Electrophysiological evidence of mediolateral functional dichotomy in the rat nucleus accumbens during cocaine self-administration II: phasic firing patterns

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Abstract

In the cocaine self-administering rat, individual nucleus accumbens (NAcc) neurons exhibit phasic changes in firing rate within minutes and/or seconds of lever presses (i.e. slow phasic and rapid phasic changes, respectively). To determine whether neurons that demonstrate these changes during self-administration sessions are differentially distributed in the NAcc, rats were implanted with jugular catheters and microwire arrays in different NAcc subregions (core, dorsal shell, ventromedial shell, ventrolateral shell, or rostral pole). Neural recording sessions were typically conducted on days 13–17 of cocaine self-administration (0.77 mg/kg per 0.2-mL infusion; fixed-ratio 1 schedule of reinforcement; 6-h daily sessions). Pre-press rapid phasic firing rate changes were greater in lateral accumbal (core and ventrolateral shell) than in medial accumbal (dorsal shell and rostral pole shell) subregions. Slow phasic pattern analysis revealed that reversal latencies of neurons that exhibited change + reversal patterns differed mediolaterally: medial NAcc neurons exhibited more early reversals and fewer progressive/late reversals than lateral NAcc neurons. Comparisons of firing patterns within individual neurons across time bases indicated that lateral NAcc pre-press rapid phasic increases were correlated with tonic increases. Tonic decreases were correlated with slow phasic patterns in individual medial NAcc neurons, indicative of greater pharmacological sensitivity of neurons in this region. On the other hand, the bias of the lateral NAcc towards increased pre-press rapid phasic activity, coupled with a greater prevalence of tonic increase firing, may reflect particular sensitivity of these neurons to excitatory afferent signaling and perhaps differential pharmacological influences on firing rates between regions.

Introduction

The putative role of the nucleus accumbens (NAcc) in reward and drug-taking behavior has prompted numerous studies in recent years that have measured various physiological changes in the NAcc during drug self-administration. Several laboratories have investigated whether NAcc neural firing patterns during cocaine self-administration are temporally linked to events such as drug infusion or appetitive behavior (Carelli et al., 1993; Chang et al., 1994; Peoples & West, 1996; Woodward et al., 1999; Carelli, 2002). Two main categories of lever press-related firing patterns have been identified: slow phasic and rapid phasic. Slow phasic patterns reflect changes in firing rate over minutes, and their time course approximates the inter-infusion interval. The pattern of locomotor behavior cannot account for slow phasic firing patterns, but the temporal pattern of approach to the lever itself nonetheless correlates with reversing slow phasic firing rate changes: approaches to the lever decrease in likelihood after the infusion, and then increase in likelihood as the inter-infusion interval proceeds (Peoples et al., 1998). This is consistent with the idea that conditioned incentive signals (via limbic afferents) probably decrease immediately after an infusion, and then gradually increase as the time for the next infusion approaches (Wise et al., 1995). The reversing slow phasic firing pattern may alternatively or additionally reflect the influence of pharmacological factors on firing rate (Nicola & Deadwyler, 2000). Specifically, the post-infusion pharmacokinetic decline in synaptic drug levels could result in gating of afferent signals such that their ability to influence NAcc firing immediately after an infusion is minimal, but increases as the interval proceeds.

Rapid phasic patterns (Carelli & Deadwyler, 1996; Peoples et al., 1997) occur within seconds of the cocaine-reinforced instrumental response. On the basis of timing alone, a relation to pharmacological factors is unlikely, and we have demonstrated that firing can be dissociated from any aspect of the cocaine infusion, including viscerosensory feedback (Peoples et al., 1997). Instead, rapid phasic patterns appear to be correlated with the animal’s instrumental response (i.e. drug seeking) and/or the tone and light cues synchronized with cocaine infusion (Carelli & Deadwyler, 1996; Peoples et al., 1997; Carelli, 2000).

Evidence for a functional dichotomy between medial and lateral NAcc subregions continues to mount (Corbit et al., 2001; Rodd-Henricks et al., 2002; Ghitza et al., 2003, 2004; Ikemoto et al., 2003).
Materials and methods

Subjects

Male Long-Evans rats (n = 32; Charles River Laboratories, Wilmington, MA, USA) were individually housed with a 12 : 12-h light/dark cycle (lights on at 10 : 00 h), handled daily, and food-restricted to maintain target body weights between 330 and 350 g (= 90% adult body weight). Rats were approximately 120–150 days old over the course of training and testing.

Surgical procedures and drug self-administration training have been described in detail previously (Fabbricatore et al., 2009). Briefly, rats were anesthetized with an injection of sodium pentobarbital (50 mg/kg body weight, i.p.; Abbot Laboratories, North Chicago, IL, USA). Animals were also injected with atropine methyl nitrate (10 mg/kg i.p.; Sigma, St. Louis, MO, USA) and Penicillin G (75,000 i.u./0.25 mL i.m.; Wyeth Laboratories, Philadelphia, PA, USA). Next, animals were fitted with a jugular catheter that exited a scalp incision and was affixed to a chronically implanted microwire array headstage anchored to the skull with acrylic cement and stainless steel screws. Precautions were taken throughout catheterization, stereotaxic surgery and postsurgical housing of rats to maintain aseptic conditions. The treatment of animals was in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Rutgers University Animal Care & Facilities Committee. Following surgery, subjects were individually housed in a steel-grid chamber for a minimum of 7 days. Thereafter, and at least 3 days prior to the start of self-administration training, animals were transferred into Plexiglas chambers (38 cm length × 20 cm width × 29 cm height) which henceforth served to accommodate housing, training and testing of subjects. Catheter patency was maintained during recovery and between training and testing sessions by quarter-hourly infusions (0.2 mL) of dilute heparinized saline solution delivered by a timer-equipped, motor-driven syringe pump (Razel Scientific Instruments, Stamford, CT, USA).

