DOSE- AND RATE-DEPENDENT EFFECTS OF COCAINE ON STRIATAL NEURONS RELATED TO LICKING

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ABSTRACT OF THE DISSERTATION

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Striatal Neurons Related to Licking

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Dissertation Director: Professor Mark O. West

To examine the role of striatal mechanisms in cocaine-induced stereotyped licking, the activity of lick-related neurons in the ventrolateral striatum of awake, freely moving rats engaged in licking was recorded before and after cocaine administration (0, 5, 10 and 20 mg/kg). Relative to zero dose, cocaine reduced lick duration and increased interlick interval, particularly at the high dose, but did not affect licking rhythm. Stereotyped licking was induced only by the high dose, evident by an increase in the number of licks in the absence of water delivery. Firing rates (FR) of striatal neurons phasically related to licking 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 FR were observed post-drug, exhibiting a dose-dependent pattern that strongly depended on pre-drug FR. At the middle and high doses relative to the zero dose group, the average FR of slow firing neurons were increased by cocaine, resulting from a general elevation of movement-related FR. By contrast, fast firing neurons showed decreased average FR only in the high dose group, with reduced FR across the entire range for these neurons. Additionally, without any drug present (prior to drug injection) FR were positively related to the strength of the correlation of FR with lick duration. Changes in behavioral correlations were demonstrated over time with no drug present

(for slow firing neurons), and following cocaine administration (for slow firing neurons at all doses, and for fast firing neurons only at the high dose). Thus, differences were observed between slow and fast firing neurons in drug effects on FR, in the strength of their behavioral correlations and with respect to changes in their behavioral correlations, suggesting that fast firing neurons may be more strongly involved in the processing of movement parameters. These 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, more global movements.

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ANCOVA	analysis of covariance
CI	confidence interval
DA	dopamine
FR	firing rate
GABA	gamma aminobutyric acid
Glu	glutamate.
GPe	external globus pallidus
GPi	internal globus pallidus
HLM	hierarchical linear model
ILI	interlick interval
i.p.	intraperitoneal
FR	firing rate
MANOVA	multivariate analysis of variance
MT1FR	mean firing rate of all matched pairs in T1, for each neuron
PETH	perievent time histogram
SNpc	substantia nigra pars compacta
SNpr	substantia nigra pars reticulate
STN	subthalamic nucleus
T1, T2, T3	pre-cocaine, cocaine and recovery time epochs, respectively
T1FR, T2FR, T3FR	firing rates of matched pairs in T1, T2 and T3, respectively

INTRODUCTION

1. Behavioral effects of cocaine

Cocaine is a potent psycho-stimulant and drug of abuse. Its main behavioral effects are reinforcement, and motor activation. The latter comprises an increase in long-sequence movements, including locomotion, at low doses and in shorter, stereotyped movements, including stereotyped head movements, sniffing and licking, at high doses (Bhattacharyya and Pradhan, 1979; Wise, 1984; Zubrycki et al., 1990; Lau et al., 1991).

A stereotyped response is defined as a response which is preformed repetitively, with little variation, and without apparent purpose (Cooper and Dourish, 1990). Lyon and Robbins (1975) argue that psychomotor stimulants increase the rate of responding in all types of behavior which have some minimal tendency. At low doses this translates into an increase in the rate of longer response sequences and a reduction in inter response intervals. At higher doses the increased rate of movement initiation causes behavioral competition among different responses such that shorter response sequences (e.g., licking) predominate. Eventually one response is initiated before the last was completed, causing the behavior to look like repetitive motor elements out of context. Psycho-stimulant induced stereotypy takes the form of increasingly higher rates of activity in a decreasing number of response categories (Lyon and Randrup, 1972).

Psychomotor stimulants at high doses induce stereotypic licking. Most studies examining the effects of psychomotor stimulants on licking used rating scales and did not measure stimulant effects on the different parameters of licking. The few studies describing those effects used amphetamine. Low doses of amphetamine (0.3-1.0 mg/kg) decreased the intervals from onset of one lick to the onset of the next (i.e., increased rate) of rats licking

water, while high doses (1.8-3.0 mg/kg) caused an increase in those intervals (Knowler and Ukena, 1973). Similarly, with monkeys licking water as an operant reinforced by food, amphetamine increased overall lick rate at low doses, and had an opposite effect at high doses (Wuttke, 1970). A more detailed analysis showed that the drug effect depended on the baseline lick rate (which changed within each session), increasing low baseline rates and not changing or decreasing high ones, corresponding to stimulant effects on other behaviors (Dews, 1958; Clark and Steele, 1966; Kelleher and Morse, 1968).

2. Striatal role in mediating psychomotor stimulant induced stereotyped behavior

Cocaine has complex effects on the central nervous system. Acting as a local anesthetic it reversibly inhibits sodium and potassium membrane conductance (Van Dyke and Byck, 1977; Fleming et al., 1990). However, its behavioral effects are thought to be linked to its effects on monoamine transmission and not to its local anesthetic action, which involves dosages 200-fold higher (Wise, 1984; Gawin, 1991; Kuhar et al., 1991; Ritz et al., 1992; Wilcox et al., 1999). Cocaine inhibits reuptake of dopamine, serotonin, and norepinephrine (Van Dyke and Byck, 1977; Fleming et al., 1990; Ritz et al., 1992). Although none of these alone can account for cocaine's behavioral effects, blockade of dopamine reuptake (by binding to dopamine uptake transporter) is the most strongly implicated (Nisenbaum et al., 1988; Gawin, 1991; Ritz et al., 1992). Cocaine-induced motor activity can be blocked by dopamine antagonists and dopamine depletion (Scheel-Kruger et al., 1977; Kovacs et al., 1990).

The striatum and nucleus accumbens are the main structures involved in the behavioral effects of cocaine and other psychomotor stimulants. The nucleus accumbens is

implicated mainly in their locomotor stimulating and reinforcing properties, and the striatum mainly in their induction of stereotyped behavior (Kelley et al., 1975; Cooper and Dourish, 1990). Cocaine has been shown to increase dopamine concentrations in the striatum and nucleus accumbens (Nicolaysen et al., 1988) via blockade of DA reuptake (Heikkila et al., 1979).

A combination of microinjection and lesion studies points to a critical role of the striatum in psycho stimulant induced stereotypy (for review see Cooper and Dourish, 1990; Robbins et al., 1990). Intrastriatal injections of amphetamine, cocaine, apomorphine, or other dopamine agonists induce stereotypy, which is blocked by dopamine antagonists (Kelley et al., 1988; Bordi and Meller, 1989; Cameron and Crocker, 1989; Delfs and Kelley, 1990). Striatal 6-hydroxydopamine lesions attenuate amphetamine-induced stereotypy (Creese and Iversen, 1974; Kelley et al., 1975). Reith et al. (1985) found a significant correlation between the potencies of cocaine congeners in inhibiting cocaine binding in the striatum and their potencies in inducing stereotyped sniffing.

Several lines of evidence suggest a specific role for the ventrolateral striatum in psychomotor-induced oral stereotypy, as well as in normal oral motor control and feeding and in orofacial dyskinesias. Microinjections of amphetamine into the ventrolateral striatum induce oral stereotypy including licking and biting (Kelley et al., 1988), which is blocked by DA antagonists (Delfs et al., 1990). Lesions in the ventrolateral striatum of rats produced impairments in tongue movements and in motor control of feeding behavior (Pisa, 1988; Pisa and Schranz, 1988; Jicha and Salomone, 1991). Dopamine depletion and cholinergic agonists in the ventrolateral striatum generate abnormal orofacial movement similar to the

ones seen in orofacial dyskinesias (Kelley et al., 1988; Kelley et al., 1989). Thus there is strong evidence that the transduction of cocaine's pharmacological effects into stereotypic oral behaviors involves the ventrolateral striatum.

Although microinjections, local lesions, and microdialysis studies are fairly localized they sum the activity of many neurons. The present single cell recording technique provides a higher level of resolution both spatially and temporally.

3. Effects of psychomotor stimulants on striatal neural activity

It is not yet clear what changes occur in striatal neurons firing during the transduction of cocaine's psycho-stimulant effects. Previous studies investigating effects of dopamine and dopamine agonists 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). Most studies in the anesthetized or immobilized preparation found inhibitions, using systemic as well as iontophoretic application of dopamine, amphetamine, or cocaine (Groves and Rebec, 1975; Rebec and Segal, 1978; Rebec et al., 1981; Nisenbaum et al., 1988; Qiao et al., 1990).

In anesthetized animals, microiontophoretic application of cocaine into the striatum caused inhibition in 92% of the cells, and excitation in 4-8% (Qiao et al., 1990). In other related areas, i.e. the accumbens and prefrontal cortex, most neurons were also inhibited (Qiao et al., 1990). Cocaine had variable and not very pronounced effects on ventral tegmental area and substantia nigra compacta dopaminergic cells, producing excitation, inhibition, or no response (Pitts and Marwah, 1987; Einhorn et al., 1988; Pitts and Marwah,

1988). Cocaine's effects on single neurons were independent of its local anesthetic action, since they were not found with procaine (Einhorn et al., 1988), in agreement with behavioral studies (Delfs et al., 1990; Lau et al., 1991).

Anesthesia has been shown not only to suppress spontaneous and sensory-evoked striatal firing (West, 1998), but also to invert the direction of drug effects on these measures (Bloom et al., 1965; Trulson and Jacobs, 1979; Wilson and Groves, 1981; Richards and Taylor, 1982). Furthermore, when dealing with a drug that exerts its effects through dopamine transmission, and in view of dopamine's most likely role in the striatum as a modulator (Rolls et al., 1984; Abercrombie and Jacobs, 1985; Wickens, 1990; Nicola et al., 2000; Horvitz, 2002), the drug's effects should be studied under the most natural conditions possible, where a *natural signal* can be modulated. Furthermore, acting as an uptake blocker and not a dopamine releaser, cocaine's effects depend on the level of activity in the striatum, which may differ substantially between the two preparations. Single cell recording from the striatum of freely moving rats allow a high resolution description of cocaine's effects in near natural conditions.

Studies on amphetamine's effects under more natural conditions, in the awake preparation, have also reported a mixture of results, with most studies showing excitations (Tschanz et al., 1991; Haracz et al., 1993; West et al., 1997), some finding inhibitions (Ryan et. al., 1989), or both excitations and inhibitions (Trulson and Jacobs, 1979).

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, 1984b; 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 lack of adequate control for movement-related changes in firing.

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, while controlling for movement-related changes in firing, demonstrated a firing rate-dependent effect (Pederson et al., 1997). Firing during movements that were normally associated with low firing rates was elevated at all doses, whereas firing during movements that were normally associated with high firing rates showed slight elevation or no change at low doses and were strongly suppressed by the high dose.

4. Anatomical connections of the striatum

The lateral striatum receives convergent and divergent topographic projections from primary somatosensory, primary motor and premotor cortices (Kunzle, 1975, 1977; McGeorge and Faull, 1989; Flaherty and Grabiel, 1993, 1994; Parent and Hazrati, 1995a). These excitatory glutamatergic cortical inputs converge on striatal medium spiny neurons which exhibit activity related to different body parts (Crutcher and Delong, 1984a; Kimura, 1990). Striatal medium spiny neurons also receive dopaminergic input from substantia nigra pars compacta (SNpc), which acts to modulate the influence of cortical input on the activity of medium spiny neurons (Wickens, 1990; Nicola et al., 2000; Bamford et al., 2004). As noted earlier, despite numerous studies aimed at

elucidating the role dopamine plays in the striatum, this role still remains difficult to understand (Wickens, 1990; Mink 1996), and thus requires continued investigation.

Medium spiny striatal neurons receive additional glutamatergic input from the thalamus, cholinergic input from the large aspiny striatal inter-neurons, and GABA (gamma aminobutyric acid), substance P, and Enkephalin input from adjacent medium spiny striatal neurons (Mink, 1996). Striatal medium spiny neurons project inhibitory GABAergic axons to the internal segment of globus pallidus (GPi) and/or substantia nigra pars reticulate (SNpr). GPi receives an additional indirect input from the striatum via the external segment of globus pallidus (GPe) and the subthalamic nucleus. GPi and SNpr further project inhibitory GABAergic axons to the thalamus, which in turn project to motor cortical areas like primary motor and premotor cortices, creating a motor loop (Alexander et al., 1986; Parant and Hazrati, 1993; Parent and Hazrati, 1995b; McFarland and Haber, 2000, Miller, 2007). Please see Figure 1 for a schematic representation of the described anatomical connections.

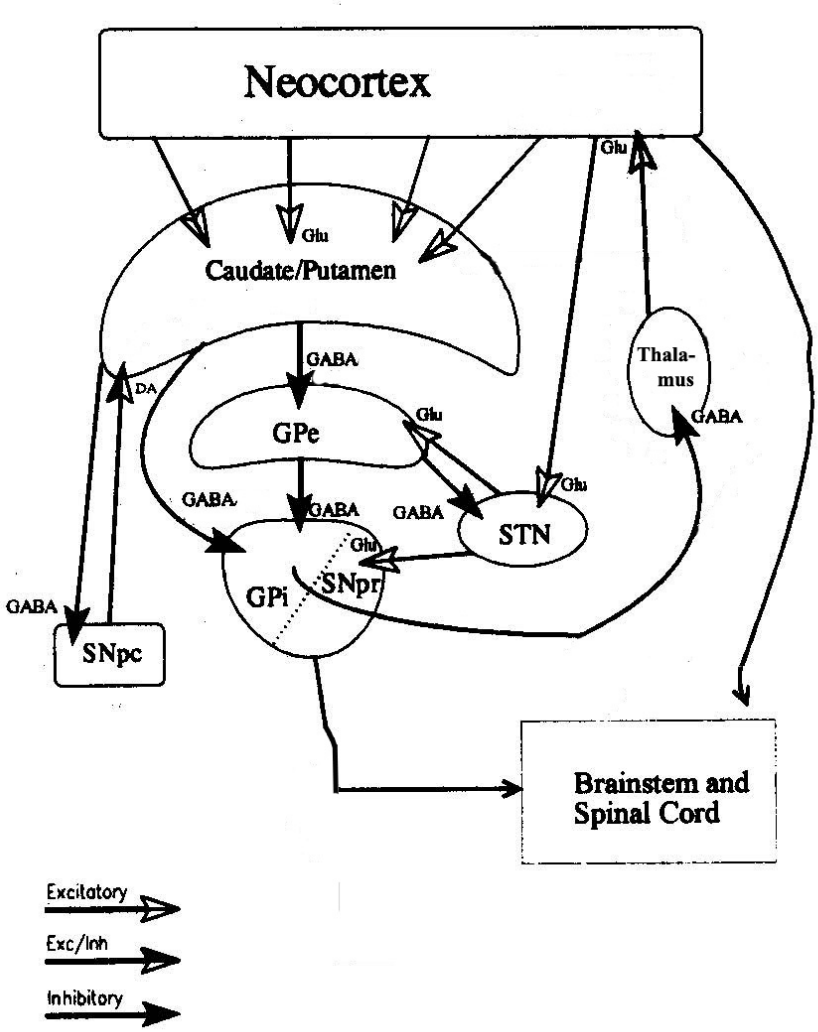

Fig. 1: Schematic representation of basal ganglia connections, adapted from Mink (1996). GPe: external globus pallidus; GPi: internal globus pallidus; SNpc: substantia nigra pars compacta; SNpr: substantia nigra pars reticulate; STN: subthalamic nucleus; DA: dopamine; GABA: gamma aminobutyric acid; Glu: glutamate. Open arrowheads represent excitatory projections, filled arrowheads indicate inhibitory connections; half-filled arrowheads represent projections that can be excitatory or inhibitory.

5. Sensorimotor related activity of striatal neurons

Electrophysiological studies in awake animals found striatal neurons to be related to sensory and motor stimuli of individual body parts. Correlations were reported with various movement parameters (most prominently direction), stages (e.g. neural activity before or after movement initiation), and the behavioral circumstances (mainly the significance of the stimuli) (Schneider and Lidsky, 1981; Crutcher and DeLong, 1984a,b; Liles, 1985; Alexander and Crutcher, 1990a,b; Crutcher and Alexander, 1990; Kimura, 1990; West et al., 1990; Apicella et al., 1991b; Carelli and West, 1991; Montgomery and Buchholz, 1991). These body-parts are somatotopically organized in accordance with the anatomical connections from sensory, motor and premotor cortices. They create a body map in the lateral striatum in which forelimbs are represented most dorsally, head and orofacial functions most ventrally, and hindlimb and axial areas between them (Kunzle, 1975; Crutcher and DeLong, 1984a; Alexander and DeLong, 1985; Selemon and Goldman-Rakic, 1985; McGeorge and Faull, 1989; Carelli and West, 1991; Mittler et al., 1994; Cho and West, 1997). These movement–related neurons are type IIb medium spiny neurons and have been demonstrated to be projection neurons (Kimura, 1990). This functional organization is further supported by lesion studies, which have demonstrated impairments of fine motor control of the forelimb or tongue after ibotenate lesions in the dorsolateral or ventrolateral striatum, respectively (Pisa, 1988; Pisa and Schranz, 1988).

