Published in final edited form as:

J Pharmacol Exp Ther. 2008 February ; 324(2): 701–713. doi:10.1124/jpet.107.129734.

Dose- and Rate-Dependent Effects of Cocaine on Striatal Firing Related to Licking

Chengke Tang¹, Taliah Mittler, Dawn C. Duke, Yun Zhu, Anthony P. Pawlak, and Mark O. West

Department of Psychology, Rutgers University, New Brunswick, New Jersey

Abstract

To examine the role of striatal mechanisms in cocaine-induced stereotyped licking, we investigated the acute effects of cocaine on striatal neurons in awake, freely moving rats before and after cocaine administration (0, 5, 10, or 20 mg/kg). Stereotyped licking was induced only by the high dose. Relative to control (saline), cocaine reduced lick duration and concurrently increased interlick interval, particularly at the high dose, but it did not affect licking rhythm. Firing rates of striatal neurons phasically related to licking movements were compared between matched licks before and after injection, minimizing any influence of sensorimotor variables on changes in firing. Both increases and decreases in average firing rate of striatal neurons were observed after cocaine injection, and these changes exhibited a dose-dependent pattern that strongly depended on predrug firing rate. At the middle and high doses relative to the saline group, the average firing rates of slow firing neurons were increased by cocaine, resulting from a general elevation of movement-related firing rates. In contrast, fast firing neurons showed decreased average firing rates only in the high-dose group, with reduced firing rates across the entire range for these neurons. Our findings suggest that at the high dose, increased phasic activity of slow firing striatal neurons and simultaneously reduced phasic activity of fast firing striatal neurons may contribute, respectively, to the continual initiation of stereotypic movements and the absence of longer movements.

The striatum has been implicated in mediating stereotyped behaviors induced by high doses of psychomotor stimulants such as cocaine and amphetamine (Cooper and Dourish, 1990). Cocaine's effects are correlated with a pharmacological increase in striatal dopamine (DA) levels (Nicolaysen et al., 1988) via blockade of DA reuptake (Heikkila et al., 1979). Injections of DA agonists into the ventrolateral striatum induce oral stereotypy (Kelley et al., 1988). Conversely, this effect is blocked by DA antagonists (Delfs and Kelley, 1990). Furthermore, striatal DA depletion attenuates amphetamine-induced stereotypy (Creese and Iverson, 1973). Thus, there is strong evidence that the transduction of these pharmacological effects into stereotypic oral behaviors involves the ventrolateral striatum.

Nonetheless, it is not yet clear what changes occur in striatal firing during this transduction. Previous studies investigating effects of psychomotor stimulants on striatal neural activity have yielded a mixture of results, including suppression (Rebec and Segal, 1978; Nisenbaum et al., 1988), excitation (Haracz et al., 1993; West et al., 1997), or both (Trulson and Jacobs, 1979; Ryan et al., 1989; Pederson et al., 1997). Studies that reported exclusively suppression

were conducted during anesthesia, which itself suppresses spontaneous and sensory-evoked striatal firing (West, 1998). Therefore, it is desirable to conduct such studies in freely moving animals. Alteration of motor behavior by psychomotor stimulants introduces the need to consider the fact that most striatal neurons naturally fire phasically in relation to movement (e.g., Crutcher and DeLong, 1984; Mittler et al., 1994; Cho and West, 1997). Trulson and Jacobs (1979) recognized the importance of assessing drug effects on firing by comparing firing during similar movements before and after injection. Because some previous studies did not adequately do so, the mixture of effects that have been reported may involve the use of anesthesia or lack of adequate control for movement-related changes in firing. Thus, consideration of these factors is critical in the design of studies assessing striatal activity associated with the use of psychomotor stimulants.

A brief report from this laboratory examining cocaine's acute effects on firing rates of striatal neurons related to head movement during stereotyped head bobbing demonstrated a firing rate-dependent effect (Pederson et al., 1997). Firing during movements that were normally associated with low firing rates was elevated, whereas firing during movements that were normally associated with high firing rates was less elevated or suppressed by cocaine. This effect was more pronounced at higher doses.

To further investigate the role of striatal neurons in cocaine-induced stereotypy, in the present study, we examined changes in firing rate of neurons related specifically to licking in the ventrolateral striatum of awake, freely moving rats during a licking task. After 1 h in the task, rats received an acute injection of 0 (saline) 5, 10, or 20 mg/kg cocaine and then continued in the task for 1 to 3 h. The design allowed for drug-induced licking movement and at the same time included several levels of strategy aimed at minimizing the influence of movement variations on the assessment of cocaine's effect on striatal firing.

The ventrolateral striatum contains neurons that fire specifically in relation to orofacial sensorimotor activities such as licking (Crutcher and DeLong, 1984; Mittler et al., 1994; Cho and West, 1997). Specific sensorimotor firing of these striatal projection neurons is mediated by inputs from primary somatosensory and motor cortices (West, 1998, and references therein), thus providing a useful model for studying corticostriatal throughput. Because neurons that fire phasically in relation to sensorimotor activity constitute at least 50 to 70% of the neuronal population in the lateral striatum (Cho and West, 1997), it is likely that lick-related neurons are key to the ventrolateral striatum's role in psychostimulant-induced oral stereotypy. It is therefore important to better clarify how their firing patterns are altered in the presence of these commonly abused drugs.

Materials and Methods

Subjects and Surgery

A total of 18 male (300–350 g) Long Evans rats (Charles River, Wilmington, MA) were randomly assigned to four groups, each with a different dose of cocaine (0, 5, 10, or 20 mg/kg i.p.). Rats at doses 5, 10, and 20 mg/kg ($n = 9$) were surgically prepared for chronic extracellular recording via a microdrive. A base for attaching a microdrive (miniature microelectrode drive; Josef Biela Engineering, Anaheim, CA) was implanted on the skull overlying the lateral striatum (centered between 0.2 and 1.5 mm anterior and between 3.1 and 4.0 mm lateral from bregma) and secured with dental cement. Rats at dose 0 ($n = 9$) were surgically prepared for chronic extracellular recording via permanently implanted microwires. An array of Teflon-coated, stainless steel microwires (Shaptek Services, Hightstown, NJ) was implanted into the ventrolateral striatum (between 2.0 mm anterior and –0.4 posterior from bregma, between 3.2 and 4.2 mm lateral from bregma, and 6.0 mm ventral from skull level) and secured with dental cement. The array consisted of 12

microwires (50- μ m diameter of each uninsulated tip) separated from one another by 0.4 to 0.55 mm, which were arranged in two parallel rows separated by 0.4 to 0.55 mm (wire center to wire center). All rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and were administered injections of atropine methyl nitrate (10 mg/kg i.p.) and penicillin G (75,000 U/0.25 ml i.m.). Anesthesia was maintained with periodic injections of ketamine hydrochloride (60 mg/kg i.p.) during the surgery.

Rats were individually housed and maintained on a reversed light/dark cycle (on, 8:00 PM/ off, 8:00 AM). After 1 week of recovery, animals were water deprived and maintained at 82% of their post-surgery weight. Food was provided ad libitum. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data in accordance with the National Institute of Health guide for care and use of laboratory animals (NIH Publication 80-23).

Microdrive Recording

For rats at doses 5, 10, and 20 mg/kg, on the day of a recording session, the microdrive was equipped with a tungsten microelectrode (10 Mohm; Frederick Haer, Brunswick, ME) and attached to the base on the rat's skull. The rat was placed in a transparent, Plexiglas recording chamber (23.5 $\times$ 17.4 $\times$ 43 cm) and connected to a harness, which was connected at its other end to a commutator allowing free movement of the rat. The electrode was lowered to the ventrolateral striatum in small increments over several hours by manual rotation of the outer cylinder of the microdrive (400 μ m/rotation) without rotation of the electrode, providing a vertical recording track. Details regarding recording procedures and on-line verification of the microelectrode's position have been described previously (Mittler et al., 1994; Cho and West, 1997). Neural signals recorded from the microelectrode were amplified and filtered (450 Hz to 10 kHz) and then were digitized (62.5-kHz sampling frequency) and stored for off-line analysis using the software of Datawave Technologies (Berthoud, CO).

Microwire Recording

For rats at dose 0, microwire recording technology was utilized, allowing simultaneous recording from chronically implanted multiple electrodes with less risk of having to discard data due to loss of single unit isolation. An array of 12 microwires was implanted into the ventrolateral striatum, where lick-related neurons are densely located in clusters (Cho and West, 1997). On the day of a recording session, the rat was placed in the recording chamber. A harness was at its one end attached to the microwire array and connected at its other end to a commutator. Neural signals were led through an amplifier that differentially amplified them against background signals from another electrode within the array that did not exhibit neural signals. Neural signals were then led through a band pass filter, and were digitized (62.5-kHz sampling frequency for each wire) and stored for off-line analysis using the Datawave software. Neural parameters, e.g., waveforms and firing rates, of striatal neurons recorded by moveable microelectrodes or microwires were similar, allowing for pooling data that were collected from both types of recordings in off-line analysis.

