AMPHETAMINE'S DOSE-DEPENDENT EFFECTS ON DORSOLATERAL STRIATUM SENSORIMOTOR NEURON FIRING

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ABSTRACT OF THE THESIS

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Dorsolateral striatum neurons were extracellularly recorded in awake behaving rats to examine amphetamine’s effects. On one hand, amphetamine has been shown to elicit motoric changes through increasing neurotransmitters such as dopamine and serotonin in the central nervous system. On the other hand, dorsolateral striatum is highly involved in motoric function and contains abundant neurotransmitter transporters that amphetamine could act upon. Therefore, it has been hypothesized that dorsolateral striatum medium spiny neurons play a role in amphetamine’s effects on motoric behavior. Although many studies have supported a role of the striatum, the involvement of its individual neurons has not been adequately characterized. Therefore, the neuronal activities of single neurons that correlate with vertical head movements in dorsolateral striatum were simultaneously recorded with head movement behavior before and after acute amphetamine injection to test the hypothesis. Behaviorally, it was observed

that amphetamine induced head movements across all doses administered. Lower doses (1mg/kg and 2 mg/kg) induced more numbers of longer movements than the higher dose (4 mg/kg). Neuronally, firing of individual head movement neurons during similar head movements defined by direction, distance, duration, velocity and apex were compared before and after administration of amphetamine. Analysis revealed that the change of firing rate induced by amphetamine was co-determined by the dose administered and the baseline firing rate of the neuron. More specifically, for all doses administered, amphetamine increased the firing rate of the slower firing neurons, but decreased the firing rate of the faster firing neurons. The magnitudes of the enhancement and reduction were greater at lower doses (1mg/kg and 2 mg/kg), but were less pronounced at the high dose (4mg/kg) of amphetamine. The parallel changes of behavior and firing pattern of dorsolateral striatum neurons support the hypothesis that dorsolateral striatum neurons participate in mediating the motor behavioral effects induced by amphetamine.

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1. Introduction

1.1 Amphetamine's drug effects

1.1.1 Molecular Mechanism

Amphetamine was first synthesized in 1887. It has various derivatives such as methylphenethylamine, phenylisopropylamine, and 2-amino-1-phenylpropane. These derivatives are similar in structure, and induce similar behavioral effects, with different efficacies (Sulzer et al., 2005).

Amphetamine increases synaptic levels of various neurotransmitters in numerous brain regions, including dopamine, serotonin and norepinephrine (Berman et al., 2009). The effects of amphetamine on reward circuitry, locomotor activity and stereotypies are thought to be mediated primarily by its actions upon the above mentioned neurotransmitters in the central nervous system (Fleckenstein et al., 2007; Sulzer et al., 2005).

One of the most studied target sites of amphetamine is the dopamine transporter (DAT), a presynaptic trans-membrane protein (Torres et al., 2003). DATs are expressed in various brain areas, including ventral mesencephalon, medial forebrain bundle, and dorsal and ventral striatum (Ciliax et al., 1995; Freed et al., 1995). Under normal conditions, DATs transport extracellular dopamine back into neurons against the dopamine gradient. Amphetamine is a substrate for DAT. It competitively binds to the extracellular component of the DAT and is transported inside the cell. This process changes the conformation of the DAT, which results

in intracellular dopamine efflux through DAT. Upon amphetamine administration, a rapid DAT internalization is observed. This is another factor that contributes to the reduced dopamine reuptake (Schmitt & Reith, 2010; Williams & Galli, 2006). Besides plasma membrane transporters, amphetamine also affects synaptic vesicles. Amphetamine acts as a weak base when it is inside the cell, which induces the release of dopamine from synaptic vesicles and raises cytosolic dopamine to a level that favors its passive diffusion outward through DATs (Fleckenstein et al., 2007; Kahlig et al., 2005).

Via similar mechanisms, amphetamine affects serotonin transporters (SERT) and induces elevation of extracellular serotonin levels. SERT is also widely distributed in the central nervous system, including caudate-putamen, amygdaloid complex, cortical areas, substantia nigra, ventral pallidum, etc (Dawson & Wamsley, 1983; Sur et al., 1996).

It is noteworthy that the brain area of interest, dorsolateral striatum, has co-localization of DAT and SERT. Its physiology could be modulated by amphetamine via both dopamine and serotonin, which will be discussed in detail in the following sections.

1.1.2 Behavioral Effects

Amphetamine has been widely prescribed for patients diagnosed with narcolepsy, obesity or ADHD. Its effects include increased attention, reduced fatigue, and euphoria. Such psychostimulants also increase activity in general and can induce repetitive motor activity that lacks variability, i.e. stereotypical behaviors

(Ellinwood & Balster, 1974; Hall et al., 2008). Low doses produce hyperactivity, but not stereotypical behavior, as might be seen at a higher dose (Sharp et al., 1987). More specifically, as theorized by Lyon and Robbins (1975), with increasing doses of amphetamine: complex behavioral chains and behaviors that require long pauses will be reduced, and as a result, behaviors with shorter duration will dominate; with further increase in dose, shorter and shorter responses will display (Lyon & Robbins, 1975).

Experimental evidence suggests that both dopamine and serotonin are involved in amphetamine induced stereotypical behavior. Lesioning the substantia nigra to deplete striatal dopamine attenuated both locomotion and stereotypical behaviors induced by amphetamine (Creese & Iversen, 1975). Also, microinjection of amphetamine into striatum induced stereotypical behavior (Fog & Pakkenberg, 1971). On the other hand, serotonin appears to counteract dopamine's effect of potentiating stereotypical behavior. Para-chlorophenylalanine, the inhibitor of the rate limiting enzyme of serotonin synthesis, decreased stereotypical behavior induced by high dose amphetamine and increased locomotion (Segal, 1976). Meanwhile, animals with raphe nuclei lesions displayed stereotyped behavior when low dose amphetamine was administered (Lucki & Harvey, 1979). The above evidence implies interactions of dopamine and serotonin might be involved in the modulation of amphetamine induced changes in behavior.