During training, each reinforced lever press resulted in a 0.2-mL intravenous infusion of cocaine hydrochloride (National Institute on Drug Abuse, Research Triangle Park, NC, USA) solution, a 7.5-s tone, which corresponded with the duration of syringe pump operation, and a 20-s time-out period, during which the cue light was off and lever presses had no programmed consequence. Training sessions (fixed ratio 1 schedule) were conducted 7 days/week, and were each limited to 80 infusions or 6 h, whichever was attained first. The average cocaine dose administered, given differences in subjects’ body weights, ranged between 0.70 and 0.91 mg/kg per infusion, with a drug dose of 0.77 ± 0.01 [mean ± standard error of the mean (SEM)] mg/kg per infusion. This resulted in an inter-infusion interval with a median ± SEM of 7.36 ± 0.01 min. After acquisition, subjects were trained for 12–18 self-administration sessions before neural recordings commenced.

Electrophysiological recording sessions

Neural recordings began 30 min before the start of the self-administration session and continued for 1 h after the session. The neural signal from individual microwires was led through a field effect transistor in the headset of an electronic harness (NB Labs, Denison, TX, USA; V. Prokopenko), and then through the Airflyte electronic swivel. From the swivel, the signal continued to a preamplifier (Riverpoint Electronics, Goldsboro, NC, USA), where it was differentially amplified against another microwire that exhibited no neural waveforms. The signal was then conducted through a bandpass (roll-off below 1000 Hz = 1.5 dB/octave; roll-off above 11 000 Hz = 6 dB/octave; gainamplifier (Riverpoint Electronics). The amplified signal was then sent to a remote computer where, using Datawave Technologies (Longmont, CO, USA) software and hardware, each waveform larger than threshold value (approximately 120% of the 25-μV noisefloor) was digitized (50-kHz sampling frequency per recorded wire), time-stamped (0.1-ms resolution), and stored for off-line analysis.

Data analysis

Post hoc analyses of the neural data were conducted using cluster analysis software (Datawave Technologies) to isolate neural waveforms.

Fig. 1. Examples of slow phasic reversal firing patterns. Each peri-event time histogram (PETH) displays the firing pattern of one neuron during the minutes before and after the lever press. The ordinate of each histogram displays the average firing rate (i.e. average discharges/s calculated as a function of 0.1-min bins). Time 0 (vertical dashed line) on the abscissa marks the occurrence of the cocaine-reinforced lever press. (A) Examples of decrease + progressive reversal firing patterns. (B) Examples of other categories of slow phasic patterns. Top, increase + progressive reversal; middle, increase + early reversal; bottom, decrease + early reversal. For each PETH, the inset depicts the corresponding neural waveform. Calibrations (bars in A, top) of waveforms: 0.25 ms, 0.10 mV (top left waveform); 0.25 ms, 0.20 mV (all other waveforms).
forms, as described previously (Peoples & West, 1996). Peri-event time histograms (PETHs) were generated on two time bases: one that displayed the approximate inter-infusion interval (slow phasic time frame, in minutes), and another that displayed the approximate time frame of the instrumental response and drug infusion (rapid phasic time frame, in seconds). Thus, in the case of phasic histograms, periods of minutes (±6 min) or seconds (±12 s) were considered when evaluating increases or decreases in firing rate in relation to the lever press. For both slow phasic and rapid phasic PETHs, the node was the electronic offset of the reinforced lever press. For each experiment, the first 10 lever presses (load-up) were excluded before generating PETHs to ensure stable behavior and drug levels, and that consecutive lever presses did not overlap in the histogram.

Histological analyses

Histological procedures and locations of all microwires have been described in detail previously (Fabbricatore et al., 2009). Briefly, rats were injected with a lethal dose of sodium pentobarbital before anodal current (50 mA, 4 s) was passed through each of the microwires in the array. After fixing, slicing, mounting, and Nissl staining, the precise location of each wire tip was estimated to be at the center of an individual lesion mark in the brain. An independent observer, who was blind to the data recorded from each wire, evaluated the histological placement of microwires. A stringent criterion required that a lesion center within 150 μm of any border be characterized as a ‘border’ neuron. Those that bordered extra-accumbal structures were eliminated from the study. Border neurons that lay between core and shell were treated as separate categories; those found at or ventral to −7.6 mm doroventral (DV) were considered to be ventral border neurons; and those dorsal to −7.6 mm DV were considered to be dorsal border neurons.

The shell was subdivided into dorsal, ventromedial, and ventrolateral regions. Wires placed in the ventromedial shell subregion were confined to the area: (i) at or posterior to 2.3 mm and anterior to 0.7 mm anteroposterior; (ii) ventral to −7.3 mm DV; and (iii) medial to 1.4 mm mediolateral. Wire tips within shell regions dorsal to this area were categorized as dorsal shell wires, and those located lateral to this region were categorized as ventrolateral shell wires. Rostral pole neurons were defined by tip locations anterior to 2.3 mm anteroposterior.

Statistical analysis of firing patterns

Slow phasic patterns

Certain neurons exhibited changes in firing rate over minutes relative to the lever press, i.e. slow phasic neurons. Analytical procedures that evaluated shifts in firing rate that occurred over the course of the inter-infusion interval have been described previously (Peoples et al., 1998). Slow phasic analyses were sensitive to firing rate change latencies relative to lever pressing, and were categorized accordingly (see Results for category definitions).

Rapid phasic patterns

Neurons whose firing rates changed within seconds of lever pressing, that is, rapid phasic neurons, were assessed on the basis of the magnitude of peri-event firing rate change as compared with the 9-s baseline period that preceded it. During the 3-s pre-press period, the first of three consecutive 200-ms bins that exhibited firing rates exceeding ±20% baseline firing defined the onset of the pre-press firing window for a given neuron. Offset was defined by the first of three consecutive bins in which firing returned to the rates observed during the baseline period. If no firing rate offset occurred by the time of the lever press, the end of the firing window was defined as the time of the lever press. Using the same 9-s baseline period, a similar analysis was conducted to determine the post-press firing window.