Apparatus

A water spout was positioned 7 mm outside the front wall of the recording chamber. The chamber and licking apparatus were designed to restrict sensorimotor variables such as body and head position in front of the spout, while still allowing for the animal's free movement. Silicon tubing (1.6 mm inside diameter) was used to connect a container filled with tap water and a solenoid valve (General Valve, Fairfield, NJ) to the stainless steel drinking spout (Small Parts Inc., Miami, FL). A small hole (7-mm diameter) in the Plexiglas wall allowed the rat's tongue access to water drops at the tip of the spout. To register each lick, a

light beam carried by fiber optic and positioned above and below the spout was fed into a diode tracker. Thus, beginning at the inside surface of the front wall, the tongue crossed the light beam at 5 mm and reached the spout at 7 mm. The presence of the light beam (absent only while the tongue was interrupting it during a lick) was recorded and time-stamped every 16.6 ms by the same clock that time-stamped the neural waveforms (Discovery software; Datawave Technologies). Water delivery was controlled by a pulse from the computer that opened the solenoid for 35 ms, delivering one drop of approximately 5 μ l through the spout (one water delivery). Time between water deliveries was pseudorandom, ranging from 6 to 12 s with a mean of 9 s. An audible tone (35 ms, 70 dB), consisting of mixed frequencies (500 Hz and 3 KHz), was presented through a speaker mounted above the chamber during activation of the solenoid to provide an audible cue corresponding to the activation of the quiet solenoid. All recording sessions were videotaped to allow for off-line monitoring of behavior. White noise (60 dB outside the Plexiglas chamber) was used to minimize any disturbances from outside the experimental room.

Video Analysis

A computerized system was used for analyzing relationships between neural activity and videotaped licking. A video camera (Panasonic WV-BL202 CCTV; Panasonic Corporation, Secaucus, NJ) with zoom lens focused on the spout and a videocassette recorder (JVC Super VHS HR S7200U; JVC Company of America, Wayne, NJ) provided a resolution of tongue movement of 30 frames/s. The clock in the computer that time-stamped neural data also sequentially time-stamped each frame via a video frame counter (Thalner Electronics VC-436; Thalner Electronic Laboratories, Inc., Ann Arbor, MI), which displayed the number of each frame on the TV monitor. Using off-line videotape analysis, a specific motor event could be isolated on a single video frame. All frames in which the event recurred were compiled and entered into the computer as nodes. Raster displays and perievent time histograms (PETHs) were constructed around the nodes to depict unit activity time-locked to the motor event. Although each video frame was 33 ms in duration, greater resolution was routinely achieved by interpolating between frames. The maximum resolution of movement employed was in increments of 11 ms, with a maximum error of ± 1 increment. Videogenerated PETHs were used to verify the accuracy of PETHs generated by the diode tracker.

Behavioral Paradigm

Prior to each recording session, a complete sensorimotor exam was conducted for every electrode that exhibited neural activity to determine whether neural firing was related to sensorimotor activity of any body part(s) (Mittler et al., 1994; Cho and West, 1997). During the exam, drops of water were manually delivered to determine whether neural firing was related to licking, as the experimenter listened through headphones to the output of the filter/amplifier. Only lick-related neurons that increased firing rate during licking and did not increase firing rate during any nonoral behavior were recorded during a recording session.

Each recording session comprised three time epochs. 1) The pre-cocaine time epoch (T1) lasted 1 h and consisted of three 15-min water-on phases (100 water deliveries in each), alternating with three 5-min water-off phases (no water-deliveries). The licking response was readily shaped during the first 2 min of the first water-on phase in T1. 2) The cocaine time epoch (T2) began with the injection of either saline (0.9%, 0.5 ml/kg i.p.) or one dose of cocaine HCl (5, 10, or 20 mg/kg, calculated according to the salt weight of the drug, injected in 0.5 ml/kg volume of 0.9% saline i.p.). T2 lasted 1 h and was programmed identically to T1 with respect to water-on/water-off phases. 3) The recovery time epoch (T3) was the 3rd or 4th h following cocaine injection. T3 was also programmed identically to T1 with respect to water-on/water-off phases. For rats at doses 5, 10, and 20 mg/kg: 1) T1

began with the injection of saline (0.9%, 0.5 ml/kg i.p.), 2) T3 was conducted to examine the recovery of licking behavior and neural firing from the effects of cocaine in T2, and 3) up to three sessions were conducted on each rat to increase the data yield, with at least 4 weeks between any two sessions. No particular dose sequence was used. For rats at dose 0 (saline), only one session was conducted on each rat.

Histology

Following the last microdrive recording session, a lethal injection of sodium pentobarbital (150 mg/kg i.p.) was given, and an electrolytic lesion was made by passing anodal current (50 μ A for 4 s) through a stainless steel, insulated wire (250 μ m) that was mounted in the microdrive and positioned at the same location at which a neural recording had been obtained. Following the last microwire recording session, a lethal injection of sodium pentobarbital (150 mg/kg i.p.) was given, and anodal current was passed through each of the 12 microwires in the array to make an electrolytic lesion at the tip of each microwire. Then, intracardial perfusion was performed on each rat using 10% formalin-saline. The brain was extracted and fixed in a solution of 30% formalin and sucrose. Coronal sections (50 μ m) through the striatum were mounted. The iron deposit at each lesion was stained with a solution of 5% potassium ferricyanide and 10% HCl, and the tissue was counterstained with 0.2% solution of Neutral Red. The location of each recorded neuron (lesion) was determined by reconstructing its three-dimensional position within the striatum according to the brain atlas of Paxinos and Watson (2005). Recorded neurons corresponding to lesions that were found outside the ventrolateral striatum were discarded.

Behavioral Analysis

Analysis of Number of Licks during Water-Off Phases—The number of licks during water-off phases was used as a measure of licking stereotypy. Cocaine-induced stereotypy was assessed as the change in the number of licks during water-off phases from T1 to T2. A standardized value, $[L_2/(L_1 + L_2) - 0.5]$, was calculated for every recording session, with L_1 equal to the number of licks during water-off phases in T1, and L_2 equal to the number of licks during water-off phases in T2. Therefore, a standardized value of zero represents no change in the number of licks during water-off phases from T1 to T2, and a positive or negative standardized value represents an increase or decrease of the number of licks during water-off phases from T1 to T2, respectively. A one-way ANOVA with α level of 0.05 was conducted to evaluate differences in this measure across doses. Post hoc Bonferroni tests were used to evaluate pair-wise differences between doses if any significant differences across doses were found by ANOVA. Furthermore, to assess reversal of licking stereotypy in T3, a similar standardized value of change in the number of licks during water-off phases between T1 and T3, $[L_3/(L_1 + L_3) - 0.5]$, was calculated for every dose group that showed a significant change between T1 and T2 relative to dose 0. Then, a within-subjects comparison was conducted using a paired Student's *t* test to compare the standardized value of change in the number of licks during water-off phases between T1 and T2 versus that between T1 and T3.

Four experimental sessions at dose 20 were excluded only from this particular analysis of stereotypy, because video analysis confirmed that these rats extensively engaged in stereotypic behaviors such as head bobbing that competed with licking the spout. This resulted in substantial reductions in licking during both water-on and water-off phases in T2. Thus, the number of licks in water-off phases was not an appropriate measure of stereotypy for these rats. Nonetheless, the presence of stereotypic behaviors in T2 and the reversal in T3 were confirmed in these four sessions via video analysis. Despite the presence of competing stereotypic behaviors, sufficient numbers of licks at the spout were exhibited to

allow the data acquired in these sessions to be included in all other analyses, including the behavioral analysis of lick parameters and the neural analysis of matched pairs (see below).

Analysis of Behavioral Parameters of Each Lick—The onset of each lick was defined as the time at which the fiber optic light beam was interrupted (blocked). The end time of each lick was defined as the time at which the light beam became unblocked. Three behavioral parameters were calculated for every lick: 1) lick duration, time between the onset and end time of a lick. Lick duration ranged from 0 to 117 ms and was divided into 7 levels with equal increments of 16.6 ms from low to high; 2) lick period, time between the onset of the present lick and onset of the next lick. Period ranged from 84 to 267 ms and was divided into 12 levels with equal increments of 16.6 ms from low to high; and 3) interlick interval (ILI), time between the end of the present lick and onset of the next lick. ILI ranged from 0 to 250 ms and was divided into 15 levels with equal increments of 16.6 ms from low to high. By definition, for each lick, period was the sum of duration and ILI.

Based on its duration and period (ILI was therefore implicitly involved), every lick of a session was sorted into one cell of a matrix having seven rows (duration) and 12 columns (period). For each session, one matrix was generated for T1, one for T2, and one for T3. Detailed video analyses of tongue movement were compared with graphic frequency distributions of all recorded periods in the present paradigm. This analysis revealed that a single or consecutive undetected lick(s) that did not reach the lick sensor to break the light beam (e.g., a short lick or lateral tongue movement) resulted in a long interval (e.g., >270 ms) between the preceding detected lick and the subsequent detected lick. Such occurrences, which were frequent, would cause an artificial period of the preceding lick that was longer than its actual period. An artificial, long period could also be caused if the rat paused between consecutive licks. Therefore, an upper limit value of 270 ms for period was used to eliminate these artificial, long intervals to obtain an accurate measurement of period.

