is slower overall in the NAcc than in the dorsal striatum (Garris & Wightman, 1994; Wu *et al.*, 2001a; Wu *et al.*, 2001b). However, there appears to be some controversy over exactly why the rates of DA clearance are different between these two brain regions. Prior studies have reported a regional difference in the $V_{\max}$, rather than the K_M , of DA clearance (Jones *et al.*, 1995; Wu *et al.*, 2001a). So, we were surprised to find no significant differences between the regional apparent $V_{\max}$ values obtained during this study (Supplementary Fig S2b). It may be

important to note that the apparent $V_{\max}$ values reported herein were obtained by increasing the stimulus intensity until a constant rate of linear DA clearance was achieved (Supplementary Fig S2), thereby confirming full saturation of the transporter. Our study is unique in this regard, as no other in vivo studies have compared the apparent $V_{\max}$ values in this exacting manner. It should also be emphasized that we are reporting apparent kinetic parameters, whereas the prior studies reported intrinsic parameters obtained by means of diffusional corrections. However, it remains to be seen if the corrections have fully accounted for the complex nature of diffusion in the brain extracellular space (Nicholson, 1995; Nicholson & Sykova, 1998; Sykova & Nicholson, 2008). In vitro studies involving radiochemical methods also found differences in $V_{\max}$ rather than K_M (Near *et al.*, 1988; Marshall *et al.*, 1990) but Andrews and coworkers (Perez *et al.*, 2007) have pointed out a number of technical challenges with such kinetic determinations.

Short-term facilitation in the NAcc

In the dorsal striatum, the evoked DA release in the fast and slow domains exhibits marked short-term depression and facilitation, respectively, especially during multiple consecutive stimuli (Moquin & Michael, 2009). This did not occur in the NAcc, where neither domain exhibited obvious plasticity during multiple consecutive stimuli (Fig 5). Thus, the short-term plasticity of evoked release in the NAcc is dramatically different from that in the dorsal striatum.

Short-term facilitation in the NAcc responses is apparent, however, during the rising phases of both the fast and slow profiles. The details of short-term facilitation of the fast profiles are most clearly evident in the responses to 200 ms stimuli (Fig 4, 60 Hz, 150–550 μ A). The response amplitude 100 ms after the stimulus begins is independent of stimulus intensity (Supplementary Fig S3). But, the response amplitude at 200 and 300 ms after the stimulus begins increases steadily with the stimulus intensity. These details are highly consistent with the restricted diffusion model we previously proposed to explain DA profiles in the dorsal striatum (Taylor *et al.*, 2012). According to this model, the diffusion of DA molecules in the extracellular space is restricted by dead space microdomains or binding sites (Nicholson, 1995; Nicholson & Sykova, 1998; Sykova & Nicholson, 2008). Conventional diffusion occurs once DA release exceeds the tissue's capacity to retain DA. This model explains the temporal features of the NAcc responses. For example, the longer initial delay in slow domains is due to the slow rate of evoked release, which causes a lower overall response amplitude but also increases the time needed to exceed the tissue's capacity to retain DA. The consistent overshoot duration in the fast and slow domains is due to DA exhibiting similar diffusion dynamics once the stimulus exceeds the tissue's retention capacity.

Our finding of differences in the short-term plasticity of evoked DA release in dorsal striatum and NAcc is consistent with the prior reports of Cragg (Cragg, 2003) and of Yavich and coworkers (Chadchankar & Yavich, 2011). Cragg (2003) reported short-term depression in tissue slices from the dorsal part of the primate striatum (putamen) and short-term facilitation in the nucleus accumbens. Using a different stimulus protocol, Yavich and coworkers reported short-term facilitation in the dorsal but not ventral striatum of the mouse.

On the role of tonic autoinhibition

Many of the distinctions between the fast and slow DA domains of the dorsal striatum appear related to the status of tonic autoinhibition, which is absent in the fast domains but dominant in the slow domains (Moquin & Michael, 2009). In contrast, tonic autoinhibition does not appear to play a similar role in the NAcc. Raclopride, the D2 antagonist, increased the overall amplitude of evoked responses in the both NAcc fast and slow domains but had

no effect during the initial 200 ms or 300 ms, respectively, of the stimuli. Thus, in the NAcc autoinhibition is observed after, but not before, the onset of evoked DA release, so there is no sign of tonic autoinhibition even in the slow domain.

Moreover, there is no clear evidence that D2 antagonist affects the kinetics of DA clearance in the NAcc (Garris *et al.*, 1994a; Benoit-Marand *et al.*, 2001). There is a slight but non-significant tendency for raclopride to increase the linear clearance rate, but this cannot be taken as a sign that autoreceptors are modulating DA uptake. At the relatively low DA concentrations involved in these experiments, the dopamine transporter appears to be operating under pseudo-first order conditions and raclopride had no noticeable impact on the overall time for needed for the DA signal to return to the baseline. So, raclopride did not affect the pseudo-first order kinetics of DA clearance.

CONCLUSION

Our findings confirm the presence of a patchwork of DA kinetics in the NAcc but also show substantial differences between the NAcc domains and those of the dorsal striatum. Diffusion effects are clearly evident in the various profiles, but these are due to diffusion *within* rather than *between* the domains. All the profiles exhibit the features of the restricted diffusion model, the mechanisms of which have been explored in detail others (Nicholson, 1995; Nicholson & Sykova, 1998; Sykova & Nicholson, 2008). The various profiles are observed with the same FSCV technique, the same carbon fiber electrodes, and the same stimulus procedures, and so appear unlikely to be due to instrumental distortions. The regional and domain-dependence of the profiles appear, therefore, to reflect the distinct DA kinetics at the various recording locations.