During the initial 3-s post-press period, the onset of the post-press firing window was defined by the first of three consecutive 200-ms bins that exhibited firing rates exceeding ±20% of firing during the baseline period. Offset times were defined as the first of three consecutive bins in which a return to baseline firing occurred, up to the limit of the post-press period (12 s). The requirement for three consecutive bins exhibiting 20% change provided a reliable and sensitive assessment of peri-event firing rate changes while excluding sporadic fluctuations in activity. The raster of every neuron was visually inspected to ensure that the pattern of discharges that formed each neuron’s PETH was representative of the discharge pattern of individual trials depicted in the raster. These firing rates were then used to calculate a ratio $B/(A + B)$, where $A$ is equal to the mean baseline firing rate, and $B$ is equal to the mean firing rate during the peri-event (pre-press or post-press) firing window.

Average onset times and average offset times of pre-press and post-press firing windows were calculated for neurons that met the > 20% change rule. These averages were then applied to any neuron for which a pre-press and/or post-press window was not defined using the above criteria (i.e. did not exhibit an identifiable change). This allowed all neurons in the study to be subjected to pre-press and post-press firing rate analyses.

After assignment of firing windows, all neurons were subjected to firing rate analyses ($B/A + B$). In a few cases (see Results), neurons exhibited particularly low firing rates during baseline and within a firing window. These neurons were assigned a $B/A + B$ value of 0.5 to more accurately reflect the lack of a difference in lever press firing relative to baseline firing.

The evaluation of firing rate changes with the $B/A + B$ ratio employed in the present study has several advantages. First, it normalizes firing rate changes across neurons with different firing rates. Second, it provides a scale that equally weighs decreases and increases in firing. Third, the $B/A + B$ ratio provides a scale on which the magnitude of firing rate change can be compared for every neuron in the sample. Fourth, this approach provides for a more comprehensive assessment of the various magnitudes of firing rate change of the entire sample of neurons in a particular region or category than an approach that selects only robust responses for analysis.

Rapid phasic duration analysis

Analyses of response onset and offset times were not possible for neurons that exhibited little or no firing rate change in the seconds before or after the lever press. Accordingly, durations of firing rate changes were calculated for neurons that exhibited substantial (i.e. twofold) increases or decreases in firing within seconds of the lever press. Pre-press and post-press firing durations were defined as the difference between offset and onset times.

Subregional comparisons of firing patterns

Slow phasic firing patterns

Chi-square tests ($2 \times 2; \chi^2 = 0.05$) (Runyon et al., 1996) were employed to determine whether differences exist in the prevalence of responsive neurons described in Peoples & West (1996) neurons between subterritories. The proportion of responsive neurons from each subregion of the shell (i.e. dorsal, ventromedial, and ventrolateral) was compared with the proportion of responsive neurons from the core. The prevalence of
responsive neurons in the core was also compared with the proportion of responsive neurons in the dorsal and ventral border regions within the NAcc. For the purpose of this analysis, differences (e.g. reversal latency and post-press directionality) in the firing topographies of phasic patterns were collapsed, allowing for a two-category analysis: responsive (any phasic pattern) vs. non-responsive.

A second, more qualitative, analysis evaluated the prevalence of slow phasic categories among subregions. As reported earlier, the majority of slow phasic neurons exhibit reversal patterns (Peoples et al., 1998), comprising either increases or decreases post-press that show reversals at various latencies as the inter-infusion interval proceeds. The possibility that these different categories of slow phasic patterns reflect distinct functional correlates warranted an analysis of their subregional distribution. Specifically, the major slow phasic categories, that is, change + reversal neurons, were compared between medial and lateral NAcc subregions.

Rapid phasic firing patterns
Planned subregional comparisons of rapid phasic patterns were undertaken using two-tailed Mann–Whitney U-tests (z = 0.05) (Castellan & Siegel, 1988), which assessed the magnitude of firing rate changes between core and each of the other subregions. To distinguish potentially behaviorally relevant neural activity (e.g. approach behavior vs. processing the completion of the lever press), separate comparisons were made between subregions using pre-press and post-press periods, as defined above.

Correlations of firing patterns between time bases
Differences among subregions in the prevalence of tonic firing patterns (i.e. average firing rate throughout the whole experiment) among NAcc subregions have been reported (Fabbricatore et al., 2009). In the present investigation, a third analysis utilized Spearman’s ρ correlations (z = 0.05) of $B/(A+B)$ values to compare relative magnitudes of the firing rate changes of individual neurons between time bases within each subregion. This non-parametric test was used because the data did not exhibit a normal distribution. Analyses included separate calculations for each neuron’s: (i) rapid phasic vs. tonic firing rates; (ii) slow phasic vs. tonic firing rates; and (iii) rapid phasic vs. slow phasic firing rates.

Results
Following acquisition of the task, behavior during experiments was typically characterized by an initial, brief period of rapid lever pressing, followed for the remainder of the self-administration phase by self-infusions at regular intervals, which ranged from 6 to 8 min. Between cocaine-reinforced lever presses, animals typically engaged in focused stereotypy, consisting of nose poking, forelimb treading and repetitive manual-orofacial behaviors (Pickens & Thompson, 1968). Focused stereotypy typically spanned the majority of the inter-infusion interval, with, in some cases, circling behavior oriented towards the lever during the last half minute before the subsequent lever press. Drug accumulation curves constructed according to the method of Yokel & Pickens (1974) showed stable drug levels during self-administration (Fabbricatore et al., 2009).