A multivariate analysis of variance (MANOVA) was performed on each of the three parameters to examine changes in that parameter between T1 and T2 across doses. For every recording session, the percentage of licks in every level of the parameter (7, 12, and 15 levels for lick duration, period, and ILI, respectively) during T1 through T2 was calculated. These percentage values were used as the dependent variable in the MANOVA for this parameter. The independent variables in the MANOVA were time (T1 and T2), dose, levels of the parameter, and two- and three-way interaction terms of these independent variables. Furthermore, if the MANOVA revealed any significant effect of cocaine on a parameter across doses, mean percentages of licks of all recording sessions in T1 and T2 were separately plotted against the levels of that parameter at different doses to illustrate the pattern of change in this parameter from T1 to T2 across doses. In addition, a second MANOVA between T1 and T3 was performed on that parameter to examine the recovery of that parameter across doses of cocaine. Of nine recording sessions at dose 20, one was excluded from the analysis of reversal because data were not available in T3 for this recording session.

We also used video analysis to measure the distance of individual licks by measuring whether or not the frontal portion of the tongue clearly and visibly extended through the hole in the front wall outside the chamber by greater than 3 mm (defined as a “long-distance lick”). For every recording session at dose 20, we examined 100 licks beginning at the 5th min of each water-on phase in T1 and T2. The percentages of long-distance licks in T1 and T2 were separately calculated by dividing the total number of long-distance licks in all three water-on phases of each phase by 300. The change of this value between T1 and T2 at dose 20 was assessed using a Wilcoxon signed ranks test.

Neural Analysis

Isolation of the Waveforms and Construction of Perievent Time Histograms for Single Neurons—After the session, a neuron's signals were isolated from background noise and from other neurons' signals using the “cluster cutting” process of the Datawave software. Waveforms of each neuron were analyzed using eight parameters: peak amplitude, valley amplitude, spike height, latency to peak, latency to valley, and voltages at three particular time points chosen based specifically on the individual waveform, as described in detail elsewhere (Tang et al., 2007). Interspike interval analysis was also used to confirm that isolated waveforms in each case corresponded to those of a single neuron (i.e., no discharges occurred within the first 2 ms in the interspike interval histogram, representing a neuron's natural refractory period). Cocaine at high concentration interferes with Na⁺ channels, which could alter extracellular action potential waveforms and result in failures to detect discharges. Despite this possibility, our analyses showed that waveforms were unchanged following the high dose (see Pederson et al., 1997).

A PETH that displayed neural firing forward and backward in time from onset of lick was constructed for each neuron recorded, using as nodes all licks from the entire session. These histograms were used to confirm that firing of each neuron analyzed was related to licking and to determine the time window in which the neuron's firing increased during the lick. In the PETHs, all lick neurons showed increased firing: some neurons increased firing before lick onset, some at onset, and some after onset. Therefore, analysis of neural firing was customized to each individual neuron by determining a time window of firing in the PETH using one of two methods: 1) a visual examination was performed on the PETH. The beginning of the time window was defined as the time (in milliseconds) at which neural activity showed a visually distinct increase in firing above the baseline preceding lick onset. If the increased firing returned to baseline at a time before the beginning of lick, then the end of the time window was set at the beginning of the lick. If the increased firing returned to baseline at a time near the end of lick, then the end of the time window was set at the end of the lick. If the increased firing rate returned to baseline at a time distant from the end of lick, then the time window ended at the time at which the increased firing returned to baseline; 2) a Wilcoxon analysis was used on the PETH. The beginning of the time window was defined as the time at which neural activity increased ($p < 0.05$, comparing two consecutive 20-ms bins at a time), and similarly the end of the time window was defined as the time at which the increased firing rate returned to the preincrease level (Peoples and West, 1996). Both methods yielded similar results. In both methods, the beginning of the time window was not allowed to exceed a maximum of 80 ms prior to the onset of the present lick to assure that the time window of the present lick did not overlap with that of the previous lick.

Matched Pairs—All assessments of cocaine's effects on firing involved comparisons of “matched pairs,” defined as follows. A neuron's firing rate (FR) during each lick was calculated by dividing the number of discharges that occurred during the specified time window of firing by the duration of that time window. Each lick from a given session was sorted into the matrix for the time epoch (T1, T2, or T3) during which the lick occurred. Mean FR was calculated for the licks (with the same duration and period) that were included in each cell of the matrix for each time epoch. To ensure an adequate sampling for a more accurate assessment of mean FR, five or more licks of a particular duration and period (i.e., five or more licks per cell) were required in each epoch to be included in the analysis. Licks with similar duration and period that occurred $\geq$ five times in each epoch were matched between T1 and T2 or between T1 and T3 and were termed a matched pair. To illustrate this approach, Table 1 presents the matched pairs between T1 and T2 and those between T1 and T3 of a representative neuron.

Duration and period were used in conjunction as matching parameters to create matched pairs to better assess firing associated with various types of licks. This is because licks with a given duration can occur at different rates of licking (i.e., different period) at different times during the recording session. Thus, lick duration alone cannot accurately represent a lick in all its varieties throughout the recording session. Indeed, the addition of period allowed dividing licks into up to 84 types of matched pairs (seven levels of duration $\times$ 12 levels of period), yielding a substantially greater number of separate measures of each neuron's firing. Another rationale of choosing duration and period for neural analysis of matched pairs was because lick-related firing occurred at the time measured by these two parameters but not during ILI.

For each neuron, the only method of comparing FR between pre- and postdrug epochs was by using matched pairs. This minimized the extent to which sensorimotor variability could influence our assessment of pre- versus postcocaine changes in firing.

Examining Changes in Mean Firing Rate of Individual Neurons from T1 to T2 across Doses with a Hierarchical Linear Model—A hierarchical linear model (HLM) is appropriate for analyzing data with a hierarchical structure (Bryk and Raudenbush, 2002). A two-level hierarchical structure existed in our neural data in which matched pairs were nested within individual neurons to which they belonged. Therefore, a two-level HLM was developed to model the two-level hierarchical neural data of matched pairs between T1 and T2.

Level 1 within-neuron model:

$$T2FR_i = \beta_{0i} + \beta_{1i} T1FR_i + e_i \quad (1)$$

In this equation, firing rates of matched pairs in T2 ($T2FR_i$) of the i th neuron were linearly regressed on firing rates of matched pairs in T1 ($T1FR_i$) of that neuron. Each neuron had a regression equation that was characterized by two regression parameters: β_{0i} represented the intercept, and β_{1i} represented the slope of the linear regression. The error term (e_i) represented the unexplained portion of within-neuron variance of the linear regression. To facilitate the interpretation of the intercept (β_{0i}) of the regression, $T1FR_i$ was centered on the mean by subtracting the mean $T1FR$ ($MT1FR_i$; for each neuron, the mean firing rate of all matched pairs in T1) from the $T1FR$ of each matched pair of that neuron. After centering, the intercept (β_{0i}) of each level 1 regression represented the predicted average firing rate of all matched pairs in T2 for an individual neuron (Bryk and Raudenbush, 2002). Regression parameters (β_{0i} and β_{1i}) that were obtained from the level 1 within-neuron model were further modeled as outcome variables in the level 2 between-neuron model.

Level 2 between-neuron model:

$$\beta_{0i} = \gamma_{00} + \gamma_{01} Dose_i + \gamma_{02} Dose_i^2 + \gamma_{03} MT1FR_i + \gamma_{04} Dose_i * MT1FR_i + \gamma_{05} Dose_i^2 * MT1FR_i + u_{0i} \quad (2)$$

$$\beta_{1i} = \gamma_{10} + \gamma_{11} Dose_i + \gamma_{12} Dose_i^2 + \gamma_{13} MT1FR_i + \gamma_{14} Dose_i * MT1FR_i + \gamma_{15} Dose_i^2 * MT1FR_i + u_{1i} \quad (3)$$

Two regression equations were included in the level 2 between-neuron model. Each had three predictor variables and their interaction terms: dose, dose², MT1FR, the interaction of dose and MT1FR, and the interaction of dose² and MT1FR. To control against multicollinearity caused by the interaction terms in the level 2 model, dose, dose², and MT1FR

were each centered around their means by subtracting the overall means from each value of these variables across all neurons (Bryk and Raudenbush, 2002). The HLM allowed us to simultaneously model changes in average FR of individual neurons and changes in slope of the within-neuron regression via between-neuron regression eqs. 2 and 3, respectively. In eq. 2, β_{0i} represented the predicted average T2FR of the i th neuron, γ_{00} represented the intercept of the regression, other γ s represented the regression parameters of corresponding variables in eq. 2, and the error term (u_{0i}) represented the unexplained portion of between-neuron variance of the regression function in eq. 2. In eq. 3, β_{1i} represented the slope of the within-neuron regression of the i th neuron, γ_{10} represented the intercept of the regression, other γ s represented the regression parameters of corresponding variables in eq. 3, and the error term (u_{1i}) represented the unexplained portion of between-neuron variance of the regression function in eq. 3. This two-level HLM was fit on the neural data of matched pairs using the SAS PROC MIXED procedure (SAS Institute Inc., Cary, NC).