1.2 Dorsolateral Striatum

1.2.1 Efferent and Afferent Connections

The dorsolateral striatum is one of the brain areas with dense DAT and SERT expression. As stated above, these two neurotransmitter transporters are the target sites of amphetamine. Also, the dorsolateral striatum is primarily involved in sensorimotor function, which amphetamine administration disturbs. Therefore, it is rational to suggest that amphetamine affects sensorimotor function through at least in part, modulating neuronal activity in dorsolateral striatum.

The dorsolateral striatum receives afferents from both primary motor (M1) and primary somatosensory cortices (S1). The afferents from both cortices are mainly ipsilateral (about 90%). Different body parts are represented topographically in S1 and M1. This topographic map is maintained in downstream areas, including striatum, pallidum, substantia nigra, and thalamus (Alexander et al., 1986; Deniau et al., 1996; McGeorge & Faull, 1989). M1 and S1 terminations that correspond to a single body part converge on clusters of dorsolateral striatal medium spiny neurons. The clusters of DLS neurons, located in matrisomes, respond during movement or tactile stimulation of the body part that is defined by its cortical inputs (Flaherty & Graybiel, 1991; Flaherty & Graybiel, 1994). Although clusters of neurons representing individual body parts are intermingled, correspondence to more than one different body part is seldom observed at the level of the cluster or the single neuron (Cho & West, 1997). Dorsolateral striatum contains a patchy somatotopy with neuronal representation of hind limb

dorsally and oral cavity ventrally (Cho & West, 1997). This somatotopy is considerably preserved along the rostrocuadal axis (Flaherty & Graybiel, 1991).

Internal and external segments of globus pallidus and substantia nigra (Beckstead & Cruz, 1986; Flaherty & Graybiel, 1993) receive innervation from the dorsolateral striatum. These sites, being the output stage of basal ganglia, are implicated in action selection and activation (Graybiel, 1997). The internal segment of globus pallidus further sends projections to the somatomotor-responsive segment of the ventrolateral nucleus of thalamus (VLo). VLo projects to supplemental motor and premotor areas, as well as M1 (Hoover & Strick, 1999) completing a “motor loop” (Alexander et al., 1986; Inase & Tanji, 1995; Kuo & Carpenter, 1973; Parent & Hazrati, 1995) .

1.2.2 Physiology

In agreement with anatomical connections, experimental evidence suggests that manipulations of striatum can modify motor behaviors. Microstimulation in dorsolateral striatum evokes similar movements to those with which the adjacent neurons are tuned (Alexander & DeLong, 1985b). Furthermore, the amplitude, speed and acceleration of the movement increase with increasing amplitude of microstimulation (Alexander & DeLong, 1985a).

Ample studies suggest that motor function at the stage of striatum is at least partially mediated via dopamine. Microinjection of dopamine agonists or amphetamine into lateral striatum induces stereotypical behavior (Kelley et al.,

1988; Sharp et al., 1987). Conversely, including a dopamine-β-hydroxylase inhibitor into the diet will reduce the locomotor stimulant effects induced by amphetamine (Thornburg & Moore, 1973).

Serotonin might also be involved in motoric behavior. Bilateral microinjection of serotonin into ventrolateral striatum induced stereotyped orofacial behaviors. Dopamine depletion attenuated those behavioral changes induced by serotonin, indicating the above mentioned effects might be mediated through interactions with dopamine (Yeghiayan et al., 1997). Moreover, microdialysis in striatum revealed elevation of serotonin in response to amphetamine administration (Hernandez et al., 1987; Kuczenski et al., 1995), and the change of serotonin level paralleled the emergence of stereotypical behavior (Kuczenski & Segal, 1989).

The somatotopy of DLS is identified by single neuron recordings in rat (Carelli & West, 1991; Cho & West, 1997; Mittler et al., 1994; West et al., 1990), cat (Malach & Graybiel, 1986) and primate (Flaherty & Graybiel, 1991; Kimura, 1990). The dorsolateral striatum in rodents can be considered the homolog of the primate putamen (Carelli and West, 1991). However, the correspondence is not perfect. The striatum is a continuous structure both in primates and in rodents. The division of caudate and putamen is structural (separated by the internal capsule, which is scattered in rodents) rather than functional for the most part. Firstly, neurons that receive M1 and S1 inputs reside in both putamen and caudate (Lidsky et al., 1985; Selemon & Goldman-Rakic, 1985). Secondly, there are two classes of neurons identified in primate striatum, type I with relatively

high spontaneous discharge rate and type II with low spontaneous discharge rate. Type II neurons are movement related neurons. More specifically, Type IIa neurons fire preceding a specific movement or movement sequence, but cease firing during the movement. On the other hand, type IIb neurons fire during a specific movement (Kimura, 1986; Kimura, 1990). Type IIa neurons are in the minority in primates and have not been identified in rodents. On the contrary, type IIb neurons that discharge during active movement, passive manipulation, and tactile stimulation of specific body parts, including tongue, face, vibrissa, forelimb, hindlimb, and neck, are well documented in both primates (Crutcher & DeLong, 1984; Liles, 1985) and rodents (Cho & West, 1997) and comprise the majority of neurons recorded in awake animals.

Interestingly, striatal movement related neurons are tuned not only to the movement of a specific body part, but also to how the movement is executed. Individual neurons preferentially fire to some aspect of the movement of the correlated body part. For instance, in rodents, some head movement neurons, which are tuned to head movement distance, may fire more intensively when the animal emits a short head movement and other head movement neurons may fire more intensively when the animal emits a longer head movement (Pawlak et al., 2010; C. Tang et al., 2007). In either case, neurons fire preferentially during movement in one direction. Similar results are observed in primates. Single neurons in primate putamen are correlated with the direction and/or force required to complete an arm movement. It is noteworthy that in putamen a lower percentage of neurons are correlated to the status of a specific muscle

(extension or flexion) in comparison to motor cortex and supplementary motor cortex. On the contrary, a larger percentage of neurons in primate putamen are tuned to the direction of the movement (Crutcher & DeLong, 1984; Crutcher & Alexander, 1990). This dissociation from M1 indicates that the putamen might be processing information about movement in a more abstract form, potentially processing S1 and M1 signals and projecting to premotor areas to help guide ongoing movements.