The recorded NAcc neural waveforms exhibited amplitudes that ranged between 100 and 300 μV. The average signal-to-noise ratio was 3.08 ± 0.08, with the majority (130/137; 95%) of waveform amplitudes being > 200% of the respective noiseband (range, 1.82–6.90). The mean NAcc firing rate during the pre-drug phase was 0.53 ± 0.12 Hz. Mean firing rates did not differ among subregions ($F_{8,135} = 0.236, P = 0.96$).

Anatomical distribution of NAcc neurons
Of a total of 297 basal forebrain neurons recorded in 32 subjects, 137 were from wires (n = 124) histologically confirmed to be located in the NAcc. Tonic firing patterns recorded from these same 124 wires and their locations were recently reported (Fabbricatore et al., 2009).

NAcc subterritorial distribution
Seventy-six per cent (104/137) of the NAcc neurons were, according to 150-μm border criterion analysis (described in Materials and methods), unequivocally located in one of the three subterritories of the NAcc. Among these, the majority were located in the shell (n = 74), and the remainder were distributed between the core (n = 25) and the rostral pole (n = 5). All rostral pole neurons proved to be in the shell subregion (Jongen-Relo et al., 1994; Paxinos & Watson, 1997).

NAcc shell subregional distribution
The majority of neurons recorded from the shell were localized to the ventrolateral shell (n = 49). The dorsal and ventromedial shell subregions yielded 12 and 13 of the recorded NAcc neurons, respectively.

NAcc border neurons
Thirty-three NAcc neurons could not be assigned a subterritorial designation, owing to their proximity to the boundary between shell and core. They were, instead, considered to be a dorsal border group (n = 19) and a ventral border group (n = 14). Thus, seven NAcc compartments were evaluated in the present study: core, dorsal shell, ventromedial shell, ventrolateral shell, rostral pole shell, dorsal border, and ventral border.

Slow phasic firing patterns
Neurons that exhibited changes in the minutes before and after lever presses, that is, slow phasic changes, represented 55% (75/137) of the total recorded from the NAcc. Patterns showing firing rate changes that approached pre-press levels within the first 4 min post-press were considered to be early reversal patterns, and those that reversed > 4 min post-press were considered to be progressive reversal neurons (Peoples & West, 1996). In rare cases (n = 3), neurons exhibited increases or decreases in firing rate that were more temporally proximal to the lever press, often beginning within the minute before the press and ending soon after it. Analyses related to phasic firing rate change categorization were conducted prior to and independently from the above histological procedures. All categories of slow phasic firing patterns had been observed in a separate data pool (Peoples et al., 1998).

Slow phasic category analysis
The decrease + progressive reversal pattern was exhibited by a majority of slow phasic neurons (60%; 45/75) (Fig. 1A). Of the remaining slow phasic categories (Fig. 1B), none represented more
Among slow phasic neurons, the majority (72/137; 96%) exhibited post-press change + reversal patterns. These included decrease + progressive reversal, decrease + early reversal, increase + progressive reversal and increase + early reversal patterns (Table 1).

Given the recent evidence for NAcc mediolateral functional dichotomy [reported and reviewed in Fabbricatore et al. (2009)], neurons that were histologically confirmed to be in either the lateral NAcc (n = 74; core/ventrolateral shell) or the medial NAcc (n = 17; dorsal shell/rostral pole shell) were evaluated in terms of whether they: (i) exhibited progressive/late reversal patterns; (ii) exhibited early reversal patterns; or (iii) failed to exhibit reversal slow phasic patterns (Table 2). Lateral NAcc neurons exhibited more than twice the percentage of progressive or late reversal patterns as compared to medial NAcc neurons (Fig. 2). In contrast, the opposite trend was observed for early reversal patterns, with more than twice the percentage being found in the medial NAcc as compared to the lateral NAcc. An analysis of these reversal patterns confirmed that they are differentially expressed mediolaterally [χ²(exact) = 4.70, degrees of freedom = 1, *P < 0.05]. Thus, over the inter-infusion interval, a longer latency in the reversal of post-press firing rates was observed in lateral NAcc than in medial NAcc neurons. Rare instances in which a subject yielded simultaneous medial and lateral recordings that exhibited early and late reversals, respectively, indicated that the different reversal latencies did not depend on differences in pharmacokinetic profiles between subjects. It is of further note that all seven ventral border neurons that exhibited slow phasic patterns were the progressive reversal type, consistent with the above-reported trend observed elsewhere (i.e. ventrolateral shell and core) in the lateral NAcc. The dorsal border group, whose distinct anatomical and physiological characteristics render it difficult to assign in terms of mediolateral categorization (Fabbricatore et al., 2009), exhibited one early reversal firing pattern and 10 progressive reversal patterns among its 11 neurons with slow phasic reversal activity.

### Rapid phasic firing patterns

Neurons that exhibited firing rate changes in the seconds proximal to the lever press either (i) spanned the lever press or (ii) were exclusive pre-press or post-press changes (Fig. 3). Mean durations of pre-press firing rate decreases (n = 23) and increases (n = 17) were 1.50 and 1.31 s, respectively. Mean durations of post-press firing rate decreases (n = 18) and increases (n = 20) were 5.30 and 2.51 s, respectively. Of the 19 neurons whose firing rate changes spanned the lever press, eight showed decreases, seven showed increases, and four showed mixed changes (i.e. exhibited post-press firing rate changes opposite to the pre-press changes).
Rapid phasic category analysis

Comparison of the firing rate changes of core neurons with those of the other subregions revealed that the magnitude of change in pre-press firing was greater in the core than in the dorsal shell ($P = 0.007$) and the rostral pole shell ($P = 0.006$). No other differences were observed ($P > 0.05$).

As the magnitude of firing rate changes in the core did not differ from the magnitude in the ventrolateral shell, whereas medial shell subregions exhibited marked differences from the core, we replicated the subregional comparison employed in Fig. 2 to assess whether rapid phasic patterns also show evidence of mediolateral compartmentalization. Analysis of variance conducted for rapid phasic firing rate changes revealed that lateral NAcc (core/ventrolateral shell) neurons exhibited greater increases in pre-press firing rate than medial NAcc (dorsal shell/rostral pole shell) neurons ($t_{26} = 1.71, P = 0.023$) (Fig. 4). Furthermore, this difference was maintained when ventral border neurons were included in the analyses ($t_{24} = 1.71, P = 0.018$). No other differences were observed ($P > 0.05$).