If the HLM on matched pairs between T1 and T2 found any significant effect of cocaine on the average FR of neurons in T2 across doses, analysis of reversal in T3 was performed by fitting another two-level HLM on matched pairs between T1 and T3 for neurons at doses 5, 10, and 20. In this HLM, the T3FR of matched pairs was used as the dependent variable in the level 1 within-neuron regression, and other predictor variables were kept the same.

Following each HLM analysis, we performed additional ordinary least squares linear regression analyses by regressing the average T2FR or T3FR on the average T1FR of neurons based on log10-transformed values at each dose. The R^2 values and regression lines were compared across doses to demonstrate any effects of cocaine on the neuron's average firing rate in T2 or T3 that were revealed by the HLM analysis.

Examining Changes in Firing Rates of Matched Pairs from T1 to T2 of

Individual Neurons across Doses—To examine the change in dispersion or variability in FR of matched pairs from T1 to T2 of individual neurons across doses, the S.D.s of T1FRs and T2FRs of all matched pairs were separately calculated for every neuron. A two-way repeated-measures ANOVA with dose as the between-neuron variable and time (T1 and T2) as the repeated-measure within-neuron variable was performed on these S.D.s.

To assess the predictability of T2FR of matched pairs from their T1FR within individual neurons, the T2FRs of matched pairs were linearly regressed on their T1FRs for every neuron. The R^2 value of each regression function represented the proportion of total variance in T2FRs of all matched pairs that could be accounted for by their T1FRs for every neuron. A one-way ANOVA was performed on R^2 values of neurons across doses.

To further examine the changes in FR of matched pairs from T1 to T2 of individual neurons across doses, two 2×4 analysis of covariance (ANCOVA) models were performed on “slow” and “fast” firing neurons, respectively. First, neurons were segregated into two categories: slow and fast firing neurons. This was done according to each neuron's average T1FR of all matched pairs, using as the cut point 1.00 impulses/s (Pederson et al., 1997), which approximated the overall median (1.02 impulses/s) of the average T1FRs of all 70 neurons in the present study. Second, all matched pairs of each individual neuron were divided into two categories of T1FR matched pairs (low and high T1FR groups), using as the cut point the middle of the neuron's range of T1FRs by calculating: [(the neuron's maximum T1FR from among all its matched pairs)/2]. Third, for every neuron, the T1FR and T2FR of each matched pair were transformed by $[(T2FR/(T1FR + T2FR)) - 0.5]$ to compute a standardized value that represented the magnitude of change in FR from T1 to T2 for each matched pair. Because this transformation was inappropriate for matched pairs with zero FR values in either T1 or T2, a constant of 0.01, the smallest decimal increment in FR

observed in the present study, was added to T1FR and T2FR of every matched pair before the transformation to include all matched pairs into this analysis (Mosteller and Tukey, 1977). Furthermore, to justify adding the constant 0.01, a thorough graphic examination was performed on every neuron to compare the patterns of change in the standardized values of its matched pairs before and after adding the constant. The results of this graphic analysis confirmed that adding the constant did not cause any differences in the patterns of change in the standardized values.

In both ANCOVAs (one for slow firing neurons and the other for fast firing neurons), the dependent variable was the standardized value of change from T1FR to T2FR of matched pairs. The independent variables were dose (0, 5, 10, and 20), T1FR group (low and high T1FR matched pairs), and the interaction between dose and T1FR group. The average T1FR of individual neurons (MT1FR) was included in each ANCOVA as a covariate to take into account the fact that matched pairs came from neurons with different average T1FRs. Post hoc Bonferroni tests were used to examine changes in FR of matched pairs at each cocaine dose relative to the saline group.

If the ANCOVA(s) revealed any significant effect of cocaine on FR of matched pairs within slow or fast firing neurons at any dose(s) in T2, analysis of reversal in T3 was performed separately for slow and fast firing neurons that exhibited stable neural activity in T3 at each dose. At each dose, matched pairs included in the analysis of reversal were those of T1 and T2 that also showed no less than five licks in T3. Similar to calculating the standardized value of change in FR between T1 and T2, a standardized value of change in FR for each matched pair between T1 and T3 was calculated. Then, for matched pairs of slow or fast firing neurons at each dose, a within-subject comparison was conducted using a Wilcoxon signed ranks test to compare the standardized value of change in FR of matched pairs between T1 and T2 versus that between T1 and T3. A significant result of this comparison would indicate a reversal of FR of matched pairs from T2 to T3 with respect to T1 at this dose. If the ANCOVA revealed a significant interaction between dose and T1FR group that indicated different effects of cocaine on low and high T1FR matched pairs at each dose, the comparison was performed separately for low and high T1FR matched pairs at each dose. If the interaction was not significant, the comparison was performed for all matched pairs by pooling low and high T1FR matched pairs at each dose.

Results

Behavioral Analysis

Cocaine-Induced Stereotypy in T2 and Reversal in T3—Changes in the number of licks during water-off phases between T1 and T2 differed across doses [$F(3,21) = 4.70$, $p < 0.05$; one-way ANOVA]. Only rats at dose 20 ($n = 5$) significantly increased the number of licks during water-off phases from T1 to T2, relative to rats at dose 0 ($n = 9$) ($p < 0.05$; post hoc Bonferroni tests). This indicates that licking stereotypy was induced only at dose 20 during the hour following cocaine injection (Fig. 1A). Reversal of licking stereotypy in T3 was then assessed for dose 20. The change in the number of licks during water-off phases in T3 relative to T1 was significantly lower than that in T2 relative to T1 (Fig. 1B; $p < 0.05$; paired Student's t test). Therefore, the number of licks during water-off phases in T3 (mean = 54) had reversed to the predrug level in T1 (mean = 83) from the increased postdrug level in T2 (mean = 343).

Dose-Dependent Changes in Lick Parameters in T2 and Reversal in T3—A MANOVA on lick duration between T1 and T2 revealed a significant three-way interaction among T1 to T2, dose, and levels of lick duration [$F(17,157) = 1.94$, $p < 0.05$; Wilks' Lambda criterion], suggesting a dose-dependent effect of cocaine on lick duration. As dose

increased, cocaine systematically increased the proportion of licks with shorter duration and concurrently decreased the proportion of licks with longer duration in T2 relative to predrug values in T1 (Fig. 2A). A separate MANOVA on lick duration between T1 and T3 revealed that there was no significant three-way interaction among T1 and T3, dose, and levels of duration ($p > 0.83$), and there was no significant two-way T1 to T3 $\times$ dose interaction ($p > 0.99$) or main effect of T1 to T3 ($p > 0.84$). This result indicates a reversal of cocaine's effect on lick duration in T3 to predrug levels.

There was also a dose-dependent effect of cocaine on ILI, indicated by a significant three-way interaction among T1 to T2, dose, and levels of ILI [$F(42,366) = 1.45$, $p < 0.05$; Wilks' Lambda criterion, MANOVA]. As dose increased, cocaine systematically decreased the proportion of licks with shorter ILI and concurrently increased the proportion of licks with longer ILI in T2 relative to predrug values (Fig. 2B). There were no significant interactions ($p > 0.61$) or main effect ($p > 0.99$) on ILI between T1 and T3 across doses, indicating a reversal of cocaine's effect on ILI in T3 to predrug levels.

In contrast, a MANOVA on period between T1 and T2 revealed there was no significant three-way interaction among T1 to T2, dose, and levels of period ($p > 0.82$), and there was also no significant two-way T1 to T2 $\times$ dose interaction ($p > 0.67$) or main effect of T1 to T2 ($p > 0.16$). Thus, cocaine did not affect period in T2 with respect to T1, which was confirmed by graphic examination. At all doses, there was a similar distribution of periods between T1 and T2 at all levels of period from 83 to 267 ms, with a small but insignificant shift following cocaine injection: a smaller proportion of long periods and a larger proportion of short periods.

Therefore, following cocaine administration, lick duration and ILI both changed in a dose-dependent fashion but in opposite directions. That is, higher doses of cocaine tended to decrease lick duration while increasing ILI, particularly during stereotyped licking at dose 20. In addition to these changes, video analysis demonstrated that rats made significantly fewer long-distance licks in T2 (median = 13.0%) relative to T1 (median = 24.3%) at dose 20 ($p < 0.05$, Wilcoxon signed ranks test).

Neural Analysis

Dose- and Rate-Dependent Effects of Cocaine on Average Firing Rates in T2 and Reversal in T3—Seventy lick-related striatal neurons were recorded in 29 recording sessions from 18 rats. All neurons were histologically verified to be located in the ventrolateral striatum (Fig. 3). Of those 70 neurons, 20, 11, 17, and 22 were obtained at doses 0, 5, 10, and 20 mg/kg, respectively. A total of 1983 matched pairs between T1 and T2 were obtained from all 70 neurons. Of those matched pairs, 771, 267, 508, and 437 were from neurons at doses 0, 5, 10, and 20 mg/kg, respectively. Thus, the numbers of neurons and matched pairs were comparable across doses. Matched pairs of a representative neuron at dose 20 are shown in Table 1 to illustrate how matched pairs were yielded for individual neurons.