Striatal neurons related to licking, swallowing and orofacial stimuli were found in the ventral putamen of primates (Crutcher and DeLong, 1984a; Rolls et al., 1984; Alexander and DeLong, 1985; Apicella et al., 1991a). Single neurons related to licking in the ventrolateral striatum of the rat were found in our laboratory (Mittler et al., 1994). These lick

related neurons increased firing rates specifically during licking but showed no change in firing rate during a full sensorimotor examination of all other body parts. Lick related neurons were found in clusters, throughout the lateral half of the striatum, significantly ventral to, and partially overlapping body-related neurons.

6. Straital lick-related neuron as a substrate for studying cocaine's effects

Several reasons make striatal lick-related neurons an appropriate substrate for examining cocaine's effects on striatal neural activity. Preservative orofacial movements, including licking are among the stereotyped behaviors induced by cocaine and other psychomotor stimulants (Lyon and Robbins, 1975; Scheel-Kruger et al., 1977; Pradhan et al., 1978; Bhattacharyya and Pradhan, 1979; Kelley et al., 1988; Cooper and Dourish, 1990). The ventrolateral striatum has been specifically implicated in these oral stereotypies. Taken together with the fact that neurons phasically related to sensorimotor activity constitute at least 50 to 70% of the neuronal population in the lateral striatum (Cho and West, 1997), lick-related neurons are the most probable target mediating cocaine induced stereotyped licking. Furthermore, specific sensorimotor firing of striatal 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.

While knowledge of the particular behavior with which the recorded neuron is correlated allows studying that neuron's activity while the drug is affecting its correlated behavior, the combination of change in movement caused by psychomotor stimulant and the phasic relation of striatal neural activity to that movement introduces a problem in interpreting drug effects on striatal firing in the awake animal. Changes in firing post-

drug could be secondary to drug-induced behavioral change (Trulson and Jacobs, 1979; West et al., 1997). For example, if firing rate of a certain neuron is *higher* the *shorter* the duration of the movement, an observed *increase* in firing rate post-drug could be due to a sensory-feedback response of that neuron to a drug-induced *decrease* in movement duration (mediated via other brain regions). Lack of adequate control for movement-related changes in firing may explain some of the mixed effects of psychomotor stimulants on striatal firing reported by previous studies.

The present study minimized possible misinterpretation of drug effects on neural firing due to secondary drug-induced behavioral modulation of firing by comparing firing which occurred during movements with similar sensorimotor parameters pre and post-drug. Studying lick related neurons provides an excellent substrate allowing such control. The topography of tongue movements can be tightly controlled by the restriction of access to the water (Halpern, 1977), providing one step in matching behaviors pre- and post-drug. Importantly, however, the present preparation simultaneously allows the manifestation of the drug's behavioral effects, providing the end-point to which neural analysis is linked. A second, more critical control can be achieved in post-hoc analysis, by comparing firing between groups of licks with similar attributes pre- and post-drug.

Thus to further investigate the role of striatal neurons in cocaine-induced stereotypy, the present study 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 one hour 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 3-4 hours. The design allowed for

manifestation of drug-induced stereotyped licking 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.

MATERIALS AND METHODS

1. Subjects and surgery

A total of 18 male (300-350g) Long Evans rats (Charles River, Wilmington, MA) were randomly assigned to four groups, each group receiving 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 miniature microelectrode drive (microdrive) assembly (Jose Biela Engineering, Anaheim, CA). A base for attaching the microdrive 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 (diameter of each un-insulated tip, 50 μm) separated from one another by 0.4-0.55 mm, which were arranged in two parallel rows separated by 0.4-0.55mm (wire center to wire center). All rats were anesthetized with sodium pentobarbital (50mg/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, intramuscular). Anesthesia was maintained with periodic injections of ketamine hydrochloride (60 mg/kg, i.p.) during the surgery.

Following surgery rats were housed individually and maintained on a reversed light/dark cycle (on 20:00/off 8:00) so that daily recording sessions were conducted during

their active period. After one week of recovery, animals were water deprived and maintained at 80-90% of their post-surgery weight. Food (Purina lab chow) 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 No. 80-23).

2. 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 (23.5cm × 17.4 cm × 43 cm) and connected to a harness, which was connected at its other end to a commutator allowing free movement of the rat. Neural signals recorded from the microelectrode were amplified and filtered (450 Hz to 10 kHz). The signals were then digitized (62.5 kHz sampling frequency) and stored for off-line analysis using a computerized system (Datawave Technologies, Berthoud, CO).

The electrode was lowered into the ventrolateral striatum in small increments over several hours by manual rotation of the outer cylinder of the microdrive (400 μm/rotation) without rotation of the electrode, providing a vertical recording track.

On-line verification of the electrode's location in the striatum was achieved by obtaining a profile of neural activity along the track. Typical tracks show large and fast firing of cortical neurons followed ventrally by a "quite zone", corresponding to the white matter, and immediately ventral to it smaller and slower firing striatal neurons, many

correlated with locomotion or sensorimotor activity of specific body parts (Carelli and West, 1991). Starting at this point neurons were tested for correlation with licking as described bellow. After exclusion of atypical waveforms neural activity was assumed to be recorded from medium-spiny neurons, which constitute 90 to 95% of striatal neurons (Kimura, 1990). Further details regarding recording procedures and on-line verification of the microelectrode's position have been described previously (Cho and West, 1997; West, 1998; Mittler et al., 1994).

The electrode was lowered slowly, allowing it to settle for a total of at least 3 hr after entering the striatum, and an additional 30 min after isolating a lick neuron, in order to permit decay of dimpling of the brain tissue and thus stable recording. During settling the animal was left undisturbed in the experimental chamber.

3. 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 (microwire recordings were possible at a later stage of this study due to advances in implementing this recording technique). An array of 12 microwires was implanted into the ventrolateral striatum where lick-related neurons are densely located in clusters (Mittler et al., 1994, Cho and West, 1997). On the day of a recording session, the rat was placed in the recording chamber. A harness was attached at its one end 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 (450 Hz to 10 kHz), and were digitized (62.5 kHz sampling frequency for each wire) and stored for off-line analysis using a computerized system (Datawave Technologies).

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.

4. Determination of neural activity related to licking

Prior to each recording session potential lick related neurons were thoroughly tested. Drops of water were manually delivered to determine if neural firing was related to licking, as the experimenter listened through headphones to the output of the filter/amplifier and viewed the neural signals on a digital storage oscilloscope (Tetronix 2230). When a potential neuron related to licking was encountered, the whole body and especially the orofacial area were thoroughly tested for possible response to other sensorimotor functions. Only lick-related neurons that increased firing rate during licking and did not increase firing rate during any non-oral behavior were recorded during this study, ensuring that all the neurons studied were specifically related to licking and no other movement or body part (Mittler et al., 1994).

5. Video analysis

All recording sessions were video taped to allow off-line monitoring of the rat's behavior. A computerized system was used for analyzing relationships between neural activity and videotaped licking. A video camera (Panasonic WV-BL202 CCTV) 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 per sec. A video frame-counter (Thalner Electronics VC-436, Thalner Electronics Labs, Inc., Ann Arbor, MI), which was synchronized by the computer clock, superimposed a frame time on the video signal. The computer clock also time stamped each recorded neuronal discharge and the lick sensor records described below.

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 peri-event time histograms (PETH) were constructed around the nodes, displaying neural activity forward and backward in time from the motor event. Although each video frame was 33 msec in duration, greater resolution was routinely achieved by interpolating between frames. The maximum resolution of movement employed was in increments of 11 msec, with a maximum error of ± 1 increment. Video-generated PETHs were used to verify the accuracy of PETHs generated by the lick sensor / diode tracker (described below).

Video analysis was also utilized for examining various behavioral parameters (such as the proportion of long distance licks), as described below.

6. Apparatus

An apparatus for generating and recording licking was developed, in order to fulfill the following requirements:

1. Induce and maintain licking for several hours, both to allow a long enough saline baseline and to obtain recovery from drug effects. The specific type of stereotyped movement induced by psychomotor stimulants is influenced by behavioral and

environmental factors present in the experimental situation (Cooper and Dourish, 1990). The present behavioral paradigm and experimental apparatus were designed to allow the development of drug-induced stereotyped licking.

2. Measure licking and use these data for assessing drug effects on behavior and for neural analysis.

3. Enable a comparison of licks with the same sensorimotor parameters pre- and post-drug, by:

a. Building the chamber and licking apparatus in a way that restricted sensorimotor variables, while still allowing for free movement and manifestation of drug effects on behavior.

b. In post-hoc analysis licks with similar attributes were grouped together so that firing could be compared between licks with similar attributes pre- and post-drug. This required acquiring the behavioral data as well as inducing a large number of licks pre- and post-drug to allow a sufficient number of matched licks pre- and post-drug.

a. Recording chamber and water delivery

A stainless steel water spout (Small Parts Inc., Miami, FL) was positioned 7 mm outside the front wall of the recording chamber (Fig. 2). The rat accessed the spout by protracting its tongue through a 7 mm diameter hole, positioned 7.5 cm above the floor, in the center of the front wall. The recording 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. The height, angle and distance of the spout from the hole could be adjusted to accommodate individual differences among rats.

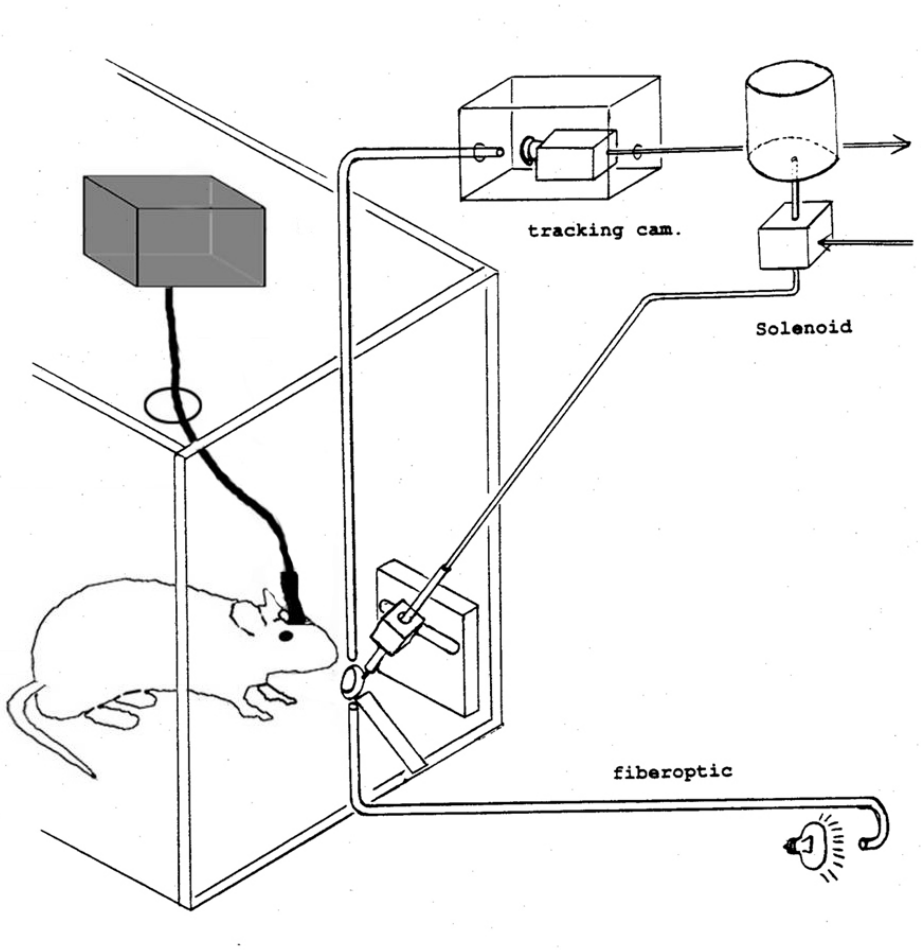

Fig. 2: Schematic illustration of the licking apparatus. The rat could accesses a water spout positioned outside the front wall of the recording chamber by protracting its tongue through a hole in the front wall (bottom center). A solenoid placed between a water container and the spout allowed computer controlled delivery of single water drops. Licks were recorded via a photo lick sensor. Light was carried by one fiber-optic which reached the bottom edge of the licking hole, and through a second fiber-optic, starting just above the upper side of the hole, to a tracking camera, which recorded presence of light or interruption of the beam by the rat's tongue on its way to the spout.

Once adjusted, set screws were tightened so that the spout's position stayed constant throughout the training and recording sessions for each animal. Transparent Plexiglas side walls, angled from floor to side (4.5 cm Height and Width, 15 cm Length, angled 45°) were added on both sides of the chamber, along the left and right flanks of the animal. These limited the position of the rat's body to the center of the chamber, directly in front of the spout while it was licking, yet still allowed free movement and the possibility to move away from the spout. An additional transparent Plexiglas wall (1.2 cm thick) was layered against the front wall, from inside the chamber. It had a slot through it, 1.7 cm wide, starting below the hole and extending to the top of the chamber, requiring the animal to place its head within this slot in order to lick. Thus the added wall limited head position, preventing the rat from angling its head laterally during licking. The height from which the slot started could be adjusted for each rat (starting from 5.8 cm above the chamber's floor), then tightened by locking screws and kept constant for that rat. At all times other then training or recording sessions (e.g. while lowering electrodes) an additional black Plexiglas wall was placed in front of the front wall, blocking access and view of the spout from the rat.

Silicon tubing (1.6 mm inside diameter) was used to connect a container filled with tap water and a solenoid valve (3-121-900, General Valve, Fairfield, NJ) to the water spout. The solenoid was placed between the water container and the tubing and was normally kept in the closed position. A pulse from the computer opened the solenoid for 35 msec, delivering one drop of approximately 5 μl through the spout (one water-delivery). Water was delivered only during “water-on” periods (see below), each consisting of 100 water deliveries. Time between water deliveries was pseudorandom,

ranging from 6 to 12 sec with a mean of 9 sec for each water-on period. An audible tone (35 msec, 70dB), consisting of mixed frequencies (500Hz and 3KHz, both at a similar amplitude), 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.

The small volume of each drop, the time between water-deliveries and their number in each water-on period were chosen to provide enough licks to analyze the neural data, maintain licking for hours (to allow a long enough saline baseline and to obtain recovery from drug effects) and yet minimize satiation.

b. Photo lick sensor

In order to overcome possible electrical disturbances in neural recording that may result from the use of lick sensors which pass a current through the animal, a photo lick sensor was chosen (based on a lick sensor described by Martonyi and Valenstein, 1971). Light from a modified slide projector was carried by one fiber optic which reached the bottom edge of the licking hole, from the outer side of the chamber, and through a second fiberoptic, aligned above the first, starting just above the upper side of the hole, to a tracking camera (Datawave Technologies) (Fig. 2). Presence of light (no interruption of the light beam) or absence of it (interruption of the beam by the rat's tongue on its way to the spout) were recorded and time stamped every 16.6 msec. in synchrony with the time stamping of neural waveforms (0.1 msec resolution) and with the time stamping of the video recording. The same computerized system was also used to control the timing of water delivery. 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 licking hole was small enough so that only the rat's tongue could pass through it (and not, e.g., its snout or chin).

To avoid interference by ambient light, required for videotape recordings, the tracking camera and the open end of the fiber it recorded were secluded in a black Plexiglas box (Fig. 2). White noise (60dB outside the Plexiglas chamber) was used to minimize any disturbances from outside the experimental room.

7. Behavioral paradigm

After reaching target weight (80%-90% of ad-lib body weight) recordings started in order to find coordinates of lick-related neurons in each rat. Once these were found, the rat was trained on two successive days. After approximately 10 min of shaping on the first day, each of the two training days consisted of four 15 min water-on periods (100 water-deliveries in each, as described above), alternating with four 5 min water-off periods, during which water was not delivered. This training was the minimum necessary to achieve stable behavior and maintain licking for several hours. A recording session was conducted on the day following the second training day.