Prevalence of neurons exhibiting firing rate changes on more than one time base

Phasic correlates within tonic groups

Slow phasic firing patterns were exhibited by 48% (30/63) of neurons exhibiting a tonic decrease and 81% (17/21) of neurons exhibiting a tonic increase [see Fabbricatore et al. (2009) for further details on tonic patterns]. A more refined analysis involving particular categories of slow phasic patterns within tonic groups is described below. Consistent with our earlier report (Ghitza et al., 2006), rapid phasic patterns were not exclusive to any particular tonic category.

Tonic correlates within phasic groups

Neurons exhibiting a tonic decrease or a tonic increase represented 41% (30/74) and 23% (17/74) of neurons exhibiting slow phasic changes, respectively.

Comparisons of firing rate changes between time bases by subregion

Correlational analyses were used to compare the relative magnitude of firing rate changes between time bases exhibited by individual neurons of each subregion. For each neuron, firing rate change values ($B/A + B$) for two time bases were entered into the analysis.

Rapid phasic vs. tonic

Pre-press firing rate changes were positively correlated with tonic changes in the core. No other subregions exhibited correlations in firing rate between these two time bases (Table 3A).

![Fig. 3. Examples of rapid phasic firing patterns.](image)

![Fig. 4. Frequency distributions of pre-press (left panel) and post-press (right panel) rapid phasic firing rate change magnitudes in the medial nucleus accumbens (M-NAcc) vs. the lateral NAcc (L-NAcc).](image)
also exhibited tonic decreases (lower left quadrant). D, dorsal; VL, ventrolateral.

It is notable that slow phasic changes were exclusively decreases in neurons that exhibited tonic decreases in dorsal NAcc (i.e. dorsal shell and rostral pole shell) and dorsal border regions, it is revealed for lateral NAcc regions, neurons of the medial NAcc (dorsal shell, rostral pole shell, and dorsal border) exhibited values that were restricted to the lower left quadrant occupied by tonic decrease neurons that exhibit slow phasic decreases. Column C contains data from statistical comparisons between rapid and slow phasic changes. Owing to the rarity of robust slow phasic patterns during cocaine self-administration, the absence of correlations here is not unexpected. D, dorsal; VM, ventromedial; VL, ventrolateral; RP, rostral pole; V, ventral.

**Slow phasic vs. tonic**

Firing rate magnitude changes were directly and significantly correlated between slow phasic and tonic time bases for dorsal shell and ventrolateral shell, including opposite signs in firing rate change between time bases (i.e. symbols in upper left and lower right quadrants). For the medial NAcc (i.e. dorsal shell and rostral pole shell) and dorsal border regions, it is notable that slow phasic changes were exclusively decreases in neurons that also exhibited tonic decreases (lower left quadrant). D, dorsal; VL, ventrolateral; RP, rostral pole; V, ventral.

**Table 3. Individual neurons exhibiting firing rate changes on more than one time base**

<table>
<thead>
<tr>
<th>Cells (n)</th>
<th>Pre-press</th>
<th>Post-press</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>24</td>
<td>0.48*</td>
</tr>
<tr>
<td>D shell</td>
<td>12</td>
<td>-0.05</td>
</tr>
<tr>
<td>VM shell</td>
<td>13</td>
<td>0.24</td>
</tr>
<tr>
<td>VL shell</td>
<td>48</td>
<td>0.01</td>
</tr>
<tr>
<td>RP shell</td>
<td>5</td>
<td>0.72</td>
</tr>
<tr>
<td>D border</td>
<td>19</td>
<td>0.32</td>
</tr>
<tr>
<td>V border</td>
<td>14</td>
<td>-0.01</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>–</td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level (two-tailed). **Significant at the 0.01 level (two-tailed). Spearman’s rho correlations (ρ = 0.05) compared relative magnitudes of the firing rate changes of individual neurons between time bases. The analyses included separate calculations for each neuron’s: (i) rapid phasic vs. tonic firing rates (column A); (ii) slow phasic vs. tonic firing rates (column B); and (iii) rapid phasic vs. slow phasic firing rates (column C). Column A indicates that, over the course of pre-press intervals in the core, the magnitudes of rapid phasic changes were correlated with that same neuron’s tonic firing changes, specifically tonic increases. This is consistent with what we have observed in a separate set of subjects reinforced by cocaine under discriminative stimulus reinforcement conditions (Gitlitz et al., 2004). Column B provides statistical details from the data in Fig. 5; what is notable is that whereas the range of slow phasic changes was manifest among tonic increases and decreases in lateral NAcc regions, neurons of the medial NAcc (dorsal shell, rostral pole shell, and dorsal border) exhibited values that were restricted to the lower left quadrant occupied by tonic decrease neurons that exhibit slow phasic decreases. Column C contains data from statistical comparisons between rapid and slow phasic data. Owing to the rarity of robust slow phasic patterns during cocaine self-administration, the absence of correlations here is not unexpected. D, dorsal; VM, ventromedial; VL, ventrolateral; RP, rostral pole; V, ventral.