The current study focused on amphetamine's acute effects on motoric function. Neuronal activity from neurons that correlate with vertical head movements in dorsolateral striatum were recorded with and without the influence of amphetamine. In order to assess how different doses of amphetamine influence head movement behavior, and more importantly, the firing rate of head movement neurons, head movements were categorized according to their direction, distance, duration, velocity and apex. Neuronal firing during similar head movements before and after administration of amphetamine was compared. Careful examination of the results showed that different doses of amphetamine differentially modified the neuronal firing rate of head movement neurons depending on their baseline firing rate.

2. Methods

2.1 Subjects

A total of 28 Male Long-Evans rats were trained and recorded in the treadmill task. Animals were kept on an 11:30 a.m./11:30 p.m. light/dark cycle with ad lib water and restricted food access. Body weight of each animal was kept at 330±3 g prior to head stage implantation surgery. A single session of sensorimotor exam followed by treadmill training were conducted a week after the surgery. During treadmill training, animals received an intraperitoneal (i.p.) dose of amphetamine, 0 mg/kg, 1 mg/kg, 2 mg/kg, or 4 mg/kg according to their randomly assigned dose group.

2.2 Surgery

Animals were initially anaesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg). Anesthesia was maintained by ketamine hydrochloride (60 mg/kg, i.p.) as necessary. Atropine methyl nitrate (50 mg/kg, i.p.) and penicillin G (75000U/0.25ml, i.m.) were also administered before surgery.

Animals in 1mg/kg and 2 mg/kg groups and six animals in 4 mg/kg group were implanted with a microdrive base (Crist Instrument Co., Inc.), 0.2 anteroposteriorally and 3.5 mediolaterally from bregma. Two connector strips (ITT Cannon, Santa Ana, CA) were cemented parallel to the mediolateral axis of the animal and 1.0 cm above the skull surface, so that the measured head

movements would be comparable between animals. Before the experimental session, a microdrive housing a tungsten microelectrode (1–10 MΩ; FHC Inc., Bowdoin, ME) was mounted onto the microdrive base. An electrode track profile was created (Carelli and West, 1991) while the microelectrode was lowering to ensure that only striatal neurons were recorded. Once a head movement neuron was encountered, a sensorimotor exam was performed (see below). If the neuron was verified as a head movement neuron, the treadmill training session would begin.

All animals in 0 mg/kg (saline) group and eight animals in 4 mg/kg group were implanted with microwire arrays. The microwire array consisted of twelve (2x6) Teflon-insulated microwires (California Fine Wire, Grove City, CA, USA, Spacing 400 micrometer wire center to wire center) and a connector strip which connected the microwires and harness during electronic recording. Each microwire array was targeted at the right dorsolateral striatum, 1.5 mm to -0.4 mm from bregma on the anterior-posterior axis, 3.2 mm to 4.2 mm from bregma on the medial-lateral axis, and between 3.5 mm-3.7 mm on the dorsal-ventral axis from skull level, where clusters of head movement neurons are densely located (Cho & West, 1997; Pawlak et al., 2010). The connector strip was mounted on the center of skull with dental cement. The top of the connector strip was 1.0 cm above skull level in every animal so that measurement of movements and movement-related firing would be comparable between subjects. A ground wire was implanted in the left hemisphere to minimize potential electrical noise. During recording, the connector strip was connected to a harness, sending

neuronal signals through a pre-amp (10X gain), filter (500-700X gain, roll off 1.5 dB/octave at 1 kHz and -6 dB/octave at 11 kHz), and recorded by a computer with a 50 Hz sampling frequency.

Parameters of waveforms of striatal neurons were similar whether recorded by stainless steel microwires or tungsten microelectrodes using the microdrive; therefore, data collected from both types of electrode were pooled.

2.3 Experimental Procedure

2.3.1 Determination of body part correlation

One to four hours before treadmill training (microdrive recording) or one day prior to treadmill training (microwire array recording), animals were placed in the training chamber (7'x12'x5' Plexiglas walls) with the harness plugged in. Electrical signals recorded from the animal were processed through a filter, a pre-amp and monitored both on oscilloscopes and with a pair of headphones. A complete sensorimotor exam was performed on the microelectrode or every microwire that showed neuronal activity (signal to noise ratio greater than 3:1) to determine possible correlations between phasic neuronal firing and sensorimotor activity of a specific body part. Neuronal activity was carefully inspected during the following three situations, (1) voluntary body movement initiated by the animal; (2) passive movement of body parts manipulated by the experimenter; and (3) tactile stimulation of each body part. Correlation was determined if substantial modulation of firing was observed (primarily by audio output from the headphones) during the three conditions described above. Head movement

correlated neurons were documented and these alone would later be recorded during treadmill training.

2.3.2 Treadmill training

Animals were connected to the harness and placed in the training chamber with the treadmill belt as the floor. Two bright LEDs facing forward on the harness were separated by 9 mm dorsoventrally. Direction, distance duration, and apex of head movements were derived by tracking the LEDs (the positions of the LEDs were digitized to a pair of numbers representing x, y coordinates, sampled and stored every 16.7 ms). A custom designed computer program (based on software provided by Datawave Technology, Longmont, CO) was used to record neuronal activity, track head movements and control the treadmill. The treadmill was activated on a 30 sec on/20 sec off cycle. Animals were forced to walk on the treadmill to avoid being pushed to the back wall of the chamber. The treadmill training session lasted for four hours. At the start of the first hour, which servd as the pre-drug baseline, referred to as T1, animals received a saline injection. One hour into the experiment, the neuronal recording and treadmill control were paused. The animals were removed from the chamber briefly and injected with saline (dose 0) or different doses of amphetamine according to the assigned dose group. After the injection, animals were placed back to the chamber. Neuronal recording and the treadmill cycle were resumed. The session continued for another 3 hours, in order to observe the peak drug effects (within an hour

after the injection; this second hour of the experiment was referred to as T2) and to allow for reversal during the last hour (typically hour 4, referred to as T3), beyond which single unit recordings could not be maintained using moveable microelectrodes.