A recording session began with a shortened water-on period (50 water-deliveries) to allow initiation of licking behavior before starting the pre-cocaine time epoch. Following these 50 water-deliveries, each recording session comprised three time epochs: 1) the pre-cocaine time epoch (T1) lasted 1 hour 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). 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.; Sigma Chemical Co., St.Louis, MO). T2 lasted 1 hour and was programmed identically to T1 with respect to water-on/water-off phases. 3) The recovery time epoch (T3) was the third or fourth hour following cocaine injection. T3 was also programmed identically to T1 with respect to water-on/water-off phases. It was conducted in order to examine the recovery of licking behavior and neural firing from the effects of cocaine in T2.

Recording sessions and the water delivery schedule described above were run continuously for 3 or 4 hours post drug injection. If stable recording could be obtained in the 4^{th} hr, recovery data was taken from hour 4; if not, recovery data was taken from hour 3.

The design of a water-on period starting immediately after drug injection was chosen in order to engage the rat in the task during the onset of drug effects, thus helping to ensure continued responding in the task. This was important especially in light of the finding that stimulant drugs strengthen the behavior which is being displayed at the onset of the drug action (Lyon and Randrup, 1972; Ellinwood and Kilbey, 1975). For rats at doses 5, 10 and 20 mg/kg, T1 began with the injection of saline (0.9%, 0.5 ml/kg, i.p.) and up to three sessions were conducted on each rat in order to increase the data yield, with at least four 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.

8. Histological verification

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 μA for 4 sec) through a stainless steel, insulated wire (250 μm) 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 μm) 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). All neurons used in the present study were verified to be located in the ventrolateral striatum.

9. Behavioral Analysis

a. Licking stereotypy: Analysis of number of licks during water-off phases

Behavioral parameters were computed by programs written specifically for this purpose (using Microsoft C ver 6.00A), and were further analyzed as well as combined with the neural data using SAS (SAS Institute Inc., Cary, NC).

Cocaine-induced licking stereotypy was assessed using the number of licks during water-off phases (consistent with the purposeless repetitive characteristics of stereotyped movements, Cooper and Dourish, 1990). The change in the number of licks during water-off phases from T1 to T2 was quantified by computing a standardized value,

$\left[L_2/(L_1 + L_2) - 0.5\right]$, for every recording session. L_1 is the number of licks during water-off phases in T1 and L_2 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, a positive standardized value represents an increase in the number of licks during water-off phases from T1 to T2, and a negative value represents a decrease.

The differences in the above measure across doses were evaluated using a one-way ANOVA (α level of 0.05). Post hoc Bonferroni tests were used to evaluate pairwise differences between doses if any significant differences across doses were found by ANOVA.

Reversal of licking stereotypy in T3 was assessed using a similar standardized value of change in the number of licks during water-off phases between T1 and T3. For every dose group that showed a significant change between T1 and T2 relative to dose 0 a standardized value of $\left[L_3/(L_1 + L_3) - 0.5\right]$ was computed for each recording session (L_3 being the number of licks during water-off phases in T3). Then, a within-subjects paired *t* test was computed, comparing 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).

b. Analysis of behavioral parameters of each lick

Onset and end times of each lick were defined as the time at which the light beam was first interrupted, and the time at which it was unblocked, respectively. Three behavioral parameters were calculated for every lick: 1) **Lick duration,** defined as the time between the onset and end time of a lick. 2) **Lick period**, defined as time between the onset of the present lick and onset of the next lick. 3) **Interlick Interval (ILI)**, defined as the time between the end of the present lick and onset of the next lick Thus, for each lick, period was the sum of duration and ILI.

Each parameter was divided into levels, utilized for both the behavioral and neural analysis. Lick duration ranged from 0 to 117 msec and was divided into 7 levels with equal increments of 16.6 msec from low to high. Lick period ranged from 84 to 267 msec (please see below for restricting the upper limit of period) and was divided into 12 levels with equal increments of 16.6 msec from low to high. ILI ranged from 0 to 250 msec and was divided into 15 levels with equal increments of 16.6 msec from low to high.

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 12 rows (period) and 7

columns (duration). For each session, one matrix was generated for T1, one for T2, and one for T3.

Detailed video analyses of tongue movement were compared to graphic frequency distributions of all recorded periods in the present paradigm. This analysis reveled 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 msec) 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 msec for period was used to eliminate these artificial long intervals in order to obtain an accurate measurement of period.

A multivariate analysis of variance (MANOVA) was performed on each of the three behavioral 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 during T1 and during 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-way and three-way interaction terms of these independent variables.

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 in order to illustrate the pattern of change in this parameter from T1 to T2 across doses. In addition, recovery of

that parameter across doses of cocaine was assessed by computing a second MANOVA between T1 and T3. Of 9 recording sessions at dose 20, one was excluded from the analysis of reversal because data were not available in T3 for this recording session.

Video analysis was used to compare the proportion of long distance licks between T1 and T2. **Long-distance licks** were defined as licks in which 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. For every recording session at dose 20, 100 licks beginning at the 5th minute of each of the 3 water-on phase in T1 and T2 were examined. 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 recording phase by 300. The change of this value between T1 and T2 at dose 20 was assessed using a Wilcoxon signed ranks test.

10. Neural Analysis

a. Isolation of the waveforms and construction of PETHs for single neurons

Waveforms of different neurons were separated from each other and from background noise (noiseband = 0.05mV) post-hoc, using the "cluster cutting" process of the Datawave software (Personal Scientific Workstation and Discovery Acquisition, Datawave Inc.). Digitized neural signals were displayed in scatter plots, each scatter plot depicting a pair of any of the following 8 parameters: peak amplitude, valley amplitude, spike height, latency to peak, latency to valley, and voltages at three particular time points chosen based on each individual waveform. Four scatter plots could be displayed on the computer screen simultaneously. In these scatter plots each point represents one

waveform, and clusters of dots represent similar waveforms. Clusters were separated from each other, defining individual neurons. Potential changes in the parameters of an identified cluster throughout the entire recording session were assessed. Waveforms whose parameters did not remain stable were either discarded, or if possible, used only until the time of loss of stability (Tang et al., 2007).

Interspike interval (ISI) histograms were constructed for each identified neuron to confirm that only isolated waveforms corresponding to single neurons were studied. If discharges occurred within the first 2 msec in the ISI histogram, representing the natural refractory period of a single neuron (Kosobud et al., 1994; Peoples et al., 1999), the recording was not used.

Cocaine at high concentration interferes with Na^+ channels, which could alter extracellular action potential waveforms and result in our equipment's failure to detect discharges. Despite this possibility, our analyses showed that waveforms were unchanged following the high dose (cf. Pederson et al., 1997). Further, such effects are not observed unless the dose of cocaine is at least 200 times greater than the highest dose used in the present study (Wilcox et al., 1999).

A peri-event time histogram (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 firing of that neuron was to be analyzed. In the PETHs, all lick neurons showed increased firing. Some neurons increased firing before lick onset, some at onset and some after onset. 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) Utilizing a visual examination of the PETH: The beginning of the time window was defined as the time (in msec) at which neural activity showed a visually distinct increase in firing above the baseline preceding lick onset. If the increased firing returned to baseline 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) Utilizing a Wilcoxon analysis: The beginning of the time window was defined as the time at which neural activity increased ($p < 0.05$, comparing two consecutive 20 msec 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 pre-increase level (Peoples and West, 1996). Both methods yielded similar results. Additionally, time windows determined by Wilcoxon analysis were confirmed to be appropriate using visual examination. In both methods, the beginning of the time window was not allowed to exceed a maximum of 80 msec prior to the onset of the present lick in order to assure that the time window of the present lick did not overlap with that of the previous lick.

Neural activity was computed, during the specified time window, for each lick. The behavioral and neural data were then combined using SAS (SAS Institute Inc., Cary, NC), so that each lick was represented by 1) the neural firing during the specified time window for that lick, and 2) the behavioral parameters of that lick. These combined measures were used for the next stage of analysis.

b. Matched pairs

In order to analyze drug effects on firing, licks with similar parameters were grouped together, so that firing could be compared between groups of licks with similar parameters pre- and post-drug. This minimized the extent to which sensorimotor variability could influence our assessment of pre- versus post-cocaine changes in firing.

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 12 rows (12 levels of period) and 7 columns (7 levels of duration). Thus all licks in a given cell have the same duration and period. For each session one matrix was generated for T1, one for T2, and one for T3 (for division into levels please see behavioral analysis above). These matrices are demonstrated in table 1.

All assessments of cocaine's effects on firing involved comparisons of "matched pairs" defined as follows (and demonstrated in table 1). 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. Mean FR was calculated for all licks that were included in each cell of the matrix for each time epoch. Thus mean firing rate was computed for groups of licks with the same duration and period in each time epoch.

Table 1: Matched pairs of a representative fast firing neuron at dose 20 mg/kg

The table illustrates the method used to compute matched pairs and the drug induced changes in firing rate between these matched pairs. Six spreadsheets illustrate the numbers of licks (left) and mean firing rates (right) of this neuron during pre-cocaine (T1; top), post-cocaine (T2; middle) and recovery (T3; bottom) time epochs of one experiment at dose 20. Each spreadsheet tabulates 7 levels of lick duration (columns) and 12 levels of lick period (rows). Each lick was sorted into a cell corresponding to its duration and period. Thus all licks in a given cell have the same duration and period. Mean firing rate (right) was computed for cells with at least 5 licks (left) in each time epoch. For example, the cell denoted by 33×167 at each spreadsheet contains all licks with duration 17-33 msec and period 150-167 msec. These licks create a matched pair between T1 and T2 and another one between T1 and T3 (rectangles). 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 pre-cocaine firing in T1. Empty cells in the spreadsheets did not contain enough licks ($n < 5$) to accurately assess firing and were thus excluded from the neural analysis of matched pairs. See Fig. 7B for waveforms and peri-event time histograms of this neuron in the three time epochs.

Table 1: Matched pairs of a representative fast firing neuron at dose 20 mg/kg

Number of Licks

Pre-Cocaine (T1)

Lick Period \ Lick Duration	17	33	50	67	84	100	117
84							
100							
117							
134	7	6					
150	31	31					
167	26	89	111				
184		26	98	77			
200	7	17	26	98			
217	6	10	15	61	163		
234			7	36			
250							
267				12			

Post-Cocaine (T2)

Lick Period \ Lick Duration	17	33	50	67	84	100	117
84							
100							
117							
134	12	6					
150	16	26					
167	14	43	47				
184		21	70	29			
200	12	16	23	35			
217	8	12	18	23	10		
234			6	11			
250							
267				6			

Recovery (T3)

Lick Period \ Lick Duration	17	33	50	67	84	100	117
84							
100							
117							
134	18	10					
150	19	25					
167	31	99	70				
184		44	133	24			
200	5	17	49	63			
217	6	19	21	33			
234			18	31			
250							
267							

Mean Firing Rate

Pre-Cocaine (T1)

Lick Period \ Lick Duration	17	33	50	67	84	100	117
84							
100							
117							
134	13.6	16.3					
150	10.0	7.8					
167	9.3	[9.5]	9.0				
184		9.5	8.7	8.7			
200	3.7	6.6	7.4	5.2			
217	5.8	6.0	4.9	6.0	4.5		
234			7.7	4.0			
250							
267				6.0			

Post-Cocaine (T2)

Lick Period \ Lick Duration	17	33	50	67	84	100	117
84							
100							
117							
134	5.8	2.5					
150	0.5	2.6					
167	4.3	[3.2]	1.7				
184		4.3	2.3	2.9			
200	2.2	5.2	3.5	1.9			
217	5.4	1.3	2.2	2.9	1.1		
234			2.2	1.6			
250							
267				1.0			

Recovery (T3)

Lick Period \ Lick Duration	17	33	50	67	84	100	117
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	7.2			
200	10.3	4.9	10.5	6.1			
217	20.1	8.3	8.6	9.1			
234			7.8	9.5			
250							
267							

The sensorimotor-related firing of striatal neurons varies from trial to trial (Prokopenko et al., 2004). Previous analyses conducted in our laboratory have revealed that 15 trials provide adequate sampling for accurate assessment of a medium spiny neuron's mean firing rate (Peoples et al., 1997). More recently, a different minimum requirements of 5, 10, 15 or 20 trials for each cell of the matrices was used to systematically compare movement-related firing of 120 striatal neurons. Graphs regressing Time 1 on Time 2 firing rates, similar to those used in the present study, revealed similar patterns across all 4 trial requirements (Pawlak, 2004). The 5-trial requirement proved to result in more accurate representation of the data than using a larger cutoff, because a threshold of 5 provided a larger sample of cells for each neuron. Thus, because the obtained average firing rates were as accurate with 5 as with more movements per cell, 5 or more licks of a particular duration and period (i.e., 5 or more licks per cell) were required in each epoch in order to be included in the analysis. Licks with similar duration *and* period that occurred ≥ 5 times in each epoch were matched between T1 and T2, or between T1 and T3, and were termed a "matched pair". Table 1 illustrates this approach, demonstrating matched pairs between T1 and T2 and those between T1 and T3 of a representative neuron. As an example of determining one matched pair, mean firing rate was calculated for all licks with duration 17-33 msec and period 150-167 msec in T1, and mean FR was computed for a matched set of licks in T2, creating a specific matched pair.

Duration and period were used in conjunction to identify matched pairs in order to provide a tight control of lick parameters for pre- vs. post drug comparisons. Licks with a given duration can occur at different rates of licking (i.e. different period). Additionally,

identifying matched pairs by both duration and period allowed dividing licks into up to 84 types of matched pairs (7 levels of duration × 12 levels of period), yielding a large number of separate measures of each neuron's firing. Since for each lick period was the sum of duration and ILI, as a by-product of clamping duration and period, ILI was also clamped.

c. Examining cocaine's effects on FR with a Hierarchical Linear Model

The neural data in the present study is hierarchical, with matched pairs nested within individual neurons to which they belonged. A Hierarchical Linear Model (HLM) is a modeling technique specifically designed to analyze a dataset with a nesting structure (Bryk and Raudenbush, 2002), and was thus utilized to analyze the neural data.

The present neural data has a two-level structure, with level 1 consisting of the matched pairs and level 2 consisting of individual neurons. Each neuron is considered a nest having many matched pairs. A two-level HLM was developed to model the two-level hierarchical neural data of matched pairs between T1 and T2.

Level 1 modeled the changes of FR of individual matched sets from T1 to T2 within neurons.

Level-1 within-neuron model:

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

In this equation, firing rates of matched pairs in T2 ($T2FR_i$) of the ith 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; 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 modeled changes in average firing rate of the neurons. That is, it models the effects of the independent variables on the average β_{0i} and β_{1i} across all neurons and is thus interpreted as the between neuron model.

Level-2 between-neuron model:

$$\beta_{0i} = \gamma_{00} + \gamma_{01}\text{Dose}_i + \gamma_{02}\text{MT1FR}_i + \gamma_{03}\text{Dose}_i^2 + \gamma_{04}\text{Dose}_i * \text{MT1FR}_i + \gamma_{05}\text{Dose}_i^2 * \text{MT1FR}_i + u_{0i} \quad (2)$$

$$\beta_{1i} = \gamma_{10} + \gamma_{11}\text{Dose}_i + \gamma_{12}\text{MT1FR}_i + \gamma_{13}\text{Dose}_i^2 + \gamma_{14}\text{Dose}_i * \text{MT1FR}_i + \gamma_{15}\text{Dose}_i^2 * \text{MT1FR}_i + u_{1i} \quad (3)$$

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. In order 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 simultaneously modeling changes in average FR of individual neurons and changes in slope of the within-neuron regression via between-neuron regression equations 2 and 3 respectively.

In equation 2, β_{0i} represented the predicted average T2FR of the ith neuron, γ_{00} represented the intercept of the regression, other γ s represented the regression parameters of corresponding variables in equation 2, and the error term (u_{01}) represented the unexplained portion of between-neuron variance of the regression function in equation 2. In equation 3, β_{1i} represented the slope of the within-neuron regression of the ith neuron, γ_{10} represented the intercept of the regression, other γ s represented the regression parameters of corresponding variables in equation 3, and the error term (u_{11}) represented the unexplained portion of between-neuron variance of the regression function in equation 3.

Modeling β_{0i} and β_{1i} allowed examining cocaine's effects as follows:

1. The intercept of each level-1 regression equation (β_{0i}) represents, after centering, the predicted average firing rate of all matched pairs in T2 for an individual neuron. Thus the

modeling results for β_{0i} represent the changes in the average firing rate from T1 to T2, and were interpreted as the between-neuron effect of cocaine on the average firing.