**Fig. 5.** Tonic firing changes in neurons that exhibit slow phasic firing patterns are differentially expressed among nucleus accumbens (NAcc) subregions. The graph depicts tonic firing data for neurons that exhibited slow phasic firing changes. Symbols indicate subregional placement of histologically confirmed NAcc microwires (see key). B/A + B values from 0.49 to 0 and from 0.51 to 1.00 reflect increasingly larger firing rate decreases and increases, respectively. A rather broad distribution of increases and decreases of slow phasic and tonic firing magnitudes is revealed for lateral NAcc neurons (e.g. core and ventrolateral shell), including opposite signs in firing rate change between time bases (i.e. symbols in upper left and lower right quadrants). For the medial NAcc (i.e. dorsal shell and rostral pole shell) and dorsal border regions, it is notable that slow phasic changes were exclusively decreases in neurons that also exhibited tonic decreases (lower left quadrant), D, dorsal; VL, ventrolateral; RP, rostral pole; V, ventral.

and dorsal border neurons (Table 3B). Interestingly, among neurons exhibiting slow phasic decreases, tonic firing rate changes in 100% of the dorsal border neurons (13/13) and 83% of the dorsal shell neurons (5/6) were also decreased. Firing rate change magnitudes were positively correlated between slow phasic and tonic component analysis of variance (ANOVA) results revealed that the prevalence of slow phasic changes was significant among tonic decreases and increases exhibited slow phasic changes (Fig. 5).

**Rapid phasic vs. slow phasic**

No relationship existed in any subregion between the magnitude of firing rate changes on the order of seconds as compared with minutes relative to lever presses (Table 3C).

**Slow phasic categories within tonic groups**

Specific categories of neurons exhibiting slow phasic changes were additionally analysed with respect to their prevalence among tonic decrease and tonic increase groups. Interestingly, a similar distribution of decrease + progressive reversal patterns was observed within groups exhibiting tonic increases (29%, 6/21) or tonic decreases (37%, 23/63). What is more, the prevalence of decrease + early reversal patterns was approximately the same between tonic increase (5%, 1/21) and tonic decrease (6%, 4/63) groups. All slow phasic NAcc neurons were analysed in terms of whether they demonstrated post-press firing rate increases (slow phasic increases) or decreases (slow phasic decreases) as a function of tonic category: increase, decrease, or no tonic change. The analysis revealed that the prevalence of slow phasic decreases was markedly different among tonic increase, tonic decrease and no tonic change groups (33%, 44%, and 43%, respectively). Slow phasic increases, on the other hand, exhibited striking differences in prevalence between tonic groups: 43% (9/21) of neurons that showed tonic increases also demonstrated slow phasic increases, as compared with only 2% (1/63) of neurons.
that showed tonic decreases (Fig. 6). As in the tonic decrease group, slow phasic increases formed a relatively small percentage [8% (4/51)] of neurons that show no tonic change. The physiological relevance of these findings with respect to NAcc functional heterogeneity is described below.

**Discussion**

Analyses of rapid and slow phasic firing patterns yielded complementary findings to tonic firing analyses, which together support functional heterogeneity in the NAcc. The more comprehensive subregional analyses in the present investigation, which expanded neural sampling into the intra-NAcc border regions and further refined subregional sampling of the shell, provided evidence of a more general mediodorsal compartmentalization of NAcc firing patterns extending beyond our prior report (Ghitza et al., 2006). The 2006 report, in which neural recordings from rats tested under discriminative stimulus conditions were combined with part of the present fixed ratio 1 data set, provided evidence for functional differences between the medial shell and the core that were similar between the two different reinforcement schedules and therefore potentially common to self-administered cocaine reinforcement. In addition to extending medial shell-core differences into broader, mediodorsal NAcc differences, the present updated observations show remarkable agreement with an emerging literature that describes the shell as a functionally heterogeneous structure (see below).

**Slow phasic patterns**

The cycle of post-press stereotypy followed, in the later minutes of the inter-infusion interval, by lever-oriented approach behavior tempers speculation about the possible involvement of slow phasic patterns in the execution of the locomotor response. We have previously reported that slow phasic patterns are present in the NAcc even when all periods of locomotion are excluded from firing rate analyses (Peoples et al., 1998). Instead, slow phasic firing changes are probably associated with the timing of appetitive responding as cocaine, and hence elevated dopamine (DA), levels decline. Indeed, evidence has been reported that phasic activity over minutes is sensitive to the systemic administration of the DA receptor antagonists eticlopride and SCH23390 (Nicola & Deadwyler, 2000). The authors found that both D1 and D2 receptor subtypes are implicated in the modulation of inter-trial NAcc firing rates, but probably via distinct mechanisms. Subsequent reports have confirmed the synergistic relationship between D1 and D2 receptor activation in the processing of drug-reinforced behavior [reviewed in Di Chiara et al. (2004)]. The present findings, in which different phasic reversal latencies were commonly found among simultaneous recordings within the same rat, are consistent with the above reports, and focus attention on the processing of reward-relevant firing changes associated with drug metabolism over the inter-infusion interval.

Differences between the prevalences of early and progressive/late reversal slow phasic patterns suggest that the firing rates of neurons in the medial (dorsal shell/rostral pole shell) and lateral (core/ventrolateral shell) NAcc may be differentially modulated by DA/drug levels over the course of the inter-infusion interval. This is consistent with an abundant literature that provides evidence for subregional differences in several NAcc DA-related parameters: differential DA immunohistochemistry (Voorn et al., 1989), higher basal DA concentrations/dopaminergic innervation in the shell (Deutch & Cameron, 1992) and a greater medial shell DA response to cocaine, as determined using both chrononanometry (David et al., 1998) and microdialysis (Pontieri et al., 1995; Barrot et al., 1999). This elevated shell DA response was recently reported to be associated with an increase in the number of phasic release events, which was shown to be sensitive to inactivation of the dopaminergic ventral tegmental area projection to the NAcc by γ-aminobutyric acid agonist administration (Aragona et al., 2008). In addition, the relative (co)expression of D1 and D2 receptor subtypes has been shown to differ between the medial shell and core (Bertran-Gonzalez et al., 2008). Taken together with the report that DA receptor subtypes have been differentially implicated in phasic neural activity occurring over the inter-response interval during cocaine self-administration (Nicola & Deadwyler, 2000), differences in DA neuromodulation are likely between the medial and lateral NAcc. This may account for differential drug level thresholds affecting slow phasic reversal latencies between medial and lateral NAcc subregions. Differences in reversal latencies may reflect the electrophysiological consequences of differential pharmacological sensitivities between neuronal populations; progressive reversal patterns may reflect neuronal processing in which a lower threshold must be attained by declining drug/DA levels before firing rates reverse over the inter-infusion interval, whereas early reversal patterns, which are sensitive to pharmacological shifts at a higher threshold, reverse with a shorter latency.