2.4 Data Analysis

2.4.1 Head Movement Profile

X and Y coordinates of LED positions were analyzed with respect to their timestamps. Each instance in which LED coordinates were continuous in time and space within the same direction was determined as a single movement. Movement parameters including duration, distance, valley, apex, velocity, slant, curvature and tilt were calculated for every recorded head movement. The above mentioned analysis was performed by MBW, a custom-designed program written by Martin B. Wolske (Pawlak et al., 2010; C. Tang et al., 2007).

2.4.2 Neuronal Signal

Neuronal data were played back from the entire session and sorted according to the shape of the waveform offline (Datawave Technology, CO). Sorting criteria involved several waveform parameters: peak time, peak amplitude, spike time, spike height, principle components and four custom cursors. Neuronal signals were considered recorded from only one single neuron when the following criteria were met: (1) Signals exhibited similar shapes, and similar waveform parameters. (2) Auto-correlation revealed a minimum inter spike interval (ISI) ≥ 2 ms (refractory period). (3) Cross-correlation was performed if several waveform

profiles were detected in the recording from one microwire. If cross-correlation revealed at least one instance of ISI smaller than or equal to 1ms, the signal was considered to originate from different neurons. Otherwise, the signals were combined and considered originating from one neuron. The purpose of spike sorting was to eliminate electronic noise and separate possible multiple units recorded on one microwire. See Figure 1 for an example.

2.4.3 Head movement Correlates

A raster and PETH (peri-event time histogram) were generated for every identified single neuron around the initiation both of upward head movements and of downward head movements. Trials on the raster plot were sorted according to the duration of head movements. A neuron had to meet the following criteria to be identified as a head movement neuron: (1) neuron showed head movement correlation during the sensorimotor exam; and (2) neuron showed significant modulation on a PETH generated around active head movement. The following criteria had to be met for a neuron to be identified as sensitive to head movement direction (up or down): (1) neuron must be identified as a head movement neuron by the above criteria; and (2) neuron was differentially modulated around upward head movement versus downward head movement (comparing PETHs around upward movements vs. downward movements). If the above criteria were not met, the neuron was excluded from further analysis. Figures 2 and 3 show example neurons that were activated during down movements or up movements respectively.

2.4.4 Amphetamine's effect on DLS neuron firing

Once a dorsolateral striatum neuron was determined to be correlated with directional head movement, the drug effect on firing was compared during similar movements (matched pairs) before and after drug administration. The rationale for assessing neural firing only during similar movements is to provide "motor equivalence". It is well established that psychostimulants, including amphetamine, change motor profiles. Comparing firing rate across similar movements eliminates the possibility that any firing rate changes might be due, for example, to feedback from altered movement parameters rather than amphetamine.

Motor equivalence was achieved by constructing matched pairs. Any curved, slanted, horizontal, or crooked movements were filtered out. Specifically, each movement had to be longer than 4 mm in distance, 30 ms in duration, and less than 18.2 degree displacement in the horizontal direction, leaving only straight, vertical head movements entered into neural analyses. The apex, duration, and distance of the latter movements were used to categorize each individual movement. Each parameter was divided in to 5 levels. Apex: 125mm-144mm, 145mm-164mm, 165-184mm, 185-204mm, 205mm-255mm (catch all). Duration: 33 ms-69 ms (designated as 67), 70 ms- 135 ms (133), 136 ms- 202 ms (200), 203 ms-269 ms (267), 270 ms and longer (catch all, 270). Distance: 4 mm-15 mm (15), 16 mm- 27 mm (27), 28 mm -39 mm (39), 40 mm-51 mm (51), 51 mm and longer (catch all, 52). The apex, duration, and distance levels define 125 (5x5x5) movement categories, where each movement fits into only one category. Movements that fell into the same category were considered similar movements.

The firing rates of all movements within one category were computed (# spikes / duration of that movement) and averaged, resulting in one average firing rate for each category. Three-dimensional matrices of firing rates were constructed with apex, duration, and distance levels comprising the three dimensions. One matrix was constructed for each time period. Movements in the same category before (T1) and after (T2) drug administration were designated as matched pairs. Average firing rates were compared in matched pairs only when ≥ five movements in that category were observed both pre and post drug administration. All assessments of drug effects on firing utilized matched pairs. Similarly, reversal was assessed by comparing the firing rates between matched pairs of T1 and T3. All matched pairs were obtained from the treadmill on condition because the treadmill off condition contained too few head movements in too few categories.

The matched pair data were considered hierarchical in nature because multiple observations, in this case the different matched pairs, were nested within neurons, in turn nested within subjects, in this case the rats. The use of standard regression or ANOVA models for hierarchical data was inappropriate because it leads to inflated Type I error rates. Therefore, a hierarchical linear model (HLM) approach was used to analyze the matched pair data because HLM is designed to take into account the hierarchical structure of data. For a more detailed exposition of the application of HLM to matched pair neural data, please see (Pawlak et al., 2010).

The matched pair data have a two-level hierarchy, in which level one consisted of the matched pairs of individual neurons and level two consisted of the individual neurons. In the HLM for the matched pair data, the level one portion will be referred to as the “within-neuron” model and the level two portion will be referred to as the “between-neuron” model.