2. The slope of each level-1 regression equation (β_{1i}) represents the degree of changes of individual matched pairs for a single neuron from T1 to T2. Thus the modeling results for β_{1i}, were interpreted as the drug effect on the firing rate of matched pairs within neurons. 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 dose 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 the other predictor variables were kept the same.

d. Demonstrating drug effects on mean FR between neurons via regression analysis

Following each HLM analysis a linear ordinary least squares regression analysis was performed by regressing the average T2FR or T3FR on the average T1FR of neurons 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 was revealed by the HLM analysis.

e. Examining change in variability of FR from T1 to T2

To examine whether there was a change in the dispersion or variability of FR of matched pairs from T1 to T2 of individual neurons across doses, the standard deviations of T1FRs and T2FRs of all matched pairs were separately calculated for every neuron. A two-way repeated-measures ANOVA was preformed on these standard deviations, with dose as the between-neuron variable and time (T1 and T2) as the repeated-measure within-neuron variable.

f. Examining drug effects on FR within-neurons via regression analysis and ANCOVA

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.

The result of the HLM suggested further examining within neuron drug effects using two 2 × 4 analysis of covariance (ANCOVA) models. The purpose of these models was to examine separately for "slow" and "fast" firing neurons, how changes in FR of matched pairs from T1 to T2 of individual neurons are effected by dose, by a dichotomous variable indicative of T1 firing rate, and by their interaction, while controlling for the average T1 FR by applying it as a covariate.

Neurons were divided into slow and fast firing neurons according to each neuron's average T1FR of all matched pairs, using as the cut point 1.00 impulse/sec

(Pederson et al., 1997), which approximated the overall median (1.02 impulse/sec) of the average T1FRs of all 70 neurons in the present study.

T1FR group ("low" and "high") was defined as follows: 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]. T1FR group was added as an independent variable to both ANCOVAs.

The dependent variable in the ANCOVAs was a standardized value representing the magnitude of change in FR from T1 to T2 for each matched pair. For each matched pair the magnitude of change was computed as [(T2FR / (T1FR + T2FR)) – 0.5]. 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 in order to include all matched pairs into this analysis (Mosteller and Tukey, 1977). Further, 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.

Thus, in both ANCOVAs (one for slow firing neurons and the other for fast firing neurons), the dependent variable was the above 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 zero dose 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 fewer than 5 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(s) 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.

g. Examining the relation of the correlations of FR with lick parameters to neurons' FR

For each neuron, Pearson's correlation between firing rate and lick duration was computed across all matched pairs in T1 as well as in T2. The same computation was repeated for lick period. To evaluate whether the strength of the correlation of T1FR with each lick parameter was related to the individual neuron's mean FR in T1, the absolute correlation values between FR and duration in T1 were regressed on mean T1FRs for all neurons at all doses. The same analysis was repeated for lick period.

Correlations were analyzed with duration and period, but not with ILI for the following reasons. For the most part, the time window for analyzing FR did not include ILI. Since striatal firing occurs *during* movement, coinciding with duration, but not with ILI (e.g., Crutcher and DeLong, 1984b; West et al., 1990), and in light of previous demonstrations of correlations of striatal firing with movement duration, but little evidence of correlations with inter response interval (e.g., Pederson et al., 1997; Tang et al., 2007), the most meaningful parameter for this analysis in the present study is duration. Additionally, while duration and period have a behavioral significance (period being a rate measure), ILI may be viewed as a measure affected by these two behavioral parameters (rate and duration), not having a behavioral significance in and of itself. Period, which includes the time window of neural firing was also analyzed, yet interpreted with caution.

h. Examining cocaine's effects on the correlations of FR with lick parameters

To examine possible effects of cocaine on the correlation of FR with duration and with period, the correlations between FR and each lick parameter in T2 were regressed on those correlations in T1 for all neurons at each dose.

RESULTS

1. Behavioral Analysis

a. Cocaine-induced licking stereotypy and its reversal

A total of 29 recording sessions were performed, 9, 5, 6 and 9 sessions at dose 0, 5, 10 and 20 mg/kg, respectively.

Cocaine-induced licking stereotypy was assessed by evaluating the effect of dose on the standardized value of change in the number of licks during water-off phases from T1 to T2 $\left[L_2/(L_1 + L_2) - 0.5\right]$. The 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). Post hoc Bonferroni tests revealed that only rats at dose 20 ($n = 5$) significantly increased the number of licks during water-off phases from T1 (mean = 83) to T2 (mean = 343), relative to rats at dose 0 ($n = 9$) ($p < 0.05$). Thus, licking stereotypy was induced only at dose 20 during the hour following cocaine injection (Fig. 3A).

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. 3B; $p < 0.05$; paired *t* test). The number of licks during water-off phases in T3 (mean = 54) had reversed to the pre-drug level in T1 from the increased post-drug level in T2.

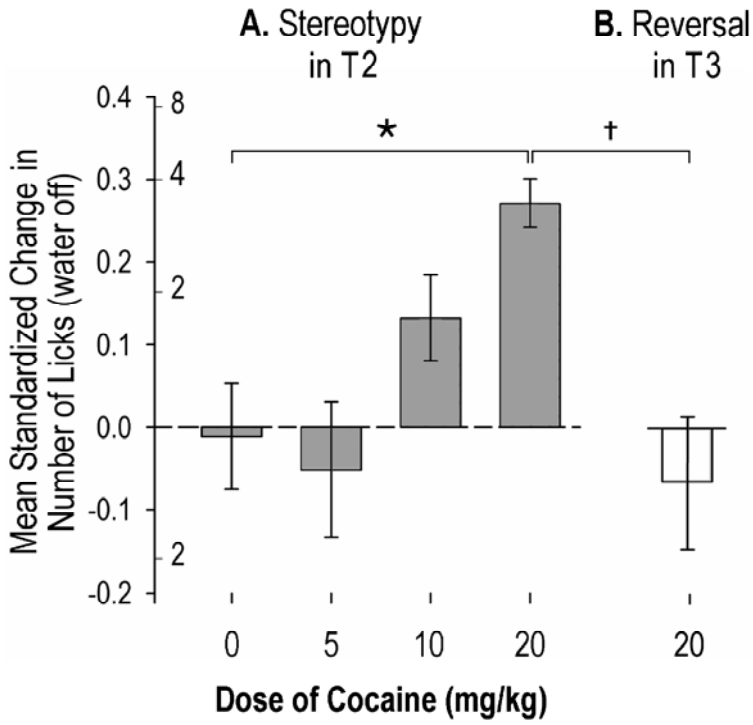

Figure 3: Cocaine induced stereotyped licking and its reversal. Stereotypic licking was induced by 20 mg/kg cocaine in T2 and subsequently reversed in T3 relative to pre-drug level in T1. Cocaine-induced licking stereotypy was assessed by evaluating the effect of dose on a standardized value of change in the number of licks during water-off phases from T1 to T2 $\left[L_2/(L_1+L_2)-0.5\right]$. A similar value computed from T1 to T3 was used to evaluate reversal. Left side of y-axis represents the standardized value of change and right side represents twofold, fourfold, 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 9, 5, 6 and 5 at doses 0, 5, 10 and 20, respectively. **A**: Rats at dose 20 exhibited significantly greater increases in number of water-off licks (mean ± S.E.M.) from T1 to T2 than rats at dose 0 (asterisk: $p < 0.05$; one-way ANOVA followed by post hoc Bonferroni tests). **B**: At 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 (cross: $p < 0.05$; paired *t* test), demonstrating reversal.

b. Dose-dependent changes in lick parameters and their reversal

Lick Duration:

Lick duration was defined as the time between the onset and end time of a lick. It ranged from 0 to 117 msec and was divided into 7 levels with equal increments of 16.6 msec from low to high.

A multivariate analysis of variance (MANOVA) was performed with the dependent variable being percentage of licks in every level of duration during T1 and during T2, and the independent variables being time (T1 and T2), dose, levels of the parameter, and two-way and three-way interaction terms of these independent variables. There was a significant three-way interaction between T1-T2, dose and levels of lick duration ($F(17, 157) = 1.94$, $p < 0.05$; Wilks' Lambda criterion), indicating a dose-dependent effect of cocaine on lick duration. As can be seen in Fig 4A, as dose increased, there was an increase in the proportion of licks with shorter duration and concurrently a decrease in the proportion of licks with longer duration in T2 relative to pre-drug values in T1. Thus cocaine induced a dose dependent shift towards shorter duration licks.

A reversal of cocaine's effect on lick duration in T3 to pre-drug levels was demonstrated by a separate MANOVA on lick duration between T1 and T3. There was no significant three-way interaction between T1-T3, dose and levels of duration ($p > 0.83$), and there was no significant two-way T1-T3*dose interaction ($p > 0.99$) or main effect of T1-T3 ($p > 0.84$).

Figure 4. Cocaine's effects on lick duration and ILI. Dose dependent decreases in lick duration and increases in interlick interval. **A**. Cocaine-induced increase in the proportion of licks with shorter duration and decrease in the proportion of licks with longer duration as a function of dose. X-axis corresponds to 7 levels of lick duration from 17 to 117 msec and 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. Number of recording sessions (N) for each dose is shown in the middle of each row. As dose increased (top through bottom), there was a significant increase in the proportion of licks with shorter durations and decrease in the proportion of licks with longer durations in T2, relative to T1 ($p < 0.05$; MANOVA, Wilks' Lambda criterion). **B**. Cocaine-induced increase in the proportion of licks with longer ILI and decrease in the proportion of licks with shorter ILI. X-axis corresponds to 8 levels of ILI from 67 to 184 msec and y-axis represents 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 was a significant increase in the proportion of licks with longer ILI and decrease in the proportion of licks with shorter ILI in T2, relative to T1 ($p < 0.05$; MANOVA, Wilks' Lambda criterion).

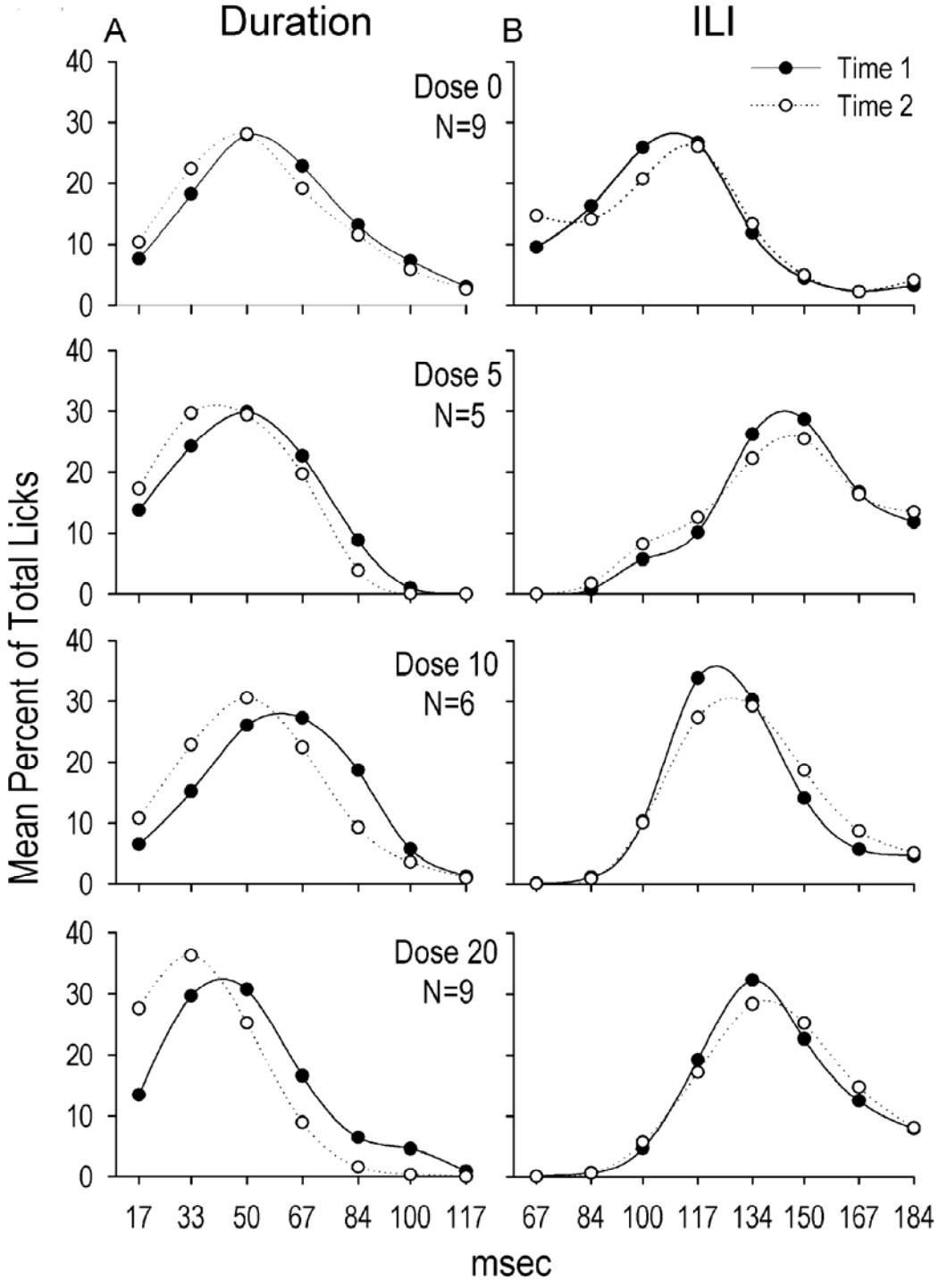
A
Duration
B
ILI
Time 1
Time 2
Dose 0
N=9
Dose 5
N=5
Dose 10
N=6
Dose 20
N=9
Mean Percent of Total Licks
0
10
20
30
40
17
33
50
67
84
100
117
134
150
167
184
msec

Figure 4

Inter-lick Interval (ILI):

Inter-lick Interval was defined as the time between the end of the present lick and onset of the next lick. It ranged from 0 to 250 msec and was divided into 15 levels with equal increments of 16.6 msec from low to high.

A multivariate analysis of variance (MANOVA) with the dependent variable being the percentage of licks in every level of ILI during T1 and during T2, and the independent variables being time (T1 and T2), dose, levels of the parameter, and two-way and three-way interaction terms of these independent variables resulted in a significant three-way interaction between T1-T2, dose and levels of ILI ($F(42, 366) = 1.45$, $p < 0.05$; Wilks' Lambda criterion). This indicated a dose-dependent effect of cocaine on ILI (demonstrated in Fig 4B). As dose increased, there was a systematic decrease in the proportion of licks with shorter ILIs and concurrently an increase in the proportion of licks with longer ILIs in T2 relative to pre-drug values. Thus cocaine induced a dose dependent shift towards longer ILIs.

There was a reversal of cocaine's effects on ILI at T3 to pre-drug levels, demonstrated by a separate MANOVA on ILI between T1 and T3. The MANOVA revealed no significant interactions ($p > 0.61$) or main effect ($p > 0.99$) on ILI between T1 and T3 across doses.

Lick Period:

Lick period was defined as time between the onset of the present lick and onset of the next lick. For each lick, period was the sum of duration and ILI. Period ranged from 84 to 267 msec (after restricting the upper limit of period to 270 msec to eliminate artificially

long periods) and was divided into 12 levels with equal increments of 16.6 msec from low to high.

In contrast to the significant effects of cocaine on duration and ILI, a MANOVA on period between T1 and T2 revealed no significant three-way interaction between T1-T2, dose and levels of period ($p > 0.82$), as well as no significant two-way T1-T2*dose interaction ($p > 0.67$) or main effect of T1-T2 ($p > 0.16$). Thus cocaine did not affect period in T2 with respect to T1. This was further 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 msec, with a small but insignificant shift following cocaine injection, showing a smaller proportion of long periods and a larger proportion of short periods.

In summary, following cocaine administration, lick duration and ILI both changed in a dose-dependent fashion in opposite directions. Higher doses of cocaine induced a shift towards shorter lick durations and concurrently towards longer ILI, particularly during stereotyped licking at dose 20. Period was not significantly altered by cocaine, reflecting a consistent licking rhythm.

Lick Distance:

Video analysis was used to compare the proportion of long distance licks between T1 and T2. At dose 20 rats made significantly fewer long-distance licks in T2 (median = 13.0%) relative to T1 (median = 24.3%) ($p < 0.05$, Wilcoxon signed ranks test).