Recent insights into the complex nature of striatal DA signaling may account for these observed differences in reversal latencies. DA functions not only on the basis of the DA receptor subtype to which it binds, but according to the activity of local glutamatergic synapses coupled with the timing of terminal DA release and diffusion. DA can act extrasynaptically as a volume transmitter, and has been hypothesized to modulate excitatory input by regulating current flow through the heads of spines in medium spiny neurons (Arbuthnott & Wickens, 2007). Excitatory neurotransmission is a critical component in striatal...
synaptic plasticity, as it has been shown that the magnitude and the direction of time-dependent shifts in excitatory postsynaptic potentials depend critically on glutamatergic interactions with DA signaling between DA receptor subtypes (Shen et al., 2008). Although D1 and D2 receptor activation have commonly been considered to have opposite roles in electrochemical signaling (Uchimura et al., 1986; Pennartz et al., 1994), the consequence of DA binding to a particular receptor subtype can be more complicated. For example, depending on the temporal sequence of binding, D1 receptor activation can either promote a continued hyperpolarized state or facilitate the maintenance of a depolarized state (Arbuthnott & Wickens, 2007). Furthermore, the extent to which D1 receptors remain activated differs relative to D2 receptors as a function of DA concentration, with D2 receptor signaling being favored at lower concentrations (Richfield et al., 1989), which may occur later in the cocaine self-administration inter-infusion interval as drug, and hence DA, levels decline. The trend towards earlier reversal latencies in the medial than in the lateral NAcc is perhaps accounted for by subtle differences in the mechanism of DA-mediated neurotransmission, including differential activation of receptor subtypes (see above), or by differential mesencephalic glutamate co-transmission, for which evidence has recently been found medially, but not laterally, in the NAcc (Chuhma et al., 2009).

**Rapid phasic patterns**

The core subregion exhibited larger pre-press firing rate increases than the dorsal shell and rostral pole shell. These differences persisted when the core was expanded into the ‘lateral NAcc’ by the inclusion of the ventrolateral shell. We have previously found medial shell–core differences in peri-event firing by using a separate set of subjects that self-administered cocaine in a discriminative stimulus paradigm (Ghitza et al., 2004), which these continuous reinforcement schedule data corroborate and expand upon by including the ventrolateral shell.

Recently, Carelli & Wondolowski (2006) extended their investigations of rapid phasic firing patterns associated with natural vs. cocaine reinforcement (Carelli et al., 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003) into a subterritorial analysis of these correlates between the shell and core. At first glance, it appeared that our report of differential subregional prevalences of rapid phasic firing was in disagreement with their findings, which had suggested that patterned discharges of NAcc neurons associated with cocaine reinforcement are distributed evenly between the shell and core. However, a closer inspection of their wire placements revealed that almost all of their shell wires were lateral placements, and consequently their subterritorial comparisons were between lateral NAcc regions. Therefore, their finding of an even distribution is consistent with what would be predicted by the present results, which show that the core and ventrolateral shell exhibit similar firing pattern prevalences. The determination of whether primary vs. drug reinforcement neuronal correlates differ mediolaterally in the NAcc would be an intriguing follow-up study, and would test the contribution of cocaine pharmacological effects to the observed mediolateral differences.

**Correlations between time bases**

Many neurons exhibited significant changes in firing on more than one time base. Determining whether a neuron that exhibits a change in firing rate on the order of seconds also does so over hours or minutes might reveal clues regarding the factors that influence its activity. Such comparisons might permit further elaboration of the relative contributions of afferent signaling and pharmacological influences on the firing patterns commonly observed within the three time bases.

**Slow phasic vs. tonic patterns**

Neurons in both the dorsal shell and dorsal border regions exhibited highly significant correlations between the magnitudes of slow phasic and tonic firing rate changes. Tonic decreases predominated in the medial NAcc, particularly in the dorsal border region (Fabbricatore et al., 2009). Interestingly, most of the dorsal shell (83%) and all of the dorsal border (100%) neurons in this analysis also exhibited slow phasic decreases. Both tonic decreases and slow phasic post-press decreases are firing patterns that are consistent with classic primary dopaminergic effects on firing rate.

Core neurons also exhibited a correlation between the magnitudes of slow phasic and tonic firing rate changes, but this trend was not restricted to any particular tonic/phasic category. It is noteworthy that tonic increase neurons are virtually exclusive to the lateral NAcc during cocaine self-administration (Fabbricatore et al., 2009) and that, unlike for tonic decrease neurons, both slow phasic increases and decreases are prevalent among them.

As stated earlier, the decrease + progressive reversal firing pattern was the predominant slow phasic category among NAcc neurons. A closer inspection of its incidence among tonic categories revealed that it was similarly prevalent in tonic increase and tonic decrease neurons [6/21 (29%) and 23/63 (37%), respectively]. Moreover, decrease + early reversal patterns also exhibited similar prevalences between tonic categories: 1/21 (5%) for tonic increase neurons, and 4/63 (6%) for tonic decrease neurons. This observation prompted a similar comparison for post-press increase slow phasic neurons.