The within-neuron model consisted of the firing rates of the individual matched pairs. For the within-neuron model, T2 firing rates were regressed on centered T1 firing rates, with a separate regression model computed for each individual neuron. Centered T1 firing rates were calculated by subtracting the average pre drug firing rate of all matched pairs for a given individual neuron from the pre drug firing rates of all the matched pairs of that neuron. This transformation rendered the intercept of the regression as the average T2 firing rate, which was advantageous because it allowed the level two portion of the HLM to model the average T2 firing rate as a function of average T1 firing rate across all neurons. The level one portion of the HLM model was expressed mathematically as:

$$T2FR_{ij} = \beta_{0j} + \beta_{1j}(T1FR)_{ij} + e_{ij}, \quad (1)$$

where $T2FR_{ij}$ is the T2 firing rate of the $_i$th matched pair for the $_j$th neuron, T1FR is the T1 firing rate of the $_i$th matched pair for the $_j$th neuron, β_{0j} is the centered intercept as well as the average T2FR value for the $_j$th neuron, β_{1j} is the slope of the regression of T2FR on centered T1FR of all the matched pairs for the $_j$th neuron, and e_{ij} is the residual error term for the $_j$th neuron.

The between-neuron portion of the HLM model utilizeed the parameters (β_{0j} and β_{1j}) generated from the within-neuron portion of the HLM model as dependent variables in separate regression equations that contain predictors associated with the neurons (not the matched pairs). For the current HLM model, the between-neuron level predictor variables were: dose, dose^2, average T1FR of each neuron, the interaction of dose by average T1FR of each neuron, and the interaction of dose^2 by average T1FR of each neuron. The dose, dose^2, and average T1FR variables were grand mean centered, which was done by subtracting the mean value of a given variable from all the individual values of that variable. The advantage of grand mean centering is that it reduces the effects of multicollinearity in the construction of the between-neuron level interaction terms. The between-neuron model was expressed mathematically as:

$$\beta_{0j} = \gamma_{00} + \gamma_{01}(\text{dose})_j + \gamma_{02}(\text{average T1FR})_j + \gamma_{03}(\text{dose}^2)_j + \gamma_{04}(\text{dose} * \text{average T1FR})_j + \gamma_{05}(\text{dose}^2 * \text{average T1FR})_j + \mu_{0j}, \text{ and (2)}$$

$$\beta_{1j} = \gamma_{10} + \gamma_{11}(\text{dose})_j + \gamma_{12}(\text{average T1FR})_j + \gamma_{13}(\text{dose}^2)_j + \gamma_{14}(\text{dose} * \text{average T1FR})_j + \gamma_{15}(\text{dose}^2 * \text{average T1FR})_j + \mu_{1j}, \text{ (3)}$$

where β_{0j} is the intercept for the $_j$th neuron and β_{1j} is the slope for the $_j$th neuron from the within-neuron model. Because of the centering procedures used in the

within- and between-neuron models, the between-neuron intercept parameters of γ_{00} and γ_{10} correspond to the average within-neuron intercept and average within-neuron slope, respectively, across all neurons in the data set. All other γ parameters are the slope coefficients associated with the between-neuron independent variables. The μ parameters are residuals that model the unexplained portion of the between-neuron variance.

2.4.5 Amphetamine's effects on head movements

In all behavioral statistical analyses, the dependent variable was the rate of head movement (counts/min) observed at all possible combinations of two levels of time, four levels of dose, two levels of movement direction, five levels of movement distance and five levels of movement duration. Pilot analysis revealed the distribution of head movement rate to be highly skewed, so a gamma distribution with a log link was specified for all statistical models stated below. A value of 0.1 was added to all individual observations of head movement rate so that the distribution of head movement rate was compatible with a gamma distribution. Pilot analysis also indicated head movement rates in the two directions did not differ significantly across distance, duration, dose, and time, so movement direction was not considered as an independent variable in the final model.

To evaluate amphetamine's effect during treadmill off periods, a nonlinear mixed ANOVA (SAS PROC GLIMMIX, SAS Institute Inc., 2005, Cary,NC) was

constructed, in which the head movement rate during treadmill off was modeled as a function of time, dose, distance, and duration (2*4*5*5). Movements were filtered less intensively than for neural analyses. Specifically, movements had to be longer than 4 mm in distance, 30 ms in duration, and have less than a 26.5 degree displacement in the horizontal direction,. Subjects were specified as a random effect. A conjugate gradient optimization algorithm was utilized. For post-hoc analysis, the twenty-five categories defined by distance and duration were divided into four quadrants, short distance-short duration (15-67,15-133, 27-67,27-133), short distance-long duration (15-267, 15-270, 27-267, 27-270), long distance-short duration (51-67,51-133,52-67,52-133), and long distance-long duration(51-267,51-270,52-267,52-270). The head movement rates in the four quadrants in dose 1, 2, and 4 across time were tested against dose 0 for interaction.

Treadmill on head movement rates with the above stated distance, duration categories were tested in a separate GLIMMIX model with the same specifications. In contrast to the treadmill off condition, the treadmill on condition contained adequate numbers and variety of head movements to create matched pairs for neural analysis. Therefore, in order to achieve a close correspondence between neural and behavioral results throughout interpretation of the present findings, treadmill on data were the focus of most behavioral analyses.

3 Results

3.1 Amphetamine's effect on dorsolateral striatum neurons

3.1.1 Amphetamine-induced firing rate changes

Twenty-eight Long-Evans rats were recorded, yielding 84 single neurons related specifically to head movement . Among the 84 neurons, 33 were from the 0 mg/kg group, 11 from the 1 mg/kg group, 12 from the 2 mg/kg group, and 28 were from the 4 mg/kg group. All neurons reported here were histologically verified to be located in the dorsolateral striatum (Figure 13).