2. Neural Analysis

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 (see Fig. 5). Of those 70 neurons, 20, 11, 17, and 22 were obtained at dose 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 at dose 0, 5, 10 and 20 mg/kg, respectively.

a. Dose- and rate-dependent effects of cocaine on average FR

(between neurons HLM results)

A two-level Hierarchical Linear Model (HLM) was developed to model the drug effects on firing rates, with matched pairs being nested within individual neurons to which they belonged. The HLM allowed examining cocaine's effects between and within neurons.

The top part of table 2 presents results of modeling the changes in average firing rates of individual neurons across doses (modeling the intercept, β_{0i}) . Thus, this part of the HLM results represents the between-neuron effects of cocaine on average firing rates. The HLM revealed a significant interaction of Dose² and the average T1FR (γ_{05} = -0.003, $p < 0.05$) on the average T2FR of individual neurons. Thus, 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.

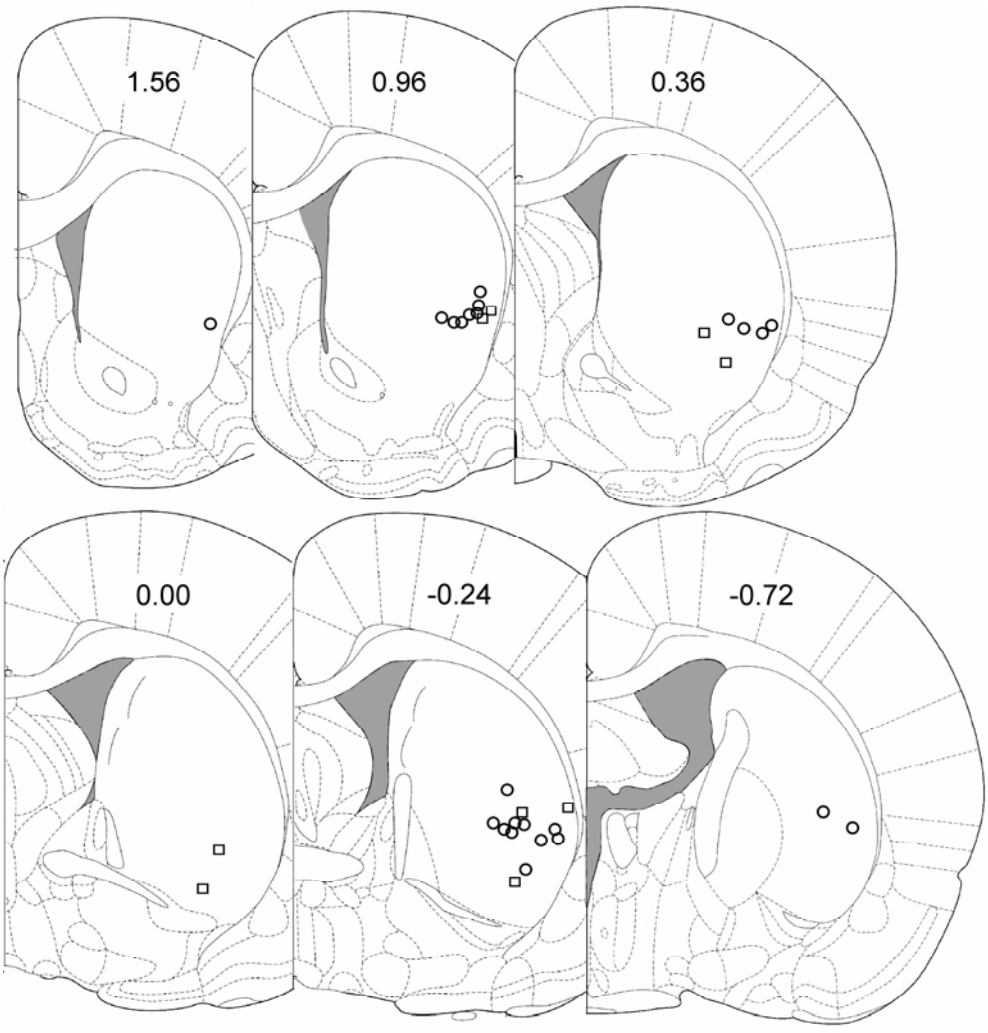

Figure 5. Histological locations of all 70 recorded neurons. Locations of all 70 recorded neurons. Every recorded striatal neuron related to licking was verified histologically to be located in the ventrolateral region of the striatum. Circles represent single neurons; squares represent several different single neurons histologically placed at the same location. Numbers on coronal plates indicate anterior-posterior distance from bregma (Paxinos and Watson, 2005).

Table 2. Results of the HLM on FR of matched pairs between T1 and T2

	Parameter	Estimate	Standard Error	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 × Average T1FR	γ_{04}	0.027	0.018	1.51
Dose^2 × 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 × Average T1FR	γ_{14}	-0.002	0.003	-0.73
Dose^2 × Average T1FR	γ_{15}	0.0002	0.0002	1.16

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Top panel presents results of modeling changes in average firing rates of individual neurons across doses. Bottom panel presents results of modeling changes in the slope of the linear regression for matched pairs within individual neurons across doses.

To illustrate these results a separate equation for modeling the intercept was obtained for each dose of cocaine by inserting the appropriate parameter values obtained from the HLM into level 2 equation for β_{0i}:

0mg/kg: $\beta 0i = 5.37 + 1.03 * MT1FRi + u0i$

5mg/kg: $\beta 0i = 5.87 + 1.09 * MT1FRi + u0i$

10mg/kg: $\beta 0i = 5.61 + 1.00 * MT1FRi + u0i$

20mg/kg: $\beta 0i = 2.85 + 0.37 * MT1FRi + u0i$

These equations reveal a much lower slope for 20mg.kg than for the other doses illustrating the significant interaction obtained by the HLM. At doses 0, 5 and 10 the slopes are all near 1, indicating that the average T2FRs of neurons at these doses remained similar to their average T1FRs. The low slope at dose 20 is further illustrated and explained in the following regression analysis.

b. Demonstrating cocaine's effects on average FR between neurons via regression analysis

The above between neuron effects revealed by the HLM were further illustrated by the following linear regression analysis. The average T2FR were regressed on the average T1FR of neurons at each dose. To accommodate a large range and uneven distribution of the average firing rates of all neurons a logarithmic transformation of average firing rates was used for this analysis. The regression lines obtained for the 4 doses are presented in Figure 6.

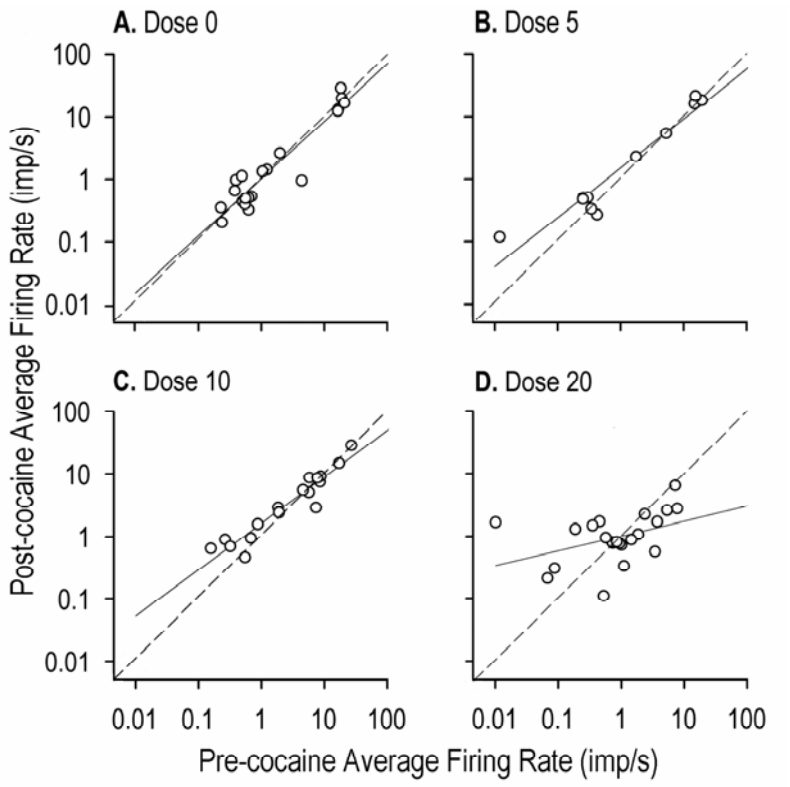

Figure 6. Regression lines on average FR between T1 and T2 across doses. Dose- and rate-dependent pattern of "clockwise" rotation of the regression lines on average firing rates of neurons between T1 and T2 across doses. Each dot represents one neuron. In each scatterplot (for each dose), the average firing rates of neurons in T2 (y-axis) are regressed on their average firing rates in T1 (x-axis). Both axes are in logarithmic scale. 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 through D), the regression lines gradually rotated clockwise away from the diagonal line of no change with decreasing slopes. This is consistent with the significant ($p < 0.05$) dose- and rate-dependent effects of cocaine on average firing rates revealed by the HLM. The strong linearity of regression at dose 0, 5 and 10 ($p < 0.001$) was absent at dose 20 ($p < 0.05$).

There was a strong linear relationship between the average T1FRs and the average T2FRs of neurons at doses 0 ($R^2 = 0.88$, $p < 0.001$), 5 ($R^2 = 0.92$, $p < 0.001$) and 10 ($R^2 = 0.94$, $p < 0.001$), 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. 6A). 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 and a distinctly low slope at dose 20 (Fig. 6B through D). These slopes reveal a "clockwise" rotation of between-neuron regression lines with increasing dose.

This dose-dependent pattern of clockwise rotation 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 impulse/sec; Fig 7A). 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 impulse/sec; Fig 7B) together determined the greater clockwise rotation of the regression line, i.e., the low slope. This low slope is consistent with the low slope revealed by the above equations obtained from the HLM.

Figure 7. Changes in FR of representative neurons following cocaine. 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 (defined as neurons with average T1FR less than 1 impulses/sec), at dose 10. The y-axis of each peri-event histogram represents average firing rate (impulses per second). Time 0 of the x-axis indicates the beginning of lick. Dashed vertical lines indicate the customized time window of firing for this neuron, applied to all three time epochs of the recording session. In T1 (top) the neuron showed lick-related activity from -20 to +65 msec, relative to beginning of lick. In T2 (middle) the same neuron showed increased activity during the same time window. In T3 (bottom) the 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 msec. **B**. Decrease in average firing rate in T2 and reversal in T3 of a representative fast firing neuron (defined as neurons with average T1FR greater than 1 impulses/sec) at dose 20. In T1 (top) the neuron showed lick-related activity from -30 msec to beginning of lick (the time window of firing, applied to all three time epochs, indicated by dashed vertical lines). In T2 (middle) the same neuron showed decreased activity during the same time window. In T3 (bottom) 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 msec. 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.

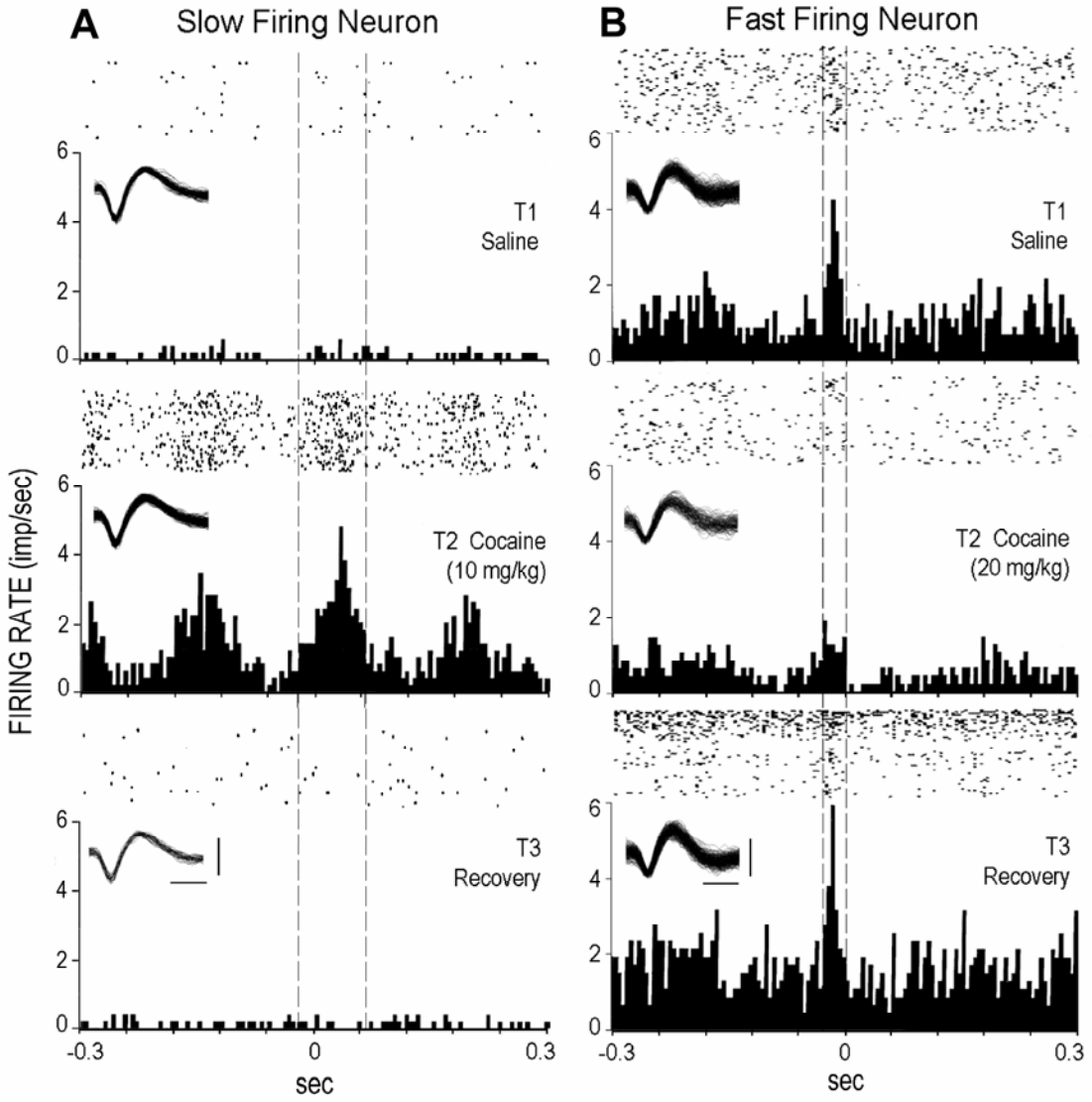
A
Slow Firing Neuron
B
Fast Firing Neuron
T1
Saline
T2 Cocaine
(10 mg/kg)
T2 Cocaine
(20 mg/kg)
T3
Recovery
FIRING RATE (imp/sec)
sec
-0.3
0
0.3

Figure 7

The slopes obtained by the regression analysis above differ from those obtained directly from the HLM results (1.03, 1.09, 1.00 and 0.37 at doses 0, 5, 10 and 20 respectively) since the regression analysis used a log transformation, while the HLM did not, and due to the range and unevenness in the original data. A linear regression analysis similar to the one described above, without a log transformation was also obtained, resulting in significant R^2 values at all doses, and the following slopes: 1.0, 1.09, 0.989 and 0.46 at doses 0, 5, 10 and 20 respectively. These slopes are very similar to the slopes obtained directly from the HLM results.

c. Reversal of dose- and rate- dependent effects of cocaine on average FR

To assess reversal of the altered average firing rates 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). A HLM on these matched pairs revealed that there were neither significant interaction effects of dose and the average T1FR (Dose*MT1FR and $Dose^2$*MT1FR; $p > 0.64$) nor significant main effects of dose (Dose and $Dose^2$; $p > 0.7$) 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. 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 (The return to baseline firing rate at T3 is illustrated for two representative neurons in Fig. 7).

d. Consistent variability of FR from T1 to T2 of individual neurons across doses

The possibility of change in the variability of firing rates of matched pairs from T1 to T2 of individual neurons across doses was examined by a two-way repeated-measures ANOVA on the standard deviations of T1FRs and T2FRs of all matched pairs computed separately for every neuron, with dose as the between-neuron variable and time (T1 and T2) as the repeated-measure within-neuron variable. There was no significant interaction between T1-T2 and dose ($F(3, 66) = 0.52$, $p > 0.67$), and no significant main effect of T1-T2 ($F(1, 66) = 0.02$, $p > 0.88$). Thus, while average firing rates of individual neurons systematically changed from T1 to T2 across doses as revealed by the HLM, the dispersion of FR of matched pairs within individual neurons did not change from T1 to T2 in each dose.

e. Lack of drug effect on FR within neurons for the pooled sample

The slope of each level-1 regression equation (β_{1i}) in the Hierarchical Linear Model represents the rate of change in firing rates of individual matched pairs from T1 to T2 (this is the slope of the regression between T1FR and T2FR). Thus the modeling results for β_{1i}, taking both levels of the HLM into account, represent the drug effect on changes in firing rate of matched pairs within individual neurons. These results are presented in the bottom part of table 2.