Table 2, top, presents results of modeling the changes in average FR of individual neurons by fitting an HLM on all matched pairs of all neurons. The HLM revealed a significant interaction of dose² and the average T1FR ($\gamma_{05} = -0.003$, $p < 0.05$) on the average T2FR of individual neurons, indicating that the changes in average firing rate of individual neurons from T1 to T2 were significantly different across doses and depended on the neuron's average T1FR. These effects are illustrated in Fig. 4 and, in addition, were further demonstrated using linear regression analysis between the average T1FRs and the average T2FRs of neurons at each dose. There was a strong linear relationship between the average T1FRs and the average T2FRs of neurons at doses 0 ($R^2 = 0.89$, $p < 0.001$), 5 ($R^2 = 0.92$, $p <$

0.001), and 10 ($R^2 = 0.90$, $p < 0.001$) but not at dose 20 ($R^2 = 0.22$, $p < 0.05$). Moreover, the slope of the regression line at dose 0 was not significantly different from 1 [slope = 0.917, 95% confidence interval (CI) = (0.757, 1.078)], indicating that the average T2FRs of neurons at dose 0 remained the same as their average T1FRs (Fig. 4A). In comparison, the slopes of the regression lines at dose 5 [slope = 0.79, 95% CI = (0.619, 0.961)], 10 [slope = 0.744, 95% CI = (0.608, 0.879)], and 20 [slope = 0.264, 95% CI = (0.031, 0.497)] were all significantly less than 1 with a decreasing trend as dose increased (Fig. 4, B–D), showing a “clockwise” rotation of between-neuron regression lines with increasing dose.

Notably, this dose-dependent pattern of clockwise rotation also demonstrates a firing rate-dependent effect of cocaine. At doses 5 and 10, the clockwise rotation of the regression lines was produced by increases in average T2FRs of slow firing neurons (i.e., neurons that exhibited average T1FRs < 1 impulses/s; Fig. 5A). At dose 20, both the increases in average T2FRs of slow firing neurons and the decreases in average T2FRs of fast firing neurons (average T1FRs > 1 impulses/s; Fig. 5B) together determined the greater clockwise rotation of the regression line.

To assess reversal of the altered average FR in T3, a total of 621 matched pairs between T1 and T3 were obtained from 28 neurons that exhibited stable neural activity in T3 at doses 5 ($n = 9$), 10 ($n = 13$), and 20 ($n = 6$). An HLM on these matched pairs revealed that there were neither significant interaction effects of dose and the average T1FR (dose $\times$ MT1FR and dose² $\times$ MT1FR; $p > 0.64$) nor significant main effects of dose (dose and dose²; $p > 0.7$) on the average T3FR of individual neurons. Additional regression analysis at each of the cocaine doses showed that none of the slopes of the between-neuron linear regression lines that regressed neurons' average T3FRs against their average T1FRs was significantly different from 1 [dose 5, slope = 0.995, 95% CI = (0.789, 1.201); dose 10, slope = 0.918, 95% CI = (0.619, 1.215); dose 20, slope = 1.913, 95% CI = (−1.078, 4.905)]. These data demonstrate that neurons' average T3FRs were not significantly different from their average T1FRs at any dose (Fig. 5). Thus, the dose- and rate-dependent effects of cocaine on the average firing rates of individual neurons observed in T2 were no longer present in T3.

Dose- and Rate-Dependent Effects of Cocaine on Firing Rates of Matched Pairs within Individual Neurons in T2 and Reversal in T3—A two-way repeated-measures ANOVA on the S.D.s of T1FRs and T2FRs of matched pairs within neurons revealed that there was no significant interaction between T1 and T2 and dose [$F(3,66) = 0.52$, $p > 0.67$] and no significant main effect of T1 and T2 [$F(1,66) = 0.02$, $p > 0.88$]. These data demonstrate that, although average FR of individual neurons systematically changed from T1 to T2 across doses as revealed by the HLM model (Fig. 4), the dispersion of FRs of matched pairs within individual neurons did not change from T1 to T2 in each dose.

Changes in firing rate of matched pairs within individual neurons were also analyzed by modeling the slope of the linear regression between T1FR and T2FR in the HLM (Table 2, bottom). Neither the interaction effects between dose and average T1FR (dose $\times$ MT1FR and dose² $\times$ MT1FR; $p > 0.24$) nor the main effects of dose (dose and dose²; $p > 0.05$) were significant, indicating that matched pairs within neurons did not exhibit a dose-dependent pattern of change in FR. Moreover, the regression R^2 values of matched pairs within neurons were low at each dose and did not differ across doses [$F(3,66) = 2.20$, $p > 0.09$; one-way ANOVA]. The average R^2 value of all neurons at all doses was 0.12 ± 0.16 (mean $\pm$ S.D.). Therefore, within individual neurons at all doses, the T1FRs of matched pairs accounted for little variance in their T2FRs and had very low predictability on their T2FRs. This is consistent with our observation that, for a given matched pair of an individual neuron, its

T2FR could be anywhere within the range of the T2FRs of all matched pairs of the neuron, regardless of the T1FR of the matched pair.

However, significant changes in FR emerged when matched pairs were separately analyzed in slow and fast firing neurons. Because cocaine's effects on average FR differed for these two types of neurons as described above, we analyzed slow and fast firing neurons separately with two ANCOVAs to test whether cocaine affected matched pairs within slow and fast firing neurons differently across doses. We further dichotomized matched pairs into low and high T1FR groups for each neuron to examine if low and high T1FR matched pairs exhibited different patterns of change in FR within individual neurons.

Thirty five neurons were categorized as slow firing, including 11, 6, 6, and 12 neurons from doses 0, 5, 10, and 20 mg/kg, respectively. At dose 0, a "regression to the mean" phenomenon, characterized by increased firing of low T1FR matched pairs and decreased firing of high T1FR matched pairs in T2, was observed within neurons (Figs. 6A and 7A). Across doses, there was a significant main effect of dose on FR of matched pairs from T1 to T2 [$F(3,822) = 8.39, p < 0.0001$, ANCOVA] but no interaction between dose and T1FR group ($p > 0.15$). Therefore, cocaine's effects on FR of the low and high T1FR matched pairs were similar across dose. Post hoc Bonferroni tests revealed significant enhancing effects on FR at doses 10 ($p < 0.001$) and 20 ($p < 0.001$) (Fig. 6A). Specifically, relative to the natural regression to the mean at dose 0, the low T1FR matched pairs showed greater increases, and the high T1FR matched pairs showed lesser decreases in firing rate from T1 to T2 at doses 10 and 20. These effects are illustrated by matched pairs of representative slow firing neurons at doses 10 and 20 (Fig. 7, E and G). Moreover, an analysis of reversal in T3 was performed separately for matched pairs at doses 10 (135 matched pairs from five neurons) and 20 (53 matched pairs from three neurons). The significant changes in FR from T1 to T2 were reversed in T3 at dose 10 ($p < 0.001$; Wilcoxon signed ranks test) but not at dose 20 ($p > 0.89$).

The remaining 35 neurons (9, 5, 11, and 10 at doses 0, 5, 10, and 20 mg/kg, respectively) were fast firing neurons. A similar regression to the mean was observed for matched pairs within neurons at dose 0 (Figs. 6B and 7B). ANCOVA revealed a significant main effect of dose on FR of matched pairs from T1 to T2 [$F(3,1139) = 59.73, p < 0.0001$] without an interaction between dose and T1FR group ($p > 0.2$), indicating a similar drug effect on low and high T1FR matched pairs across doses. However, in contrast to the enhancing effect on matched pairs within slow firing neurons, the high dose of cocaine suppressed both low and high T1FR matched pairs within fast firing neurons (Fig. 6B, $p < 0.001$, post-hoc Bonferroni tests). Specifically, relative to the regression to the mean at dose 0, low T1FR matched pairs showed decreases rather than increases in firing rate, whereas high T1FR matched pairs showed greater decreases in firing rate in T2 at dose 20. These effects are illustrated by matched pairs of a representative fast firing neuron at dose 20 (Fig. 7H). In addition, in T3, a reversal of cocaine's effect was found ($p < 0.05$; Wilcoxon signed ranks test) on 54 matched pairs between T1 and T3 obtained from three fast firing neurons at dose 20.

Discussion

Our question was: "Following cocaine injection, what changes in striatal activity accompany changes in motor behavior that involve cocaine's effects in the striatum?"

Behavioral Effects

Psychomotor stimulants cause increased long-sequence movements at low doses and shorter, stereotyped movements at high doses (Bhattacharyya and Pradhan, 1979). In the present paradigm, although licking was induced by water delivery, the highest dose of cocaine

nonetheless induced stereotyped licking. This was evidenced by increased licking in the absence of water delivery, consistent with stereotypic effects observed in other studies (Lyon and Robbins, 1975; Bhattacharyya and Pradhan, 1979). This pattern of purposeless, repetitive movement is a defining characteristic of drug-induced stereotypy (Cooper and Dourish, 1990).