Our findings reveal two substantial differences between the NAcc and dorsal striatum. First, we find no clear evidence of any autoinhibitory tone in the NAcc. Second, and likely a consequence of the first, there are no clear signs of short-term plasticity in the NAcc, where multiple consecutive stimuli evoked consistent DA release. The functional significance of the patchworks themselves, the short-term plasticity in the dorsal striatum, and the lack thereof in the NAcc, remains to be established.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

DA	dopamine
NAcc	nucleus accumbens core
MFB	medial forebrain bundle
PBS	phosphate buffered saline
PFA	paraformaldehyde
FSCV	fast-scan cyclic voltammetry

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Fig. 1. Two types of evoked dopamine responses in NAcc

A brief stimulus (200 ms, 60 Hz, 250 μ A) evokes both a fast (solid line) and slow (dashed line) profile at two recording sites within the NAcc of a single rat. A longer stimulus (1 s, 60 Hz, 250 μ A) evokes a slow profile after a 200 ms initial delay (dash dot delay). In this and subsequent figures, solid circles and triangles mark the start and end of the stimulus, respectively.

Fig. 2. Comparison of fast and slow responses during a 1-s stimulus

In this and subsequent figures, the solid lines and symbols report the average of profiles recorded in multiple animals and the dotted lines report the confidence interval based on the SEM of each data point. (a) The profiles are the average of 8 fast (diamond) and 8 slow (square) responses. The difference between fast and slow profiles is significant (all data from 0.1 to 2.9 s, two-way ANOVA with a repeated measures design: domains $F_{(1,14)} = 12.051$; $n = 16$; $p < 0.005$). The maximum amplitude (Fig 2b, independent-samples t-tests, $*p < 0.005$), initial rate of overflow (Fig 2c, independent-samples t-tests, $**p < 0.001$), and rate of DA clearance after the stimulus (Fig 2d, independent-samples t-tests, $*p < 0.005$) are significantly profile-dependent. The overshoot duration is not significantly (Fig 2e, independent-samples t-tests, $p > 0.5$) different between fast and slow profiles.

Fig. 3. Effects of current intensity on evoked DA overflow

Average fast ($n=4$) and slow ($n=6$) profiles as a function of stimulus intensity (3 s, 60 Hz, 150–550 μA). Error bars and symbols for individual data points omitted for clarity. The maximum DA amplitude (independent-samples t-test, $p < 0.05$) and initial rate (0–0.3 s, independent-samples t-test, $p < 0.05$) are significantly profile-dependent across all 150–550 μA intensities. See Supplementary Fig S2b for information on the rate of linear clearance after the stimulus.

Fig. 4. Short-term facilitation in NAcc fast domains

Average profiles recorded in fast ($n = 4$) and slow ($n = 6$) domains during brief stimuli as a function of stimulus intensity (200 ms, 60 Hz, 150–550 μ A). Error bars omitted for clarity.

The difference between fast and slow response maximum DA amplitude at all current intensities is significant (one sample t-test, 150–300 μ A, $p < 0.05$; independent-samples t-test, 350–550 μ A, $p < 0.05$). See Supplementary Fig S3 for information on the amplitude of release 100, 200, and 300 ms after the stimulus begins.

Fig. 5. Evoked dopamine overflow during multiple consecutive stimuli

(a) Average ($\pm$ SEM) of individual responses: fast (solid line; $n = 8$ rats) and slow (dashed line; $n = 8$ rats) during four 1-s stimulus trains separated by 2-s intervals (60 Hz, 250 μ A). (b) The difference between fast and slow response in maximum DA amplitude in multiple stimulus trains is significant (two-way ANOVA with a repeated measures design; $F_{(1,14)} = 17.058$; $n = 16$; $**p < 0.001$). (c) When the response amplitudes were measured with respect to the signal at the beginning of each train, there was a slight but not significant decrease in the amplitudes normalized with respect to the amplitude during the first train (two-way ANOVA with a repeated measures design; stim sequence $p > 0.05$ and domains $p > 0.05$).

Fig. 6. Effects of raclopride on fast profiles

(a) Average ($\pm$ SEM, $n = 6$) of individual responses during 0.2 s stimulus (60 Hz, 250 μ A). (b) Raclopride significantly (paired samples t-test; $p < 0.05$) increased the maximum DA amplitude. (c) Raclopride had no significant effect on the first 100 ms of the fast profile (paired samples t-test; $p < 0.05$, only at 0.2 s). (d) Raclopride did not significantly affect the linear clearance rate or (e) overshoot duration of the fast profile.

Fig. 7. Effects of raclopride on slow profiles

(a) Average ($\pm$ SEM, $n = 6$) of individual responses during 1-s stimulus (60 Hz, 250 μ A). (b) Raclopride significantly (paired samples test; $p < 0.05$) increased the maximum DA amplitude. (c) Raclopride had no significant effect on the first 200 ms of the slow profile (paired samples test; $p < 0.05$, only at 0.3 s). (d) Raclopride did not significantly affect the linear clearance rate. (e) Raclopride significantly (paired samples test; $p < 0.0005$) increased overshoot duration of the slow profile.