The HLM results for modeling β_{1i} reveal that neither the interaction effects between dose and average T1FR (Dose*MT1FR and Dose²*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.

The predictability of T2FR of matched pairs from their T1FR within individual neurons was assessed by linearly regressing T2FRs of matched pairs on their T1FRs for every neuron, and performing a one-way ANOVA on the resulting R^2 values across doses. 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 ± standard deviation).

The low average R^2 values indicate that 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 the 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.

f. Cocaine's effects on FR of matched pairs within neurons, with slow and fast firing neurons analyzed separately

The between neuron effects revealed by the HLM and demonstrated by the regression analysis indicated that cocaine affects the average firing rates of slow and fast firing neurons differently. Hypothesizing that the drug exerts different effects on matched pairs for slow and fast firing neurons, drug effects on these two groups of neurons were assessed separately. To further examine if drug effects differ for matched pairs with low

and high T1 firing rate, a dichotomous variable indicative of T1FR of each matched pair was added to the analysis.

Thirty five neurons were categorized as slow firing (defined as neurons with average T1FR less than 1 impulses/sec), including 11, 6, 6 and 12 neurons from dose 0, 5, 10 and 20 mg/kg, respectively. The remaining 35 neurons were fast firing neurons (average T1FR greater than 1 impulses/sec), including 9, 5, 11 and 10 at dose 0, 5, 10 and 20 mg/kg, respectively.

Two 2 × 4 analysis of covariance (ANCOVA) models were obtained, one for slow firing neurons and the other for fast firing neurons. In both the dependent variable was the standardized value of change from T1FR to T2FR of matched pairs [(T2FR / (T1FR + T2FR)) – 0.5]. 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. In order to control for the average T1FR of each neuron (MT1FR), MT1FR was included in each ANCOVA as a covariate.

Separate drug effects on slow and fast firing neurons are presented in Figures 8 and 9. For slow firing neurons, across doses, there was a significant main effect of dose on firing rates of matched pairs from T1 to T2 ($F [3, 822] = 8.39$, $p < 0.0001$, ANCOVA), but no interaction between dose and T1FR group ($p > 0.15$). Thus cocaine's effects on firing rates of the low and high T1FR matched pairs of slow firing neurons were similar across dose.

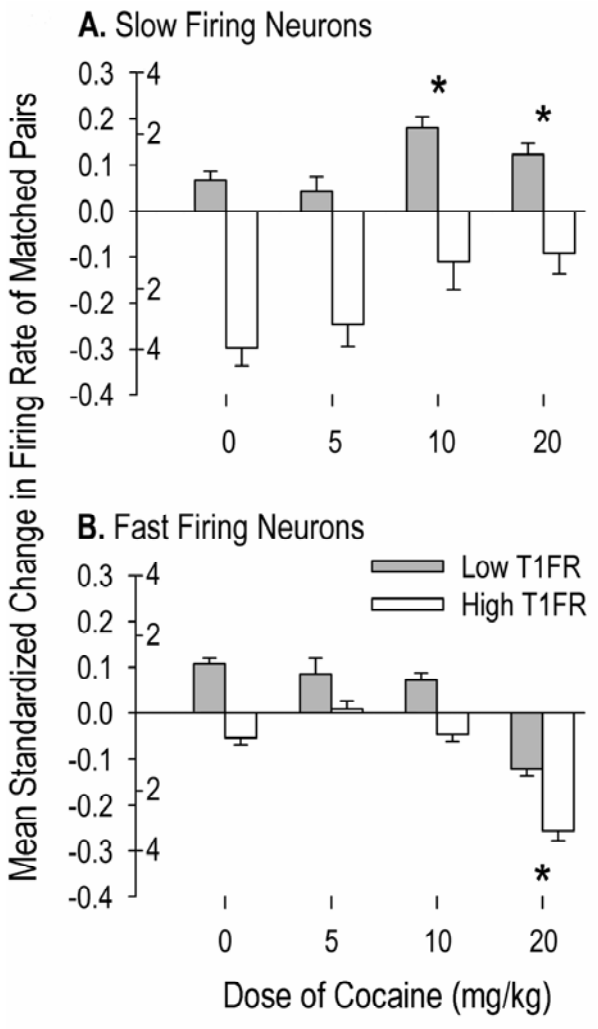

Figure 8. Cocaine's effects on the FR of matched pairs within neurons. Dose- and rate-dependent effects of cocaine on the firing rates of matched pairs within neurons. The top and bottom graphs illustrate the modeling results of ANCOVAs for matched pairs of slow and fast firing neurons, respectively. Left side of the y-axis represents the standardized value of change in firing rate of each matched pair [(T2FR / (T1FR + T2FR)) – 0.5]). The right side of the y-axis represents twofold, fourfold, etc., changes in FR in T2, relative to T1. Horizontal line at 0 represents no change in FR from T1. Black and white bars show the mean of standardized changes in FR of low and high T1FR matched pairs across neurons, respectively. Error bars represent the standard errors of the means. *$p < 0.001$, post hoc Bonferroni tests compared with dose 0.

Figure 9. Changes in FR of matched pairs from T1 to T2 for 8 representative neurons. Each row consists of two neurons from one dose: a slow firing neuron in the left, and a fast firing neuron on the right. 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. Dots above it correspond to matched pairs showing increased FR from T1 to T2, dots below it correspond to matched pairs showing decreased FR from T1 to T2.

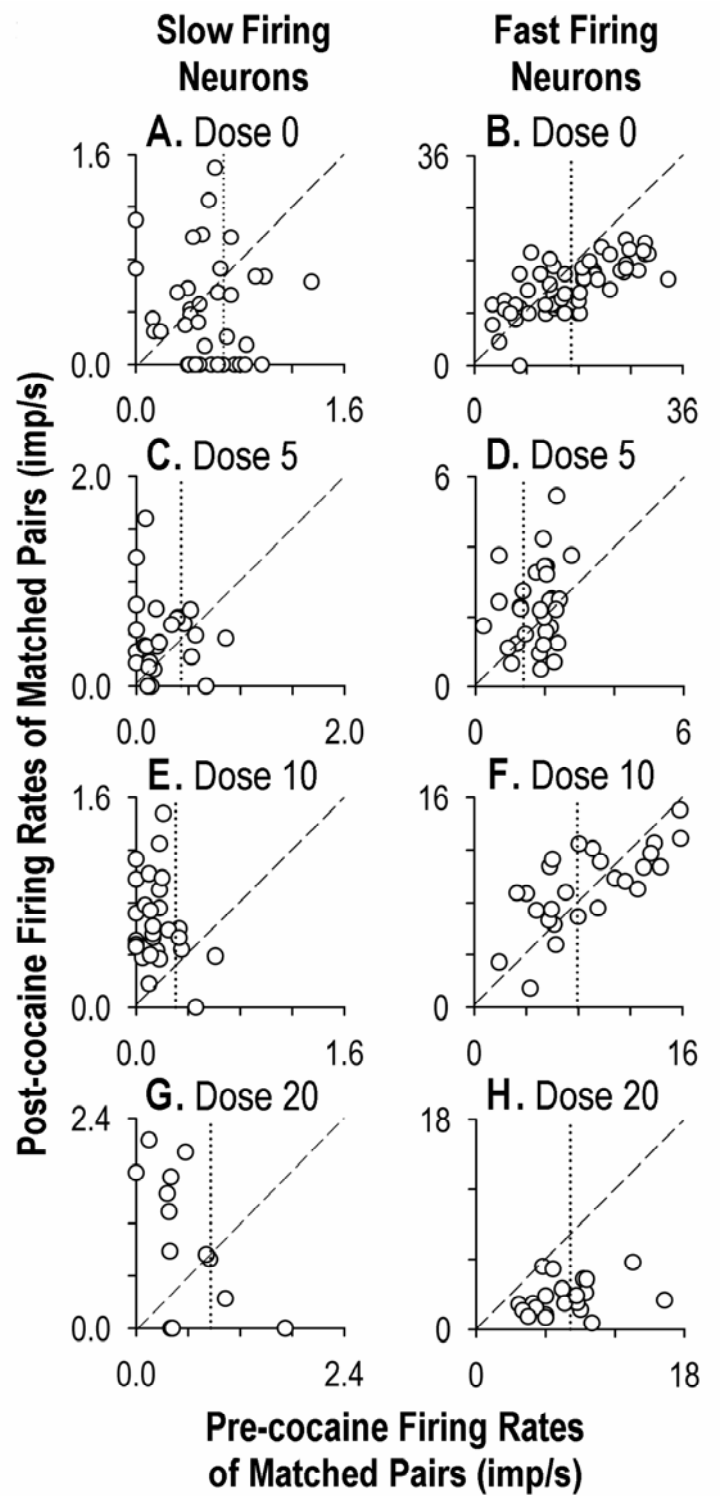
Slow Firing Neurons
Fast Firing Neurons
A. Dose 0
B. Dose 0
C. Dose 5
D. Dose 5
E. Dose 10
F. Dose 10
G. Dose 20
H. Dose 20
1.6
0.0
36
2.0
6
16
2.4
18
Post-cocaine Firing Rates of Matched Pairs (imp/s)
Pre-cocaine Firing Rates of Matched Pairs (imp/s)

Figure 9

At dose 0, there was "regression to the mean", characterized by increased firing of low T1FR matched pairs and decreased firing of high T1FR matched pairs in T2 (Figs. 8A and 9A). While the other doses also showed increased firing of low T1FR matched pairs and decreased firing of high T1FR matched pairs in T2, post hoc Bonferroni tests revealed significant *enhancing* effects on firing rates at doses 10 ($p < 0.001$) and 20 ($p < 0.001$) (Fig. 8A). Relative to the natural regression to the mean at dose 0, at doses 10 and 20 low T1FR matched pairs showed greater increases and the high T1FR matched pairs showed lesser decreases in firing rate from T1 to T2. These effects are illustrated by matched pairs of representative slow firing neurons at doses 10 and 20 (Figs. 9E and 9G).

An analysis of reversal in T3 was performed separately for matched pairs at doses 10 (135 matched pairs from 5 neurons) and 20 (53 matched pairs from 3 neurons). The significant changes in firing rates form T1 to T2 were reversed in T3 at dose 10 ($p < 0.001$; Wilcoxon signed ranks test), but not at dose 20 ($p > 0.89$).

For fast firing neurons ANCOVA revealed a significant main effect of dose on firing rates 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 (similar to the effects revealed for the slow firing neurons).

A regression to the mean was observed for matched pairs within neurons at dose 0 (Figs. 8B and 9B, similar to the one seen for slow firing neurons). 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. 8B, $p < 0.001$, 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 while 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. 9H).

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 3 fast firing neurons at dose 20.

g. Correlations of FR with lick parameters are related to FR in T1

Regression analysis revealed that in T1 there was a significant positive relationship between the absolute correlation values of FR with lick duration and the mean FR of individual neurons for all neurons at all doses ($R^2= 0.10$, $p=0.009$). This relationship was also significant for lick period ($R^2= 0.13$, $p=0.003$). These results indicate that a neuron with higher mean FR tended to have stronger correlations of FR with duration and period in the pre-drug T1 phase.

h. Cocaine's effects on the correlation of FR with lick parameters

For each neuron, Pearson's correlation between firing rate and lick duration, as well as period, was computed across all matched pairs of this neuron in T1 as well as in T2. The mean of the absolute correlation values of FR with duration in T1 across all neurons and all doses was 0.33 ± 0.25 (mean ± SD), with 46% of the 69 neurons exhibiting absolute correlations above 0.3. The mean of the absolute correlation of FR with period in T1 across all neurons and all doses was 0.32 ± 0.22 (mean ± SD), with 46% of neurons exhibiting absolute correlations above 0.3.

To examine possible effects of cocaine on the correlation of FR with each lick parameter, the correlation values between FR and each of the two lick parameters in T2 were regressed on those in T1 for all neurons at each dose. For duration and for period, when all lick neurons were analyzed, there was a significant relationship between the correlations in T2 and those in T1 at dose 0, 5 and 10, but not at dose 20 (for duration, the p-values for the regression models for doses 0, 5, 10 and 20 were 0.002, 0.003, <0.0001 and 0.69, respectively; for period they were 0.016, 0.002, 0.02 and 0.34, respectively).

When neurons were divided into slow and fast firing neurons a different pattern was found for the two groups. The regression results for duration are presented in figure 10, with the accompanying R-square and p-values. For slow firing neurons there was no significant relation with duration at any dose. That is, the correlation of T1FR with duration changed post drug at all doses. In contrast, for fast firing neurons the regression models for duration where significant at doses 0, 5 and 10, but not at 20mg/kg. That is, for fast firing neurons at the low and medium doses the behavioral correlations were maintained at T2. Only at dose 20 did the correlation of FR with duration change post-drug for these fast firing neurons.

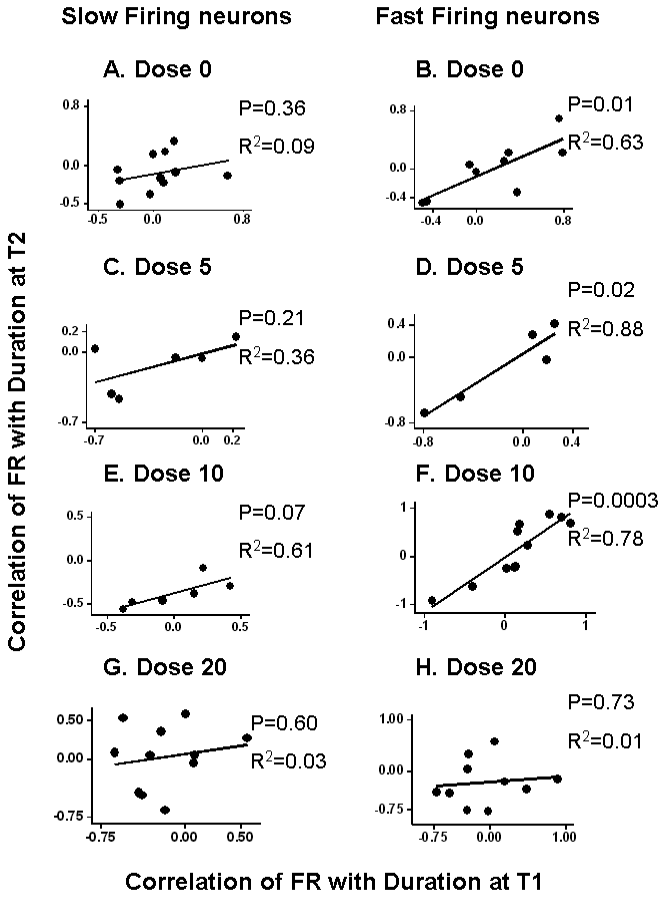

Figure 10: Regression of correlations of FR with duration between T1 and T2. The left row represents slow firing neurons, the right fast firing neurons. Each dot represents one neuron. In each scatterplot the correlations between FR and each lick parameter in T2 (y-axis) were regressed on those correlations in T1 (x-axis) for all neurons at that dose. The solid line represents the linear regression line. In each scatterplot, the x- and y-axis are equivalent in scale, customized to the ranges of correlations of neurons in that dose. Accompanying each plot are the R-square and P-value for that regression model. For slow firing neurons the correlation of FR with duration at T1 changed post drug at all doses. For fast firing neurons the regression models where significant at doses 0, 5 and 10 (i.e., the behavioral correlations at T1 were maintained at T2), but not at 20mg/kg.

The results for period followed the same pattern as those for duration. For slow firing neurons the correlation of T1FR with period changed post drug at all doses (The R-square values for doses 0, 5, 10 and 20 were 0.04, 0.25, 0.57 and 0.001, respectively; The p-values for the regression models for the same doses were 0.55, 0.31, 0.08 and 0.9, respectively). For fast firing neurons the correlation of T1FR with period were maintained post drug at doses 0, 5 and 10, but changed at dose 20, similar to the correlations with duration (The R-square values for doses 0, 5, 10 and 20 were 0.59, 0.91, 0.46 and 0.08, respectively; The p-values for the regression models for the same doses were 0.015, 0.012, 0.022 and 0.44, respectively).

DISCUSSION

The present study aimed to answer the question: Following cocaine injection, what changes in striatal activity accompany changes in motor behavior that are attributed to cocaine's elevation of striatal dopamine?