Compared with predrug levels, cocaine simultaneously: 1) decreased the proportion of long lick durations while increasing the proportion of short durations and 2) increased the proportion of long ILI while decreasing the proportion of short ILI. These shifts in the frequency distributions of duration and ILI were more pronounced with increasing dose and subsequently reverted to predrug levels. Corresponding to decreased lick duration was a significant decrease in the distance of licks at the high dose. Reduced lick distance and duration are consistent: 1) with our previous finding that cocaine (20 mg/kg) decreased the proportion of long head movements (Pederson et al., 1997), 2) with findings in other laboratories (Fowler and Mortell, 1992), and 3) with the fact that striatal neurons phasically related to movement exhibit strong correlations with such parameters (Crutcher and DeLong, 1984; Pederson et al., 1997; Tang et al., 2007).

Period was not altered by cocaine, reflecting the complementary, opposing changes in its two components, i.e., decreased lick duration and increased ILI. The consistent licking rhythm, which was not affected even by a dose (20 mg/kg) high enough to induce stereotypic licking, suggests that cocaine did not exert any apparent effects on brain stem mechanisms controlling licking rhythm (Wiesenfeld et al., 1977; Brozek et al., 1996; Travers et al., 1997). Nonetheless, our observed trend (though not significant) toward fewer long periods and more short periods within bursts is in the same direction as Knowler and Ukena (1973) observed at low doses in their sample of three rats. In another sample of three monkeys, amphetamine-induced increases in licking rate were inversely related to control rate (Wuttke, 1970). However, lick rate is not simply the inverse of within-burst period. We analyzed period only within bursts of licking (period ≤ 270 ms) but did not assess overall rate due to the fact that a small percentage of licks failed to break the light beam.

Dose- and Firing Rate-Dependent Effects on Firing

At zero dose (saline), average firing rates did not change after injection, represented by a regression line between T1 and T2 average firing rates that was not different from a line of no change. At low and moderate doses, slow firing neurons exhibited increased firing rates, resulting in a clockwise rotation of the regression line. At the high dose, which induced stereotypic licking, the regression line rotated clockwise to the greatest degree, determined by both the increased firing of slow firing neurons and the decreased firing of fast firing neurons. These dose-dependent changes in firing were not present during the 3rd h following cocaine administration, at which time behavior also returned to predrug levels.

Moreover, cocaine's effects on firing associated with matched pairs of individual types of licks were also dose- and rate-dependent, underlying the observed changes in average firing rate. At zero dose, matched pairs with lower firing rates in T1 tended to increase firing in T2 and those with higher firing rates in T1 tended to decrease firing in T2. These combined to yield no change in average firing rate after saline injection. This regression to the mean was observed in matched pairs of both slow and fast firing neurons, consistent with the natural and spontaneous fluctuations in phasic firing exhibited by sensorimotor striatal neurons (Prokopenko et al., 2004). In contrast, significant and differential effects were observed on matched pairs of individual slow and fast firing neurons following cocaine injection.

For slow firing neurons at the middle and high doses, matched pairs with lower predrug firing rates exhibited a greater increase, and those with higher predrug firing rates exhibited

a lesser decrease in firing, compared with the saline group. Thus, firing of all matched pairs was elevated above control at these two doses, underlying the increased average firing rate of slow firing neurons.

For fast firing neurons, however, the high dose of cocaine suppressed firing of all matched pairs, especially those with higher predrug firing rates, underlying the decreased average firing rate of these neurons at this dose. Thus, suppressed firing rates were observed only for fast firing neurons, especially for their highest firing rates, and only at the high dose.

Potentially analogous to the observation of Dews (1958) of rate-dependent effects of stimulants on behavior, cocaine's effect depended on an individual neuron's normal firing rate. Slow firing neurons exhibited a generally greater tendency to discharge at moderate and high doses, their low firing rates more elevated and their high firing rates less reduced than predicted by the regression to the mean observed for controls. Fast firing neurons exhibited the opposite, i.e., a generally lower tendency to discharge at the high dose, both their low and high firing rates strongly reduced compared with controls. Notably, their highest firing rates exhibited the greatest reductions.

The present dose- and rate-dependent patterns of striatal activity during cocaine-induced stereotypic licking help to sort out the mixture of increases and decreases in firing rates observed in previous studies of effects of psychomotor stimulants on striatal neurons during behavior (Trulson and Jacobs, 1979; Gardiner et al., 1988; Haracz et al., 1989; Ryan et al., 1989; Pederson et al., 1997; West et al., 1997). It appears that predrug firing rate is an important factor to consider in such studies. According to the present findings, dose-dependent changes in a neuron's average firing rate would be predicted by its predrug average. Slow firing neurons would on average exhibit elevated firing rates at all doses. Moreover, an individual slow firing neuron would exhibit dose-dependent elevations only for its normally low, but not its high, firing rates. For a fast firing neuron, both its normally low firing rates, and to an even greater extent its normally high firing rates would be suppressed by high doses capable of inducing stereotypic movement. Indeed, initial firing rate may be important in a more general sense, as demonstrated by our finding that the initial firing rate of striatal neurons is correlated with their rate of change in firing during motor habit learning (Tang et al., 2007).

We previously reported that striatal head movement neurons exhibited similar rate-dependent changes in movement-related firing after cocaine administration (Pederson et al., 1997). Recently, we found that these drug effects were also dose-dependent when compared with a saline group (unpublished data). These studies and the present one independently demonstrate consistent firing rate- and dose-dependent effects of cocaine on movement-related striatal firing in different populations of striatal neurons associated with different stereotyped behaviors. Cocaine's effects on both slow and fast firing neurons may be involved in producing stereotypy. At a high dose, the elevation of low striatal firing rates may stimulate motor and premotor areas to initiate movements, whereas the simultaneous suppression of high firing rates may reduce the likelihood of long-sequence or exploratory movements. Other psychomotor stimulants (e.g., amphetamine) may also influence striatal firing during stereotypy in a similar dose- and rate-dependent manner (Trulson and Jacobs, 1979; West et al., 1997). This hypothesis needs to be validated in future studies.

The present elevation of low average firing rates and reduction of high average firing rates at the high dose suggests that, under conditions of elevated striatal dopamine transmission by cocaine (Nicolaysen et al., 1988; Czoty et al., 2000; Stuber et al., 2005), there is a reduced range in overall sensorimotor output. We have previously reported that under opposite conditions the range of striatal sensorimotor firing increased. That is, following

microinjection of apomorphine into the substantia nigra to reduce striatal dopamine transmission via autoreceptor-mediated inhibition of dopamine cell firing, striatal firing in response to somatosensory stimulation exhibited greater than normal fluctuations (Prokopenko et al., 2004). Together, these findings suggest that a regulatory role of striatal dopamine may be to restrict the range of corticostriatal throughput. This is inconsistent with hypotheses that striatal dopamine acts to weaken throughput of weak cortical inputs and strengthen throughput of strong cortical inputs (Nicola et al., 2000; O'Donnell, 2003; Bamford et al., 2004). Indeed, the fastest firing rates (presumably reflecting strong cortical input) were the most reduced by cocaine in the present study. It must be noted that these findings do not reflect a singular action of cocaine on striatal dopamine but also likely reflect the summation of peripheral and central effects exerted by systemic cocaine, which may include attenuation of strong somatosensory thalamocortical signaling (Rutter et al., 2005).

Acknowledgments

We thank our departed colleague, Volodimir Prokopenko, for intellectual and technical support and Linda King for technical assistance.

This study was supported by National Institute on Drug Abuse Grants DA 06886 and DA 04551.

ABBREVIATIONS

DA	dopamine
T1	precocaine time epoch
T2	cocaine time epoch
T3	recovery time epoch
ANOVA	analysis of variance
ILI	interlick interval
MANOVA	multivariate analysis of variance
PETH	perievent time histogram
FR	firing rate
HLM	hierarchical linear model
ANCOVA	analysis of covariance
CI	confidence interval

References

- Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, Schmauss C, Zakharenko SS, Zablow L, Sulzer D. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron*. 2004; 42:653–663. [PubMed: 15157425]
- Bhattacharyya AK, Pradhan SN. Interactions between motor activity and stereotypy in cocaine-treated rats. *Psychopharmacology*. 1979; 63:311–312. [PubMed: 113821]
- Brozek G, Zhuravin IA, Megirian D, Bures J. Localization of the central rhythm generator involved in spontaneous consummatory licking in rats: functional ablation and electrical brain stimulation studies. *Proc Natl Acad Sci U S A*. 1996; 93:3325–3329. [PubMed: 8622936]
- Bryk, AS.; Raudenbush, SW. *Hierarchical Linear Models: Applications and Data Analysis Methods*. Sage Publications; Thousand Oaks, CA: 2002.