Table 1 lists the HLM results modeling the relationship between average T1 firing rate and average T2 firing rate of individual neurons. For the within neuron intercept β_{0j}, i.e. average time 2 firing rate, the HLM revealed a significant interaction between dose and average T1 firing rate, γ_{04} =-0.163286, t(78)= -3.287, p=0.002<0.01, and a significant interaction between dose^2 and average T1 firing rate, γ_{05}=0.124873, t(78)= 3.942, p<0.001. These results indicate T2 firing rate of individual neurons depended on the dose of drug administered and the average baseline (i.e. T1) firing rate of the neuron. Specifically, amphetamine elevated average firing rates of slow firing neurons but reduced average firing rates of faster firing neurons (Figure 5, 6, 7, 8). For within neuron slope β_{1j}, HLM failed to reveal significant effects of dose, dose^2, T1 firing rate, or their interactions, indicating a lack of predictable firing rate dependence on a within neuron level (Figure 9). That is, at a within-neuron level, the tendency for a neuron's low firing rates in T1 to be higher in T2 and its high firing rates in T1 to

be lower in T2 (i.e., regression to the mean, observed at dose 0) was not altered or enhanced by amphetamine.

Figure 10 shows the OLS regression of each neuron's empirical Bayesian estimated (BE) average T2 firing rate on its average T1 firing rate. Pearson's correlation values of individual dose groups were calculated and labeled on the graph. Each scatter plot also shows a diagonal reference line of no change. A dot below the reference line indicates the neuron represented by the dot exhibited a higher average T1 firing rate than its BE average T2 firing rate. On the contrary, a dot above the reference line indicates that the neuron represented by the dot exhibited a lower average T1 firing rate than its BE average T2 firing rate. The slope and intercept with 95% confidence interval of the OLS regression for individual doses were: dose 0, 0.3572 (0.2041, 0.5102) and 0.5016 (0.3182, 0.685); dose 1, -0.1024 (-0.2483, 0.04351) and 1.033 (0.807, 1.259); dose 2, -0.2796 (-0.4685, -0.09064) and 1.395 (1.175, 1.614); dose 4, 0.04562 (-0.1903, 0.2815) and 1.067 (0.8075, 1.326). Both the regression graph and the slope coefficients showed a dose-dependent and firing rate-dependent T1-T2 firing rate relationship. In Dose 0, the slope was positive yet smaller than 1, implying average firing rates during head movement decreased with the passage of time. On the contrary, dose 1, 2 and 4 all displayed a slope significantly smaller than that of dose 0, indicating the average firing rates of neurons with a faster T1 firing rate were reduced by amphetamine, whereas the average firing rates of neurons with a slower T1 firing rate were reduced by amphetamine. Further, at doses 1

and 2, the slopes exhibited a clock-wise turn relative to dose 0, whereas the dose 4 slope exhibited a lesser clock-wise turn. This curvilinear relationship observed on the graph was verified by the significant interaction between dose^2 and average T1 firing rate.

3.1.2 Reversal

A separate HLM was utilized to model the relationship between average T1 firing rate and average T4 firing rate of individual neurons to assess the reversal of drug effects. For the within neuron intercept β_{0j}, i.e. average time 3 firing rate, the HLM failed to reveal a significant interaction between dose and average T1 firing rate, γ_{04} =-0.069972, t(78)=-0.849, p=0.399, but revealed a significant interaction between dose^2 and average T1 firing rate, γ_{05}=0.134731, t(78)= 2.498, p<0.015. Figure 11 illustrates the results. In comparison to figure 10, the slopes for dose 0 are nearly identical, whereas all the three groups that had been administered amphetamine showed a counter-clock wise turn in the T1-T3 slope compared with the T1-T2 slope, indicating a reversal of drug effects. The slope and intercept with 95% confidence interval of the OLS regression for individual doses were: dose 0, 0.4461 (0.2715, 0.6206) and 0.2424 (0.03326, 0.4516); dose 1, 0.01272 (-0.1217, 0.1417) and 0.4215 (0.2134, 0.6297); dose 2, 0.01581 (-0.3289, 0.3605) and 0.7143 (0.3142, 1.114); dose 4, 0.5178 (0.1459, 0.8896) and 0.461 (0.05262, 0.8693).

3.2 Amphetamine's effect on behavior

3.2.1 Drug induced head movement during treadmill off

Head movements during the treadmill off period were examined to assess amphetamine's influence on behavior across T1 and T2. Individual head movements were classified according to their distance and duration. Five categories of distance and five categories of duration were defined yielding a total number of 25 behavioral categories. The GLIMMIX model of treadmill off behavioral uncovered a 4 way interaction between dose, time, distance and duration, $F(48,4655)=2.36$, $p<0.0001$. Further, the 25 movement categories defined by distance and duration were blocked into the four quadrants, short distance-short duration, short distance-long duration, long distance-short duration, long distance-long duration. Post hoc tests were conducted between dose 0 and all other drug doses for interaction of dose, time, and quadrants. For dose 0 versus 1, $t(4655)=2.18$, $p=0.0296$; for dose 0 versus 2, $t(4655)=3.94$, $p<0.0001$; for dose 0 versus 4, $t(4655)=2.31$, $p<0.0001$. These results indicate that all doses of amphetamine were sufficient to induce head movements in the absence of the moving treadmill's innate ability to induce head movements.

3.2.2 Drug induced head movement during treadmill on

During treadmill on, head movements were induced possibly by both the moving treadmill and different doses of amphetamine. The GLIMMIX model of treadmill on behavior across T1 and T2 revealed a 4 way interaction between dose, time, distance and duration, $F(48,4754)=3.84$, $p<0.0001$, implying that different subtypes of head movement behaviors defined by 5 categories of distance and 5

categories of duration were differentially modulated by different doses of amphetamine. To more specifically test how distance and duration of movements changed across time and dose, post hoc comparisons were made: the 25 movement categories defined by distance and duration were blocked into the four quadrants. The post hoc tests revealed a significant interaction of time and quadrants between 0 and all other dose groups. For dose 0 versus 1, $t(4754)=2.2$, $p=0.0282$; for dose 0 versus 2, $t(4754)=4.72$, $p<0.0001$; for dose 0 versus 4, $t(4754)=6.61$, $p<0.0001$. Graphically, Figure 10 visualizes the head movement rates of a specific category in different dose groups during T1 and T2. Compared to the dose 0 group, which exhibited a rather stable response pattern in all movement categories across T1 and T2, all groups administered amphetamine in T2 showed increased numbers of head movements in general. More specifically, the 1mg/kg and 2 mg/kg groups showed an intensive induction of longer movements after drug administration. The dose 4 group, while exhibiting significantly more head movements, showed fewer long movements compared to the lower dose groups (Figure 12).