To this end, the activity of lick-related neurons in the ventrolateral striatum of awake, freely moving rats was recorded before and after administration of four doses of cocaine (0, 5, 10 and 20 mg/kg) while animals were engaged in licking. The paradigm succeeded in replicating classic behavioral effects of psychomotor stimulants (Lyon and Robbins, 1975). Simultaneously, cocaine's effect on single neuron firing was examined by comparing the firing rates associated with licks that were matched in movement parameters pre and post drug ("matched pairs"). Cocaine was found to exhibit dose-dependent effects on neural activity that strongly depended on pre-drug firing rate.

1. Effects of cocaine on behavior

Psychomotor stimulants cause increased occurrence of long-sequence movements (e.g., locomotion) at low doses and shorter, stereotyped movements characterized as purposeless, repetitive movement, at high doses (Bhattacharyya and Pradhan, 1979; Cooper and Dourish, 1990). In the present paradigm, although licking was induced by water delivery, the highest dose of cocaine caused stereotyped licking. Stereotypy was evident by a significant increase in the number of licks during water-off phases (that is, in the absence of water delivery), at the high dose, followed by a subsequent reversal to pre-drug levels. This effect is consistent with stereotypic effects observed in other studies

(Lyon and Robbins, 1975; Bhattacharyya and Pradhan, 1979). The above pattern of purposeless, repetitive movement is a defining characteristic of drug-induced stereotypy (Cooper and Dourish, 1990).

The specific type of stereotyped movement induced by psychomotor stimulants is influenced by behavioral and environmental factors present in the experimental situation (Cooper and Dourish, 1990). Additionally, stimulant drugs have been shown to strengthen the behavior which is being displayed at the onset of the drug action (Ellinwood and Kilbey, 1975). The present behavioral paradigm and experimental apparatus were designed with the intention of allowing the development of stereotyped licking. It was thus important to ensure that the rats were engaged in licking pre-drug. In addition, the continuation of licking immediately after drug injection and beyond was necessary for obtaining all measures of drug effects on firing. These procedures have proved effective, and allowed the manifestation of cocaine-induced stereotyped licking.

Cocaine caused a dose dependent change in lick parameters. As dose increased, cocaine systematically decreased the proportion of licks with longer duration while increasing the proportion of licks with shorter duration, compared to pre-drug levels. These shifts in the frequency distributions of duration were more pronounced with increasing dose, and subsequently reversed to pre-drug 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 with the previous finding that a high dose of cocaine (20 mg/kg) decreased the proportion of long-distance head movements (Pederson et al., 1997). Importantly, they are both consistent with the characteristics of stereotyped movements following high doses of psychomotor stimulants, which consist

of a shift towards shorter responses (Lyon and Robbins, 1975; Cooper and Dourish, 1990).

Additionally, cocaine increased the proportion of long ILI while decreasing the proportion of short ILI. As for duration, the shifts in the frequency distribution of ILI were more pronounced with increasing dose, and subsequently reversed to pre-drug levels. However, ILI was calculated by subtracting duration from period and it can be viewed as a variable affected by these two behavioral parameters: rate (period) and duration. Thus the shifts towards longer ILI are likely due to the shifts towards shorter durations combined with the constant period (see below). As such the change in ILI would not signify any behaviorally important effects on its own.

Period was not significantly altered by cocaine, reflecting a consistent licking rhythm, which was not affected even by a dose (20 mg/kg) high enough to induce stereotypic licking. This is consistent with clear evidence that lick rhythm is controlled by an oscillator in the hypoglossal nucleus and nearby areas of the brain stem, including the reticular formation (Travers et al., 1997; Wiesenfeld et al., 1977; Brozek et al., 1996), and suggests that cocaine did not affect these areas at doses that exerted striatal-mediated effects on behavior. It should be noted that period was analyzed only within bursts of licking (period $\leq$ 270 msec). The overall rate was not assessed since a small percentage of licks failed to break the light beam. Thus the consistent licking rhythm observed in the present data reflects the rhythm of licking within bursts.

Additionally, there was a non-significant trend in the present data toward fewer long periods and more short periods within bursts (i.e., a faster rhythm). This trend is in the same direction as the well documented faster rate of short behaviors following

psycho-stimulants (Lyon and Robbins, 1975; Cooper and Dourish, 1990). While the lack of a significant drug effect on lick period is likely due to brain stem mechanisms and not the striatum having the main control over lick rhythm, the trend towards a faster rhythm could reflect some influence of the striatum over lick rhythm. Accordingly, the striatum is suggested to have some effect over brain stem nuclei controlling lick rhythm via the substantia nigra, indirectly via the superior colliculus, or also possibly via thalamic projections to cortex which in turn influences the described brain stem nuclei (Travers et al., 1997).

Most studies examining the effects of psychomotor stimulants on licking used rating scales and did not measure the different parameters of licking. The few studies describing those effects used amphetamine. Low doses of amphetamine were reported to increase lick rate whereas high doses decreased it (Wuttke, 1970; Knowler and Ukena, 1973). While the results of the high doses in these studies are inconsistent with the present study, methodological problems may have affected these results. Wuttke (1970) studied only 3 subjects whose control rates of licking were drastically different from each other and amphetamine's effects reported by Knowler and Ukena were based on only 3 rats, which were repeatedly injected with many doses of two different drugs. In contrast, the present design used a pre-drug control period and a post-drug period for each of four dose groups, including a zero-dose group, with 5 to 9 subjects per group.

When comparing the stimulant effects on lick rate to their effects on the rate of other behaviors it is important to note that licking rate can be described in terms of lick rate within bursts and rate of burst initiation (Hulse and Suter, 1968). It is possible that the two would be influenced differently by stimulants, e.g., if the striatum affects the rate

of burst initiation, while brainstem mechanisms are more correlated with lick rate within bursts, cocaine would be predicted to increase the rate of burst initiation more than lick rate within bursts. The present study as well as the study by Knowler and Ukena (1973) measured lick rate within bursts, while Wuttke (1970) measured overall lick rate. The rate of burst initiation could not be accurately measured in the present study due to undetected licks that did not reach the lick sensor to break the light beam, which caused the apparent initiation of a new burst. A lickometer more sensitive to short licks could solve this, allowing examination of burst initiation rate.

Amphetamine has been shown to exert rate dependent affects on the rate of different operant behaviors in different species. Responses with low control rates were increased by amphetamine, while responses with high control rates showed only little change after low doses of amphetamine and were decreased by high doses (Dews, 1958; Cook and Kelleher, 1962; Clark and Steele, 1966; Wuttke, 1970; for review see Kelleher and Morse, 1968). The present paradigm was not designed to assess rate dependent effects on behavior. This would require using an operant behavior with several control rates (accomplished, for example, via different reinforcement schedules).

A series of studies examining the effects of systemic injections of different dopamine-blocking drugs such as haloperidol, clozapine, and olanzapine decrease tongue force and number of licks, and showed either no effects or a decrease in lick rhythm (Fowler and Mortell, 1992; Fowler and Das, 1994; Das and Fowler, 1995, 1996; Fowler & Wang, 1998). The smaller influence of dopamine-blockers on lick rhythm relative to their stronger effects on other lick parameters, as well as the direction of the small effects on lick rhythm (reduction) are in line with the present results.

2. Effects of cocaine on neural activity

a. Dose and rate dependent effects

The present study revealed dose- and firing rate-dependent effects of cocaine on firing rate of striatal neurons, at different levels of analysis. Several advantages of the present paradigm allowed revealing these effects. Focusing on neurons correlated with a specific movement (licking) allowed studying that neuron's activity while the drug is affecting its correlated behavior. It enabled designing an experimental paradigm that ensured a large number of licks pre- and post-drug, while measuring specific lick parameters synchronized with the neural recordings. This detailed data allowed examination of cocaine's effect on firing by comparing the firing rates associated with licks that were matched in movement parameters pre and post drug, minimizing any influence of sensorimotor variables on changes in firing. Furthermore it allowed examination of drug-effects on the correlations of striatal neurons with lick parameters.

The Hierarchical Linear Model results for modeling the intercept (top part of table 2) indicate that changes in *average* firing rate of individual neurons from T1 to T2 were significantly different across doses and depended on the neuron's average pre-drug firing rate. This effect is illustrated by the clockwise rotation of the regression lines depicted in Figure 6. At dose zero (saline), average firing rates of individual neurons 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, as would be expected. At low and moderate doses, cocaine caused an increase in the firing rates of slow firing neurons, resulting in a clockwise rotation of the regression line. At the high dose, which induced stereotypic licking, cocaine caused both an increase in the firing rates of slow firing

neurons and a decrease in the firing rates of fast firing neurons, resulting in the regression line rotating clockwise to the greatest degree (i.e., having the lowest slope). These dose-dependent changes in firing were not present during the last hour following cocaine administration, at which time behavior also returned to pre-drug levels.

A within neuron analysis on the entire pooled sample revealed no significant within neurons drug effects.

Once the sample was separated into slow and fast firing neurons, a separation suggested by the between neuron rate dependent effects, a significant difference in drug effect was uncovered between these two groups.

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).

By 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 pre-drug firing rates exhibited a *greater increase* and those with higher pre-drug firing rates exhibited a *lesser decrease* in firing, compared to the zero dose 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. Matched pairs with low pre-drug firing rate decreased firing rate at dose 20, as opposed to increasing it at dose zero. Matched pairs with high pre-drug firing rates decreased their firing rate at dose 20 to a greater degree than the decrease observed at dose zero. These effects underlie the decreased average firing rate of these neurons seen at the high dose. Thus, suppressed firing rates relative to the zero dose group were observed only for fast firing neurons, especially for their highest firing rates, and only at the high dose.

Thus, 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 a generally lower tendency to discharge at the high dose, both their low and high firing rates strongly reduced compared to 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 can potentially explain the mixture of increases and decreases in firing rates observed in previous studies examining the 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). This mixture of effects is not surprising in light of dopamine's modulatory effects on corticostriatal synapses. According to the present findings, the direction and degree of drug-induced changes in a neuron's firing rate would be predicted by its pre-drug firing

rate, which is thus an important factor to consider in such studies. Slow firing neurons would on average exhibit elevated firing rates at all doses. Moreover, an individual slow firing neuron would exhibit dose-dependent elevations for its normally low firing rate, and smaller decreases in FR, relative to saline, for 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. Additionally, initial firing rate may be important in a more general sense, as demonstrated by the 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).

Striatal head movement neurons were reported to exhibit similar rate-dependent changes in movement-related firing after cocaine administration (Pederson et al., 1997). These drug effects were also found to be dose-dependent when compared with a saline group (Pawlak et al., 2001). 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.

The present elevation of low average firing rates at all doses and reduction of high average firing rates at the high dose (see figure 8) suggest 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 striatal output. Consistent with this result, 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 fluctuations in magnitude (Prokopenko et al., 2004). Together these findings suggest that a regulatory role of striatal dopamine may be to restrict the range in amplitudes of corticostriatal throughput.

b. Changes in behavioral correlations in slow and fast firing neurons

The present study found several differences between slow and fast firing neurons, suggesting the possibility of different types of neurons with different functions and possibly different biological properties. In addition to the different drug effects on the firing rate of slow versus fast firing neurons, firing rates at T1 were found to be related to the strength of the correlation of FR with lick duration as well as lick period. Neurons exhibiting higher mean T1FR showed higher absolute correlations of T1FR with duration as well as with period. Thus an important functional difference between slow and fast firing neurons appears to be the strength of their correlation with movement parameters. Additionally, for slow but not for fast firing neurons the correlations with duration and period at T1 were altered at T2, even for the zero dose group. Without any drug present, across time, slow firing neurons changed their correlations with lick parameters, while the behavioral correlations of fast firing neurons remained consistent. This further suggest that fast firing neurons may be more strongly related to movement parameters, and possibly more involved in the control of movement parameters, relative to slow firing neurons.

Another significant and interesting difference between slow and fast firing neurons appeared when the effects of cocaine on the correlation of FR with lick parameters pre-and post-drug were examined. For slow firing neurons the correlations of

FR at T1 with duration as well as with lick period were altered at T2 for all doses including zero, and thus occur in the absence of cocaine. In contrast, fast firing neurons maintained the correlation of FR with duration and with period pre- and post-drug for doses 0, 5 and 10mg/kg, and changed their correlations only at the high dose. These results further support a functional difference between slow and fast firing neurons. Cocaine was found to affect both FR and the strength of the correlation with movement parameters differently for these two groups of neurons.

This putative difference between slow and fast firing neurons could be examined in a separate study investigating the correlation of slow versus fast firing striatal neurons with different movement parameters. Examining other movement parameters in addition to the ones examined in this study, like force, distance and velocity, would be valuable in better understanding how different neurons are related to movement parameters, and would be important in order to examine whether slow firing neurons are truly not related to any of these parameters. Relations of different neurons to other movement characteristics like movement initiation (see below) should also be examined. Since cocaine had different effect on these two groups of neurons a more detailed examination of their function and behavioral correlates would help clarify the mechanisms by which stimulants influence the striatum to induce stereotyped behavior.

It should be noted that when interpreting the correlations with behavioral parameters, the results relating to correlation with duration are likely the most meaningful, while the results of the correlation analysis with period must be interpreted with caution. As described earlier, striatal firing occurs *during* movement, coinciding with duration (e.g., Crutcher and DeLong, 1984b; West et al., 1990). Additionally striatal

firing has been demonstrated to be correlated with movement duration, but showed little evidence of correlation with period (Pederson et al., 1997; Tang et al., 2007). While period includes the time window of neural firing, it includes an additional time when for the most part the neurons were not firing (coinciding with ILI). Furthermore, lick rhythm is controlled by brain stem mechanisms (Wiesenfeld et al., 1977; Brozek et al., 1996; Travers et al., 1997), and appropriately was not affected by cocaine in the present study. An additional concern when interpreting the correlation results for period is the natural correlation between period and duration. In a separate study of the same task, duration and period were found to be strongly positively correlated (Teixeira et al., 2006).

The regression models results for period followed the same pattern as for duration. That is, for both parameters the regression models were significant for fast firing neurons at doses 0, 5 and 10, but not at 20, and were not significant at any dose for slow firing neurons. Due to all the above, and especially to the correlation between duration and period, it is possible that the results of the present analysis for the correlations with period were at least in part secondary to the correlations with duration, and may not have a significant implication on their own. Thus firing showed correlations with duration and period, and although they were not dissociable, our interpretation is that these correlations likely reflect neuronal relationships to lick duration.

Changes in behavioral correlations with striatal FR were previously observed for a different population of striatal neurons in a different instrumental learning paradigm (Tang et al., 2007). Striatal neurons related to head movement were recorded over 14 days as an instrumental head movement became habitual. Neurons exhibiting higher

initial firing rates showed a decrease in firing rate during habit formation, while a small percent of the total neurons showed lower initial firing rates as well as an increase in FR during habit formation. These changes in firing rate were accompanied by a change in the correlation of FR with movement parameters. Faster firing neurons showing a decrease in FR across session tended to lose their behavioral correlation across sessions (similar to the direction of the effects of a high dose of cocaine on fast firing neurons in the present study), while slower firing neurons showing increases or no change in FR across sessions tended to increase or maintain their behavioral correlation across session. These results together with the present findings suggest than when responding to an altered environment, whether with drug administration or habitual training, fast and slow firing neurons exhibit different changes in FR as well as in the correlations of their FR with behavioral parameters.

Finding changes in correlations of striatal FR with movement parameters accompanying changes in FR caused by other paradigms and other drugs would further support the suggestion of an association between these two effects. A change in the receptive field of some striatal neurons (48% of the recorded neurons) was observed following intranigral apomorphine injections (Prokopenko et al., 2004). This could be viewed as an altered correlation (in this case with sensory input). Consistent with the above suggestion, firing rate was significantly changed following apomorphine injection. Thus following drugs that cause very different changes in striatal dopamine levels there was a change in FR as well as in motor or sensory correlations of striatal neurons.

The described reduction in firing rate of fast firing neurons following habit forming suggests that reduced striatal firing rate is linked to performing habitual

movements (Tang et al., 2007). A similar reduction in firing rate of forelimb striatal neurons was found following acquisition of a lever pressing task (Carelli et al., 1997). Cocaine induced stereotyped movements share some characteristics with habitual movements, both being more automated and less influenced by ongoing processing of new sensory-motor information. In both the head movement habit formation study and the present study there was a decrease in firing rate of fast firing striatal neurons accompanying more automated movements. The loss of behavioral correlation of these neurons in both cases may further be linked to more automated movements, which possibly require less input from striatal neurons to control their execution.