- Cho J, West MO. Distribution of single neurons related to body parts in the lateral striatum of the rat. *Brain Res.* 1997; 756:241–246. [PubMed: 9187338]
- Cooper, SJ.; Dourish, CT. An introduction to the concept of stereotypy and a historical perspective on the role of brain dopamine. In: Cooper, SJ.; Dourish, CT., editors. *Neurobiology of stereotyped behavior*. Oxford University Press; New York: 1990. p. 1-24.
- Creese I, Iverson SD. Blockade of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res.* 1973; 55:369–382. [PubMed: 4145950]
- Crutcher MD, DeLong MR. Single cell studies of the primate putamen: I. Functional organization. *Exp Brain Res.* 1984; 53:233–243. [PubMed: 6705861]
- Czoty PW, Justice JB Jr, Howell LL. Cocaine-induced changes in extracellular dopamine determined by microdialysis in awake squirrel monkeys. *Psychopharmacol (Berl)*. 2000; 148:299–306.
- Delfs JM, Kelley AE. The role of D₁ and D₂ dopamine receptors in oral stereotypy induced by dopaminergic stimulation of the ventrolateral striatum. *Neuroscience.* 1990; 39:59–67. [PubMed: 1982467]
- Dews PB. Studies on behavior: IV. Stimulant actions of methamphetamine. *J Pharmacol Exp Ther.* 1958; 122:137–147. [PubMed: 13502838]
- Fowler SC, Mortell C. Low doses of haloperidol interfere with rat tongue extensions during licking: a quantitative analysis. *Behav Neurosci.* 1992; 106:386–395. [PubMed: 1590957]
- Gardiner TW, Iverson DA, Rebec GV. Heterogeneous responses of neostriatal neurons to amphetamine in freely moving rats. *Brain Res.* 1988; 463:268–274. [PubMed: 3196918]
- Haracz JL, Tschanz JT, Wang Z, White IM, Rebec GV. Striatal single unit responses to amphetamine and neuroleptics in freely moving rats. *Neurosci Biobehav Rev.* 1993; 17:1–12. [PubMed: 8096071]
- Haracz JL, Tschanz JT, Greenberg J, Rebec GV. Amphetamine-induced excitations predominate in single neostriatal neurons showing motor-related activity. *Brain Res.* 1989; 489:365–368. [PubMed: 2743163]
- Heikkila RE, Cabbat FS, Duvoisin RC. Motor activity and rotational behavior after analogs of cocaine: correlation with dopamine uptake blockade. *Commun Psychopharmacol.* 1979; 3:285–290. [PubMed: 575770]
- Kelley AE, Lang CG, Gauthier AM. Induction of oral stereotypy following amphetamine microinjection into a discrete subregion of the striatum. *Psychopharmacology.* 1988; 95:556–559. [PubMed: 3145527]
- Knowler WC, Ukena TE. The effects of chlorpromazine, pentobarbital, chlordiazepoxide and D-amphetamine on rates of licking in the rat. *J Pharmacol Exp Ther.* 1973; 184:385–397. [PubMed: 4688176]
- Lyon, M.; Robbins, T. The action of central nervous system stimulant drugs: a general theory concerning amphetamine effects. In: Essman, WB.; Valzelli, L., editors. *Current Developments in Psychopharmacology*. Spectrum Publications; New York: 1975. p. 79-163.
- Mittler T, Cho J, Peoples LL, West MO. Representation of the body in the lateral striatum of the freely moving rat: single neurons related to licking. *Exp Brain Res.* 1994; 98(1):163–167. [PubMed: 8013585]
- Mosteller, F.; Tukey, JW. *Data Analysis and Regression: A Second Course in Statistics*. Addison-Wesley Publishing Co. Inc; Menlo Park, CA: 1977.
- Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci.* 2000; 23:185–215. [PubMed: 10845063]
- Nicolaysen LC, Pan H, Justice JB Jr. Extracellular cocaine and dopamine concentrations are linearly related in rat striatum. *Brain Res.* 1988; 456:317–323. [PubMed: 3208083]
- Nisenbaum ES, Orr WB, Berger TW. Evidence for two functionally distinct subpopulations of neurons within the rat striatum. *J Neurosci.* 1988; 8:4138–4150. [PubMed: 3183715]
- O'Donnell P. Dopamine gating of forebrain neural ensembles. *Eur J Neurosci.* 2003; 17:429–435. [PubMed: 12581161]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic; New York: 2005.

- Pederson CL, Wolske M, Peoples LL, West MO. Firing rate dependent effect of cocaine on single neurons of the rat lateral striatum. *Brain Res.* 1997; 760:261–265. [PubMed: 9237544]
- Peoples LL, West MO. Phasic firing of single neurons in the rat nucleus accumbens correlated with the timing of intravenous cocaine self-administration. *J Neurosci.* 1996; 16:3459–3473. [PubMed: 8627379]
- Prokopenko VF, Pawlak AP, West MO. Fluctuations in somatosensory responsiveness and baseline firing rates of neurons in the lateral striatum of freely moving rats: effects of intranigral apomorphine. *Neuroscience.* 2004; 125:1077–1082. [PubMed: 15120867]
- Rebec GV, Segal DS. Dose-dependent biphasic alterations in the spontaneous activity of neurons in the rat neostriatum produced by D-amphetamine and methylphenidate. *Brain Res.* 1978; 150:353–366. [PubMed: 567085]
- Rutter JJ, Devilbiss DM, Waterhouse BD. Effects of systemically administered cocaine on sensory responses to peri-threshold vibrissae stimulation: individual cells, ensemble activity, and animal behaviour. *Eur J Neurosci.* 2005; 22:3205–3216. [PubMed: 16367787]
- Ryan LJ, Young SJ, Segal DS, Groves PM. Antidromically identified striatonigral projection neurons in the chronically implanted behaving rat: relations of cell firing to amphetamine-induced behaviors. *Behav Neurosci.* 1989; 103:3–14. [PubMed: 2923676]
- Stuber GD, Roitman MF, Phillips PE, Carelli RM, Wightman RM. Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. *Neuropsychopharmacology.* 2005; 30:853–863. [PubMed: 15549053]
- Tang C, Pawlak AP, Prokopenko V, West MO. Changes in activity of the striatum during formation of a motor habit. *Eur J Neurosci.* 2007; 25:1212–1227. [PubMed: 17331217]
- Travers JB, Dinardo LA, Karimnamazi H. Motor and premotor mechanisms of licking. *Neurosci Biobehav Rev.* 1997; 21:631–647. [PubMed: 9353796]
- Trulson ME, Jacobs BL. Effects of D-amphetamine on striatal unit activity and behavior in freely moving cats. *Neuropharmacology.* 1979; 18:735–738. [PubMed: 492495]
- West MO. Anesthetics eliminate somatosensory-evoked discharges of neurons in the somatotopically organized sensorimotor striatum of the rat. *J Neurosci.* 1998; 18:9055–9068. [PubMed: 9787009]
- West MO, Peoples LL, Michael AJ, Chapin JK, Woodward DJ. Low-dose amphetamine elevates movement-related firing of rat striatal neurons. *Brain Res.* 1997; 745:331–335. [PubMed: 9037428]
- Wiesenfeld Z, Halpern BP, Tapper DN. Licking behavior: evidence of hypoglossal oscillator. *Science.* 1977; 196:1122–1124. [PubMed: 558653]
- Wuttke W. The effects of D-amphetamine on schedule-controlled water licking in the Squirrel monkey. *Psychopharmacologia.* 1970; 17:70–82. [PubMed: 4986984]

Fig. 1.

At dose 20, stereotypic licking was induced by cocaine in T2 and subsequently reversed in T3 relative to predrug level in T1. Cocaine-induced stereotypy was assessed as the standardized change in the number of licks during water-off phases in T2 or T3 relative to T1. The left side of the y-axis represents the standardized value of change and right side represents 2-, 4-fold, etc., increase (>0) or decrease (<0) in T2 or T3 relative to T1. Horizontal line at 0 represents no change from T1. Numbers of recording sessions included in this analysis were nine, five, six, and five at doses 0, 5, 10, and 20, respectively. A, stereotypy in T2. Rats at dose 20 exhibited significantly greater increases in number of water-off licks (mean $\pm$ S.E.M.) from T1 to T2 than rats at dose 0 (*, $p < 0.05$). B, reversal in T3. Relative to T1, rats at dose 20 exhibited significantly lower number of water-off licks in T3 than in T2 (†, $p < 0.05$).

Fig. 2.

Dose-dependent changes in lick duration and interlick interval. A, cocaine-induced shift of licks toward shorter duration as a function of dose in T2. The x-axis represents seven levels of lick duration from 17 to 117 ms, and the y-axis represents percentage of licks in each level. Each dot represents mean percentage of licks in each level across all recording sessions at each dose in T1 (closed circle) or T2 (open circle). For each dose, two spline curves were fitted for data points in T1 and T2 separately. As dose increased (top through bottom), there were significantly increased licks with shorter durations and simultaneously decreased licks with longer durations in T2, relative to T1 ($p < 0.05$). B, cocaine-induced shift in proportion of licks toward longer ILI as a function of dose in T2. The x-axis represents eight levels of lick ILI from 67 to 184 ms, and the y-axis represents the percentage of licks in each level. For better display, licks in levels shorter than 67 and in levels longer than 184 were merged into the levels of 67 and 184, respectively, due to very low proportions of licks in these levels. As dose increased, there were significantly increased licks with longer ILI and simultaneously decreased licks with shorter ILI in T2, relative to T1 ($p < 0.05$). Number of recording sessions is shown in the middle of each row (dose).