Among the 63 tonic decrease neurons, the slow phasic pattern of only one neuron (2%) was a post-press increase, as compared with 43% (9/21) among tonic increase neurons. Tonic increase neurons shared with the tonic decrease and no tonic change categories similar percentages of post-press decrease slow phasic patterns. Returning to the question of whether pharmacological factors influence tonic firing rates, additional evidence is herein presented suggesting that drug effects alone cannot account for the firing pattern profiles observed over the course of the self-administration session. If the sole influence on firing rate were strictly pharmacological, then tonic increase neurons would be expected to exhibit only post-press increase slow phasic patterns, consistent with the directionality of tonic firing rate change during the self-administration phase of the experiment. The fact that a substantial percentage of tonic increase neurons show post-press decrease slow phasic patterns suggests that some other factor, perhaps mediated by afferent signaling, is effecting this opposite firing rate directionality. As mentioned earlier, tonic increase neurons are restricted to lateral NAcc regions, so the influence of these ‘non-pharmacological’ factors on firing rate are not homogeneously distributed in the NAcc, but are specific to lateral regions.

**Rapid phasic vs. slow phasic patterns**

No NAcc subregion exhibited a significant correlation between the magnitudes of rapid and slow phasic firing rate changes of individual neurons. Rapid phasic patterns probably do not reflect pharmacological influences on firing rate (Carelli & Deadwyler, 1996; Peoples et al., 1997), whereas the time course of firing rate changes over the inter-infusion interval is not incompatible with pharmacological contributions (Wise et al., 1995; Nicola & Deadwyler, 2000).
Rapid phasic vs. tonic patterns

Core neurons exhibited a positive correlation between the magnitudes of rapid phasic and tonic firing rate changes. Not surprisingly, tonic increase core neurons accounted for this correlation: among all NAcc subregions, tonic increases were most predominant in the lateral NAcc, the core in particular [see Fig. 6 in Fabbricatore et al. (2009)]. The finding that pre-press firing increases and tonic increases co-exist preferentially in the lateral NAcc, where pharmacological firing rate suppression appears to be mitigated relative to the medial NAcc (see above), tempts speculation that one or both firing patterns reflect afferent processing related to the instrumental response.

Floresco et al. (2003) reported that independent tonic and phasic modulation may occur at the level of the ventral striatal region, wherein population firing and bursting firing (and hence tonic and phasic accumbal DA eflux, respectively) are regulated by distinct afferent pathways. This and more direct cortical–limbic signaling (Goto & Grace, 2005) are means by which the mesolimbic DA system and its target neurons can be influenced simultaneously by both pharmacological effects and signaling related to limbic–motor processing.

We have previously presented evidence of non-pharmacological effects in both the tonic increase and the tonic decrease categories (Fabbricatore et al., 1998) in experiments similar to those mentioned above, but with an extinction phase replacing the post-self-administration phase. Interestingly, evidence for non-pharmacological effects on firing rate among neurons that exhibited tonic decreases appeared during late extinction, when calculated drug levels had waned. Late extinction reversals in firing rate that paralleled changes in response rate, but not declining drug levels, were present in more than one-third of both tonic categories. Thus, in the case of neurons exhibiting reduced tonic firing, the throughput of behavioral signaling may be impeded by pharmacological factors during self-administration but become manifest in late extinction when drug levels are low.

A review by Di Chiara (2002) examined the impact of differential DA transmission between the core and shell. The author described the differential responsivity to DA transmission in the shell and core (tolerance and sensitization, respectively) after chronic drug exposure, and concluded that each neuroadaptation is a likely mechanism in driving the persistent drug-seeking behavior that ultimately leads to addiction. The present findings, derived from animals with regular, 6-h self-infusions were not maintained in either region.

The present findings show remarkable agreement with an emerging literature that: (i) describes the shell compartmentally, in terms of its position and connectivity in the basal forebrain circuitry [described in Fabbricatore et al. (2009)]; and (ii) advances the notion that shell compartmentalization subserves functional heterogeneity. For example, Ikemoto (2003), Ikemoto et al. (2005) have demonstrated, using intracranial self-administration, that drug (cocaine and amphetamine) self-administration is maintained by medial, but not lateral, shell infusions, extending earlier reports (Carlezon et al., 1995; Rodd-Henricks et al., 2002; Sellings & Clarke, 2003) that implicated the medial shell in the primary reinforcing effects of psychomotor stimulant administration. The core was similar to the lateral shell in that self-infusions were not maintained in either region.

Conclusions

Mediolateral firing rate differences likely can be attributed to both: (i) distinct subterritorial connectivities, in light of the finding that rapid phasic (and hence non-pharmacological) pre-press increases are elevated in the lateral NAcc and not elsewhere; and (ii) pharmacological differences, as reversal latencies are shorter in the medial NAcc, possibly because of differential DA neurotransmission in this region. The lateral NAcc tends to exhibit more excitation, with greater prevalences of both tonic increases and rapid phasic pre-press increases, whereas the medial NAcc exhibits more inhibitory firing patterns across time bases, possibly reflecting higher neuronal drug sensitivity.

These data extend our previous observation (Fabbricatore et al., 1998) that direct pharmaceutical factors alone are insufficient to account for longer time frame firing patterns: opposing firing rate changes within individual neurons when tonic and slow phasic categories are evaluated together suggests that both pharmacological and non-pharmacological factors contribute to signaling in neurons that are critical to drug self-administration behavior. During drug self-administration, pharmacological factors apparently predominate in the medial NAcc, given the ubiquitous firing rate suppression observed here in both tonic and slow phasic firing patterns. The interaction of pharmacological effects and afferent signaling likely mediates lateral NAcc firing changes during drug self-administration, as opposite signs among tonic and slow phasic firing patterns are observed here. Future investigations into the capacity for chronic disinhibition of medial NAcc targets to drive lateral NAcc output and any resultant neuroplastic changes in basal forebrain circuitry that may occur over prolonged drug exposure may be crucial in understanding the shift from casual use to drug addiction.

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Abbreviations

DA, dopamine; DV, dorsovelentral; NAcc, nucleus accumbens; PETH, peri-event time histogram; SEM, standard error of the mean.

References

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