3.2.3 Reversal

Head movements during T1 and T3 were tested against each other to assess reversal of drug effects on behavior during treadmill on. There was a significant 4 way interaction between dose, time, distance, and duration, $F(48,4754)=2.54$, $p<0.0001$. Post hoc comparison of dose 0 against dose 1 showed that the interaction between dose, time, and quadrants was not significant, $t(4754)=0.1$,

$p=0.9213>0.05$. However, when comparing dose 0 against doses 2 and 4 respectively, the interaction between dose, time, and quadrants was significant: dose 0 against dose 2, $t(4754)=4.61$, $p<0.0001$; dose 0 against dose 4, $t(4754)=3.53$, $p=0.0004$. The above mentioned relationship is shown in Figure 12. Graphically, both doses 1 and dose 2 showed fewer head movements during T3 relative to T2, especially the number of longer head movements, whereas dose 4 did not exhibit a visible difference between T2 and T3. Thus, amphetamine's induction of head movements in T2 reversed (dose 2) or recovered (dose 1) by T3 except at the highest dose.

During treadmill off across T1 and T3, there was a significant 4 way interaction between dose, time, distance, and duration, $F(48,4655)=2.40$, $p<0.0001$. Post hoc tests revealed a significant interaction for dose 0 against all drug doses: dose 0 versus 1, $t(4655)=2.28$, $p=0.0225$; dose 0 versus 2, $t=2.71$, $p=0.0067$; dose 0 versus 4, $t=2.79$, $p=0.0053$, indicating that amphetamine's induction of head movements during treadmill off in T2 had not recovered in T3.

4 Discussion

Changes in firing rate of dorsolateral striatum neurons related to vertical head movements were assessed simultaneously with spontaneous head movements before and after different doses of amphetamine administration. Effects of three drug doses (1mg/kg, 2 mg/kg, and 4 mg/kg) were tested. Induction of movements, especially head movements that were longer both in distance and duration, was observed at lower doses, but less obvious at the high dose. These results are in agreement with earlier research (Kuczenski & Segal, 1989; Sharp et al., 1987). Moreover, the induction by the high dose of significantly more head movements than dose 0, but fewer long head movements compared to lower doses supports Lyon and Robbins' theory, which states, with increasing doses of amphetamine, while the frequency of movement initiation will increase, the frequency of longer duration behaviors will decrease (Lyon & Robbins, 1975).

Numerous early studies aiming to clarify neuronal mechanisms underlying the behavioral changes induced by amphetamine focused on striatum. Amphetamine iontophresis in striatum inhibited the activity of neurons (Kiyatkin & Rebec, 1997; Stone, 1976) . On the other hand, dose-dependent modulations of striatal neuron firing rates were reported both in anesthetized (Ewing et al., 1983; G. V. Rebec & Segal, 1978; G. V. Rebec et al., 1981) and awake behaving animals (Kish et al., 1999; G. V. Rebec et al., 1997; G. V. Rebec & Groves, 1975) following systemic administration of amphetamine. These studies reported an inhibition of striatal neuron firing at lower doses (1mg/kg, 2.5mg/kg) of amphetamine and an excitation at higher doses (5 mg/kg, 7.5 mg/kg). However, differential neuronal

responses were found in striatum when ongoing motoric activity was taken into account. Both inhibition and excitation of firing were manifested in striatal neurons that were sensitive to motoric activities after low and high doses of amphetamine administration (Gardiner et al., 1988; Haracz et al., 1989). These findings indicate that striatal activity observed in the above metioned studies after amphetamine injection reflect an interaction of the ongoing behavior and pharmacological effects of amphetamine. The present study sought to tease these two factors apart by comparing the firing rate of striatal neurons during similar movements before and after amphetamine administration.

Given the differential sensitivity of individual dorsolateral striatum neurons to different types of movement, head movements were sorted into categories, defined by distinct levels of direction, apex, distance, duration and velocity (calculated as distance/duration) for neuronal analysis. Firing rates were compared only within the same movement category using matched pairs before and after injection, and also between different dose groups. Thus the resulting difference in firing rate could be interpreted as pharmacological effects of amphetamine, rather than altered sensory processing of the motoric changes induced by amphetamine. Imposing constraints by movement categories is one of the key advances over earlier studies. This restriction reduced sensorimotor variability within a category but was applied post hoc, so that movements of the animal were not confined by any means, providing the possibility to assess amphetamine's effects on firing during voluntary, unconditioned movements. A second advance over previous studies was that adequate numbers of matched

pairs were obtained, without conditioning head movements to increase their frequency. This allowed the present study to verify amphetamine's actual induction of head movements, whereas previous studies observed stimulant effects on ongoing, conditioned movements (Tang et al, 2008; Pawlak et al, 2010). An additional advance provided by these prior studies and reaffirmed by the present study is the importance of taking into account T1 firing rate when predicting T2 firing rate in response to a stimulant drug. The significant interactions between dose by T1 firing rate and dose^2 by T1 firing rate indicate that T2 firing rate is co-determined by the drug dose administered and the neuron's baseline firing rate.

At the between neuron level, amphetamine disrupted the pattern of firing rate change induced by the passage of time (Figure 10). At dose 0, T2 average firing rates were diminished compared to T1, which indicates a natural decline in firing rate of dorsolateral striatum neurons during treadmill walking for two hours. This finding differs from previous findings from this laboratory, in which dorsolateral striatal neuron firing rate remained highly stable through 2 hours when movements were conditioned (Pawlak et al., 2010). The difference might be due to: (1) in the present study, the movement was unconditioned, and the animal could make any type of movement voluntarily, while in the previous studies the movement was conditioned; water was delivered as reward only if the criterion movement was performed. (2) In the previous studies, animals were conditioned to emit thousands of movements continuously and with little variation; on the other hand, in the unconditioned case, the variation from movement to movement

and among different body parts was spontaneous, within the context of treadmill walking.