Fig. 3. Locations of all 70 neurons. Every striatal neuron related to licking was verified histologically to be located in the ventrolateral region of the striatum. Circles, single neurons; squares, several different single neurons histologically placed at the same location. Numbers on coronal plates indicate anterior-posterior distance from bregma (Paxinos and Watson, 2005).

Fig. 4.

Dose- and rate-dependent pattern of clockwise rotation of the regression lines on average firing rates of neurons between T1 and T2 across doses. In each scatterplot, the average firing rates of neurons in T2 (y-axis, log10-transformed) are regressed on their average firing rates in T1 (x-axis, log10-transformed) for each dose. Each dot represents one neuron. The solid line represents the linear regression line, and the broken diagonal line represents no change of the average firing rates from T1 to T2. As dose increased (A–D), the regression lines gradually rotated clockwise away from the diagonal line of no change with decreasing slopes, consistent with the significant ($p < 0.05$) dose- and rate-dependent effects of cocaine on average firing rates revealed by HLM. The strong linearity of regression at doses 0, 5, and 10 ($p < 0.001$) was absent at dose 20 ($p < 0.05$).

Fig. 5. Changes in average firing rates of representative slow and fast firing neurons following cocaine administration. A, increase in average firing rate in T2 and reversal in T3 of a representative slow firing neuron at dose 10. Slow firing neurons were defined as those with average firing rate in T1 less than 1 impulses/s. The y-axis of each perievent histogram represents average firing rate (impulses per second). Time 0 of the x-axis indicates the beginning of lick. Top, in T1, the neuron showed lick-related activity from -20 to +65 ms, relative to beginning of lick. Dashed vertical lines indicate this customized time window of firing for this neuron that was applied to T1, T2, and T3. Middle, in T2, the same neuron showed increased activity during the same time window. Bottom, in T3, the same neuron's activity showed reversal to T1 level during the same time window. The average firing rate of this neuron increased from 0.16 in T1 to 0.64 in T2 and then reversed to 0.29 in T3. Each histogram displays neural activity associated with identical number of 1000 licks in each time epoch. Calibration, 0.15 mV; 0.2 ms. B, decrease in average firing rate in T2 and reversal in T3 of a representative fast firing neuron at dose 20. Fast firing neurons were defined as those with average firing rate in T1 greater than 1 impulses/s. Top, in T1, the neuron showed lick-related activity from -30 ms to beginning of lick. This time window of firing (indicated by dashed vertical lines) applied to all three time epochs of the recording session. Middle, in T2, the same neuron showed decreased activity during the same time window. Bottom, in T3, the same neuron's activity showed reversal to T1 level during the

same time window. The average firing rate (impulses per second) of this neuron decreased from 7.73 in T1 to 2.75 in T2 and then reversed to 10.02 in T3. Each histogram displays neural activity associated with identical number of 941 licks in each time epoch. See Table 1 for matched pairs of this neuron. Calibration, 0.1 mV; 0.2 ms. For both representative neurons, the overlaid waveforms in each time epoch are shown on top left of each histogram. Raster above each histogram displays neural activity on a trial-by-trial basis in chronological order from the bottom to the top of each raster. T3 rasters (Recovery) illustrate that firing rates continually approached T1 levels as T3 progressed.

Fig. 6.

Dose- and rate-dependent effects of cocaine on the firing rates of matched pairs within neurons. The standardized value of change in firing rate of each matched pair was calculated by $[(T2FR/(T1FR + T2FR)) - 0.5]$. The top and bottom graphs illustrate the modeling results of ANCOVA for matched pairs of slow and fast firing neurons, respectively. In each graph, at each dose, the black and white bars show the mean of standardized changes in firing rates of low and high T1FR matched pairs across neurons, respectively. Error bars, represent the S.E.M.s. Left side of y-scale represents the standardized change in firing rate, and the right side represents 2-, 4-fold, etc., changes in firing rate in T2, relative to T1. Horizontal line at 0 represents no change in firing rate from T1. *, $p < 0.001$, post-hoc Bonferroni tests compared with dose 0.

Fig. 7.

Changes in firing rates of matched pairs from T1 to T2 within individual neurons. Eight neurons are presented. Each row consists of two representative neurons from one dose; one represents slow firing neurons (left column), and the other represents fast firing neurons (right column). Each scatterplot represents one neuron, and each dot represents one matched pair. In each scatterplot, the *x*- and *y*-axis are equivalent in scale, customized to the ranges of firing rates of matched pairs in T1 and T2 for each neuron. The vertical dotted line indicates the middle of the range of firing rates of matched pairs in T1, used as the cut point to dichotomize matched pairs into low and high T1FR groups within each neuron. The diagonal broken line indicates a reference line of no change; thus, above it increased FR, and below it decreased FR from T1 to T2.

Spreadsheets showing the matched pairs of a representative fast firing neuron at dose 20 mg/kg and the changes in movement-related firing after cocaine administration

Six separate spreadsheets were used to show the numbers of licks (left) and mean firing rates (right) of this neuron during T1 (top), T2 (middle), and T3 (bottom) time epochs of one experiment at dose 20. On each spreadsheet, the entire ranges of lick duration and period were respectively divided into 7 (columns) and 12 (rows) of equal levels. Each lick was sorted into the spreadsheet on the basis of these two parameters, and each cell in the spreadsheet corresponds to a set of movements that exhibited the same lick parameters. For cells with at least five movements (left) in each time epoch, mean firing rate (right) was calculated and shown for each cell. For example, all movements of 17- to 33-ms duration and 150- to 167-ms period were sorted into a unique cell (33×167) of each spreadsheet, creating a specific matched pair between T1 and T2 and another one between T1 and T3 (boldface). In total, there were 22 matched pairs between T1 and T2 and 20 matched pairs between T1 and T3 for this neuron. At dose 20, firing rates associated with all matched pairs of this fast firing neuron decreased in T2 and subsequently reversed in T3, relative to precocaine firing in T1. Empty cells in the spreadsheets did not contain enough movements ($n < 5$) to accurately assess firing, and their neural data were thus excluded from the analysis of matched pairs. See Fig. 5B for waveforms and perievent time histograms of this neuron in the three time epochs.

TABLE 1

Experimental Epoch	Lick Period	Lick Duration							Lick Period	Lick Duration							mean firing rate (imp/s)
		17	33	50	67	84	100	117		17	33	50	67	84	100	117	
Pre-cocaine (T1)																	
Post-cocaine (T2)																	

Experimental Epoch	Lick Period	Lick Duration	Lick Period	Lick Duration
Recovery (T3)	150	16	26	
	167	14	43	
	184	21	70	29
	200	12	16	23
	217	8	12	18
	234			23
	250			10
	267			6
				11
				6
				1.1
				2.2
				1.6
Recovery (T3)	84			
	100			
	117			
	134	18	10	
	150	19	25	
	167	31	99	70
	184		44	133
	200	5	17	49
	217	6	19	21
	234			33
	250			18
	267			31
				6
Recovery (T3)	150			
	167			
	184			
	200			
	217			
	234			
	250			
	267			
Recovery (T3)	150	0.5	2.6	
	167	4.3	3.2	1.7
	184		4.3	2.3
	200	2.2	5.2	3.5
	217	5.4	1.3	2.2
	234			2.9
	250			1.9
	267			1.1
				2.2
				1.6
				1.0
Recovery (T3)	84			
	100			
	117			
	134	11.9	12.0	
	150	10.4	10.5	
	167	13.6	10.9	8.9
	184		10.9	8.4
	200	10.3	4.9	7.2
	217	20.1	8.3	6.1
	234			9.1
	250			7.8
	267			9.5

TABLE 2

Results of the two-level hierarchical linear model on firing rates of matched pairs between T1 and T2. Top presents results of modeling changes in average firing rates of individual neurons across doses. Bottom presents results of modeling changes in the slope of the linear regression for matched pairs within individual neurons across doses.

	Parameter	Estimate	S.E.	t Value
For Average Firing Rate in T2 (β_{0i})				
Intercept	γ_{00}	4.944	0.287	17.23***
Dose	γ_{01}	0.174	0.118	1.47
Dose ²	γ_{02}	-0.015	0.006	-2.41*
Average T1FR	γ_{03}	0.889	0.053	16.82***
Dose $\times$ average T1FR	γ_{04}	0.027	0.018	1.51
Dose ² $\times$ average T1FR	γ_{05}	-0.003	0.001	-2.46*
For slope of within-neuron regression (β_{1i})				
Intercept	γ_{10}	0.190	0.057	3.33***
Dose	γ_{11}	0.043	0.022	1.93
Dose ²	γ_{12}	-0.002	0.001	-1.65
Average T1FR	γ_{13}	0.031	0.009	3.50***
Dose $\times$ average T1FR	γ_{14}	-0.002	0.003	-0.73
Dose ² $\times$ average T1FR	γ_{15}	0.0002	0.0002	1.16

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.