Following habit formation there is likely less requirement of striatal involvement in ongoing control of the now automated movements, resulting in lower FR of fast firing neurons (which are speculated to be involved in the ongoing control of less automated movements). The altered correlations would reflect this change, since striatal fast firing neurons are now less involved in other brain areas' execution of the habitual movements. On the other hand, cocaine's pharmacological effects resulted in reduced firing rate of fast firing neurons, which may in turn lead to stereotyped movements. The present study found decreases in FR of fast firing neurons as well as altered correlations of their FR with movement at the dose inducing stereotypy, and not at lower doses which did not result in stereotypy. The reduced FR of fast firing neurons following cocaine may be viewed as an abnormal signal, interfering with the ongoing control of more variable and possibly longer sequence movements. This may result in movements that are more automated, shorter, and less responsive to sensorimotor feedback, and furthermore could be reflected in the altered correlations with movement parameters. In both cases striatal

firing rates were altered, either by cocaine's pharmacological effects or by overtraining. In either case, premotor areas receive this altered striatal signal, along with inputs from all the other afferents, and continue to process movements. In both cases, for different reasons, striatal fast firing neurons possibly become less involved in the control of movement parameters (relative to saline or to earlier sessions), reflected in their altered correlations.

The altered correlations found in the studies described above, all following substantial changes in environment or pharmacology (habitual training, cocaine and apomorphine), point to the importance of further examining the mechanisms underlying changes in striatal correlations with sensory and motor parameters and their functional implications.

c. Possible functional differences between slow and fast firing neurons

Lick related neurons recorded in any experiment are part of a large population of striatal lick related neurons, which together with all other striatal neurons, as well as other brain areas, take part in the final output determining movement. This is in accord with the low percentage of licks during which a single lick-related neuron fires. A large population of lick neurons contributes to the final output determining movement. Thus the combined activity of slow and fast firing lick neurons is predicted to influence the final pattern of licking and the combined effects of cocaine on these neurons would be predicted to influence drug-induced stereotyped licking.

At a high dose, the elevation of low striatal firing rates may stimulate motor and premotor areas to increase the rate of movement initiation. As striatal medium spiny

neurons increase slow firing rates, the resulting signals from thalamus to cortex may result in cortical areas initiating movements at a faster rate, a defining characteristic of stereotyped movement. The altered correlations of slow firing neurons even at dose 0 (i.e., with time, and no drug present) as well as at all other doses suggest that these neurons do not encode movement parameters such as duration. Based on the role of the striatum in movement initiation, and the present elevation of their FR by cocaine, it is possible that slow firing neurons play a role in movement initiation.

The results of the correlation analysis with behavioral parameters suggest that fast firing neurons may have a stronger relation to movement parameters. A reduction in firing rate of these neurons may interfere with making longer movements, since execution of these movements may require more guidance and feedback related to their different parameters. A reduced FR of fast firing neurons related to different body parts would thus be predicted to result in a reduced likelihood of long-sequence or exploratory movements. Additionally, a reduced FR of fast firing neurons related to licking may result in a reduced likelihood of long licks, consistent with the present behavioral results. The reduced FR of fast firing neurons may be viewed as an abnormal signal. Thus the usual scaling of movement parameters provided by fast firing neurons is abnormal under a high dose of cocaine. While at this point a complete picture of how these effects translate into shorter movements is not clear yet and requires further studies, the present results provide an important portion of this picture.

It should be noted that while the present data were analyzed and discussed using dichotomous terms of slow versus fast firing neurons, it is possible that neurons vary on a continuum with a range of biological and functional differences, from the slowest to

fastest firing neurons. The HLM and regression analysis results support the possibility of a continuum (e.g., in figure 6D, especially for the slower neurons: the lower T1FR, the more T2FR was elevated). Studies examining differences between these neurons, as described above, would be required to discern whether these are two groups of neurons or a continuum. For ease of discussing the results they are described here using the terms slow versus fast firing neurons.

Rate dependent effects could result from the drug affecting neurons with different characteristics. Alternatively, the drug effects may depend on the actual T1FR, not on the neuron in question (i.e., it is not the characteristics of the neuron but the FR that matters), or both of the above could be involved. Finding between but not within neuron rate dependent effects in the present study supports the first alternative, i.e., that the rate dependent effect result from the drug affecting neurons with different characteristics, and are not due to differences in firing rate per se.

In the ANCOVA results, there were no dose*T1FR group interaction effects, indicating that the drug effects on FR of the low and high T1FR matched pairs of slow firing neurons were similar across doses for slow firing neurons, and the same is true for fast firing neurons. As can be seen in figure 8, for slow firing neurons cocaine increased firing of all matched pairs relative to the zero dose group (The smaller decreases of high T1FR matched pairs are in effect a higher FR relative to dose zero). For fast firing neurons at 20mg/kg cocaine decreased firing of all matched pairs. While low and high firing matched pairs behave differently at zero dose, the drug effects on all matched pairs are in the same direction (increases) for slow firing neurons, and in the same direction

(decreases) for fast firing neurons, regardless of T1FR of the individual matched pair. Furthermore, the lack of interaction in the ANCOVA results indicates that there was no significant difference in the magnitude of the drug effects based on T1FR.

Thus, all the above, in addition to the lack of significant within neuron effects in the HLM results, support the interpretation that the present rate dependent effect result from cocaine exerting different effects on neurons with different characteristics. Yet it should be noted that further analysis of data from a study examining the effects of cocaine on head movement-related neurons (Pederson et al., 1997) revealed both between and within neuron rate-dependent effects (Pawlak et al., 2001). More studies are required to further resolve this issue.

The present study was not designed to assess the possible mechanisms that may explain the different drug effects between slow and fast firing neurons. The following possible scenarios could be suggested, yet at this point they are merely speculative. Further studies would be necessary to examine them.

Activation of D1 and D2 receptors (the terms D1 and D2 are used here to refer to D1-like and D2-like receptors) has been shown to exert different effects on striatal firing. Activation of D1 receptors was shown to have predominantly excitatory effects on medium spiny neurons, while activation of D2 receptors results in predominantly inhibitory effects (Wickens, 1990; Horvitz, 2002; O'Donnell, 2003; Nicola et al., 2004; Surmeier et al., 2007). If slow firing neurons have a larger proportion of D1 versus D2 receptors, while fast firing neurons have a larger proportion of D2 relative to D1 receptors, this would explain how an increase in striatal dopamine level results in an

increase in FR of slow firing neurons, and a decrease in FR of fast firing neurons. The larger proportion of D2 receptors affecting fast firing neurons could be post- and/or pre-synaptic (O'Donnell, 2003; Bamford et al., 2004; Surmeier et al., 2007).

Medium spiny neurons have been shown to move between two membrane potential ranges, referred to as "down" and "up" states (Wilson and Groves, 1981; Wilson and Kawaguchi, 1996). At rest, neurons are in the down-state, near -80 mV, and do not generate action potentials. In response to temporally coherent, convergent excitatory cortical input, neurons move to a more depolarized membrane potential (the upstate), near -55 mV, staying at that state for hundreds of milliseconds or seconds, during which further cortical input can trigger firing. D1 receptors are suggested to sustain the up-state (Nicola et al., 2000; O'Donnell, 2003). Thus their activation following cocaine administration would result in an increased duration of the up-state, and thus the potential for increased FR. If slow firing neurons have more D1 receptors relative to fast firing neurons (consistent with the suggestion above) this would result in an increase in the duration of the up-state in these neurons following cocaine administration, possibly explaining their increased firing rate post-drug.

Additionally, based on their FR, fast firing neurons spend more time in the up state relative to slow firing neurons. If following cocaine the up state is maintained for a longer duration, this may have a smaller effect on fast firing neurons, which are already spending a larger percent of the time in the upstate, yet would cause slow firing neurons to spend relatively more time post-drug in the up state, thus increasing their FR.

In support of the above suggestion of a different distribution of D1 and D2 receptors on slow versus fast firing neurons, the relative amounts of D1 and D2 receptors

have been shown to vary among different striatal medium spiny neurons. Striato-pallidal GABAergic neurons containing enkephalin were found to express relatively more D2-like receptor, whereas striato-nigral GABAergic neurons containing dynorphin and substance P were found to express relatively more D1-like receptors (Gerfen et al., 1990, 1992; Le Moine and Bloch, 1995; Yung et al., 1995). Applying antidromic stimulation from these nuclei while recording from striatal slow and fast firing neurons could help examine this suggested difference between slow and fast firing striatal neurons.

d. Relation of the present data to the hypothesis of dopamine increasing striatal signal to noise ratio

The present results seem 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). Yet, as will be explained below, the present rate dependent effects may be conceptually different from dopamine's effects on weak and strong cortical inputs referred to by the increase in signal to noise theory.

Rolls et al. (1984) suggested that dopamine increases the signal to noise ratio in the striatum following recording of lick-related as well as other striatal neurons in behaving monkeys. Iontophoretically applied DA decreased spontaneous firing rate and movement related firing rate of striatal neurons by a similar magnitude, resulting in an increase in the ratio of movement-related firing rate to spontaneous firing rate.

In a series of studies examining the effects of amphetamine as well as iontophoreticaly applied dopamine Rebec et al. found dopamine and amphetamine to

excite striatal neurons related to movement and inhibit striatal neurons not related to movement, suggesting that dopamine increases the signal to noise ratio of striatal neural activity (Haracz et al., 1993; Pierce and Rebec, 1995; Rebec et al., 1997; for review see Rebec, 2000, 2006). Yet while recording from freely moving animals, these studies lack adequate clamping of behavior. In some no clamping was done (e.g., Rebec et al., 1997). In others (e.g., Haracz et al., 1993) behavior was clamped by matching 8 second periods for which behaviors received similar rating scores pre- and post-drug, providing a very crude matching of behaviors, which does not take into consideration the specific movement the recorded neurons are correlated with. In order to minimize the possibility that the examined neural effects were secondary to the behavioral effects of the drug, clamping must focus on the specific behavior correlated with the recorded neural activity, matching movements with as similar parameters as possible and analyzing the recorded activity during time windows relevant to the related firing and movement. Thus, rather than pharmacological effects on firing, differences in sensorimotor processing of drug-induced movements, which were not adequately controlled for, could instead account for the reported larger drug effects on movement related neurons.

Based on reviewing a large body of literature Nicola et al. (2000, 2004) suggest that dopamine, acting on D1 receptors, may increase firing rates of neurons receiving highly convergent (presumably most important) excitatory input while decreasing firing rates of neurons receiving less temporally coherent inputs. Dopamine would thus facilitate selection among competing neurons by enhancing the contrast between stronger and weaker excitations.

While the results of the present study seem inconsistent with the above hypothesis, since the fastest firing rates (presumably reflecting strong cortical input) were the ones most reduced by cocaine, it is important to note that the studies suggesting an increase in signal to noise ratio looked at movement-related firing versus spontaneous or non-movement related firing, while the present study examined drug effects on fast versus slow movement-related firing. Dopamine's effects on slow movement-related firing may be different from its effects on spontaneous or non-movement-related firing, even if they are all of a slow rate. Additionally, there is no reason to regard slow movement-related firing as "noise". The present results suggest that different characteristics of slow and fast firing neurons may cause dopamine to exert different effects on their firing rates. Based on this explanation, cocaine's increase of movement-related firing of slow firing neurons does not suggest an increase in spontaneous firing or firing of neurons related to movements not exhibited at the moment, which are also slow. Thus the present conclusions regarding dopamine's rate dependent effects on movement-related firing are conceptually different from dopamine's effects on movement-related firing versus spontaneous or non-movement related firing.

Prokopenko et al. (2004) examined the effects of microinjected intranigral apomorphine (to decrease striatal dopamine release) on baseline versus somatosensory-evoked firing rate of striatal neurons. They found no consistent change post drug in the evoked to baseline firing rate ratio, arguing against the hypothesis that dopamine increases the signal to noise ratio in the striatum. Further studies examining the effects of increased striatal dopamine on movement-related versus spontaneous firing would be required to resolve this. Such studies would have to apply adequate control necessary for

matching behavior pre- and post-drug. Baseline firing could be assessed during periods with no movement, but this would not exclude possible unobserved changes post-drug, e.g., in tongue movements inside the mouth, or in muscle tension. Another possible strategy, which was applied by Prokopenko et al. (2004) is to record from neurons with only cutaneous responsiveness and little or no correlation to movement, while ensuring no somatosensory stimulation when recording baseline firing.

e. Potential involvement of other brain areas and neurotransmitters in the present drug effects

The effects found in the present study are the result of systemically administered cocaine. Thus, while they primarily reflect the effects of an increase in dopamine acting upon striatal neurons, they also reflect the consequence of all peripheral and central effects of the drug.

Systemic cocaine was found to have a rate dependent effect on the response of ventral posterior medial (VPM) thalamic neurons to vibrissa stimulation (Rutter et al., 1998, 2005). Cocaine increased the response of neurons with low control firing rate, and decreased the response of neurons with high control firing rates. Cocaine's rate dependent effects on thalamic neurons could have contributed to the rate dependent effects observed in the present study in the striatum. Nonetheless, it is not clear how much impact would be exerted across di- or trisynaptic routes to the striatum.

Furthermore, cocaine was shown to modify firing rate of cortical neurons (possibly via enhancement of norepinephrine and/or serotonin levels). High doses of systemic cocaine suppressed both evoked and spontaneous activity of cortical

somatosensory neurons, while low doses selectively enhanced stimulus-evoked discharges (Jimenez-Rivera and Waterhouse, 1991). The facilitating effects were also observed following iontophoretically applied cocaine into the cortex. Additionally, systemic cocaine enhanced long-latency responses of barrel field cortical neurons to vibrissae stimulation in anesthetized rats (Bekavac and Waterhouse, 1995). At least some effects of systemic cocaine on cortical firing were blocked by a selective noradrenergic toxin, suggesting a role for norepinephrine in these effects (Waterhouse et al., 1991). Potential changes in cortical firing post-drug likely contribute to the observed changes in striatal firing. Striatal firing may be also influenced by the enhancement in serotonin levels following cocaine. These effects could me mediated via striatal cholinergic interneurons which respond to serotonin (Blomeley and Bracci, 2005), or via changes in firing in the Dorsal Raphe Nucleus, which can influence striatal firing rates (Rebec and Curtic, 1983).

Employing local microiontophoresis of dopamine agonists directly into the striatum would help to better determine the effects of an increase in striatal dopamine on striatal firing rates. Examining the impact of locally applied selective D1 and D2 antagonists on the effects of locally or systemically applied cocaine can further discriminate between the drug-effects mediated by the elevation in striatal dopamine versus other effects, as well as between the effects mediated by different dopamine receptors.

Finding effects consistent with the present study following microinjected intranigral apomorphine (Prokopenko et al., 2004) lends support to the likelihood that the present results are at least to a large part the consequence of an increase in striatal

dopamine levels. The strong evidence from the literature described earlier, pointing to the causal involvement of an increase in striatal dopamine in the induction of stereotyped behavior, and specifically in oral stereotypies, further support the interpretation of the present finding focusing on involvement of striatal dopamine, yet they do not eliminate possible contributions of other central and peripheral influences on the present results.

The local anesthetic effects of cocaine are not likely to explain the present results, since they involve dosages 200-fold higher than the ones employed here (Wise, 1984; Gawin, 1991; Kuhar et al.1991; Ritz et al.1992; Wilcox et al., 1999). Comparing the present results to the effects of procaine, a local anesthetic with chemical similarities to cocaine would verify this (see Bekavac and Waterhouse, 1995).

In summary, the present study replicated the classic behavioral effects attributed to stimulant actions in the striatum while identifying the changes in striatal neural activity accompanying these effects. Cocaine was found to exert dose-dependent effects that strongly depended on pre-drug firing rate. At the middle and high doses relative to the zero dose group the average firing rates of slow firing neurons were increased by cocaine, resulting from a general elevation of movement-related firing rates. By 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. Importantly, this is the same dose at which the drug induced stereotyped licking. These 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 more global, longer movements.

While aiming to identify cocaine's effects on striatal firing, the present study revealed two additional interesting findings with potentially important implications to the understanding of striatal processing: 1) Changes in behavioral correlations of striatal neurons were demonstrated over time with no drug present (for slow firing neurons), and following cocaine administration. 2) Differences were observed between slow and fast firing neurons in drug effects on FR, in the strength of their behavioral correlations and with respect to changes in their behavioral correlations. These differences suggest that fast firing neurons may be more strongly involved in the processing of movement parameters. Further studies are required to examine the implications of these findings.

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