The main finding of the present study is that, after different doses of amphetamine were administered, in general, the firing rates of dorsolateral striatal neurons were modified such that neurons exhibiting lower average T1 firing rates showed strong increases in firing rate, whereas neurons exhibiting higher average T1 firing rates showed decreases in firing rate. Elevation of firing rates of striatal head movement neurons by a low dose of amphetamine is in agreement with our initial study (West et al., 1997), which became the prototype for the present approach. The neurons recorded in the present study were dorsolateral striatum medium spiny neurons (MSNs) exclusively, based on electrophysiological criteria (Kimura, 1990), and the fact that MSNs constitute 95% of the striatal neuron population. The MSNs not only receive inputs from cortical areas, they also receive modulatory signals from various striatal interneurons, and also from dopamine, serotonin and norepinephrine neurons (Tepper & Bolam, 2004). Cortical inputs from M1 and S1 define the receptive field of a given striatal neuron; inputs from striatal interneurons might integrate signals from several MSNs and coordinate MSNs activity; inputs from dopamine, serotonin and norepinephrine neurons might contain global information regarding the psychophysiological state of the organism. All these signals containing local information as well as global information may simultaneously modulate the activity of MSNs. The particular neurochemical balance influencing a given striatal neuron likely is reflected in its firing rate and by extrapolation, the

differential firing rates of individual neurons, in the absence of the drug (T1). After amphetamine was administered, the existing neurochemical balance was likely shifted by the drug's several pharmacological effects (see below). This resulted in a differential modulation of firing rate which nonetheless remained linked to each neuron's neurochemical balance in T1, in that it depended on the neuron's T1 firing rate.

More specifically, at lower doses (1 mg/kg, and 2 mg/kg), T2 firing rate differed from T1 firing rate in a dose-dependent manner. As the dose increased from 0 mg/kg to 2 mg/kg, the relationship between average T1 and T2 firing rates showed a systematic clockwise turn, which suggests as dose of amphetamine increases, there was more enhancement of firing rate for neurons that exhibited a slower average T1 firing rate, but more reduction in firing rate for neurons that exhibited a faster average T2 firing rate. This clockwise trend is the same as that observed previously with increasing doses of cocaine (Pawlak et al., 2010; Pederson et al., 1997). However, when given 4 mg/kg amphetamine, the clockwise trend did not hold; instead, the average firing rate of slower firing neurons increased less compared to lower doses, and the average firing rate of faster firing neurons also decreased less. This result was different from those observed in cocaine studies, where the slope of regressing average T2 firing rate on average T1 firing rate continued to decrease (rotate clockwise) as the dose of cocaine increased (Pawlak et al., 2010). The different results from the study by Pawlak et al (2010) were possibly related to a difference between conditioned versus unconditioned head movements, or could have been determined by the

different pharmacological effects of cocaine versus amphetamine. Cocaine blocks DAT, SERT, and norepinephrine transporters, whereas amphetamine's major effect is reversing transport through those transporters (Kahlig et al., 2005; Ramamoorthy & Blakely, 1999). Administration of either cocaine or amphetamine increases dopamine and serotonin levels in striatum (Bradberry et al., 1993; Hernandez et al., 1987). However, manipulation of the serotonin system selectively disrupted amphetamine self-administration but not cocaine administration, indicating that serotonin might be more involved in amphetamine induced pharmacological effects (Porrino et al., 1989).

The involvement of striatal dopamine and serotonin in amphetamine induced behavioral changes has been demonstrated by several experiments (Hernandez et al., 1987; Kuczenski et al., 1995; Yeghiayan et al., 1997). Further, serotonin may be more involved in amphetamine's induction of stereotypical behaviors (Kuczenski & Segal, 1989), characterized by induction of shorter rather than longer movements, as observed in animals at dose 4 in the present study. The above mentioned effects of neurotransmitters on behavior could be meditated through dorsolateral striatum MSNs, more than half of which are related specifically to focused movements of particular body parts. Movement related neurons showed enhanced firing during the specific movements to which the neurons are tuned when dopamine was administered (Pierce & Rebec, 1995; Rolls et al., 1984). In contrast, serotonin was shown to impose an inhibitory influence on striatal dopamine function (Kuczenski, 1979). Thus, the firing rate dependence observed in the present study (i.e., slower firing neurons exhibited

increased firing rate, while faster firing neurons exhibit decreased firing rate after drug administration) implies a relatively heavier influence of serotonin or lighter influence of dopamine on the faster firing neurons after drug administration. Further study is required to clarify differences between slow versus fast firing striatal neurons, a distinction found to be important in several recent studies (Pawlak et al., 2010; C. Tang et al., 2008; C. C. Tang et al., 2009; C. Tang et al., 2007).

The parallel changes in behavior and neuronal activity observed here are consistent with the pivotal role of dorsolateral striatum in mediating amphetamine induced behavioral changes. Neuronal information from the dorsolateral striatum, a critical component in the “motor loop”, relays through globus pallidus, thalamus, and reaches premotor and motor cortex (Alexander et al., 1986). Amphetamine induced firing rate changes in dorsolateral striatum movement correlated neurons likely alter the signals sent to premotor and motor cortex, resulting in changes in motoric activity. Strong elevations of the large population of slower firing neurons may be responsible for inducing movements more frequently, while reduced firing of faster firing neurons may contribute to stimulant-induced stereotypies (cf. Tang et al, 2008; Pawlak et al, 2010).

Fixed Effect		Coefficient	Standard Approx. Error	T-ratio	d.f.	P-value
For INTRCPT1, β0						
INTRCPT2,	γ00	1.083036	0.094126	11.506	78	0.000
CDOSE,	γ 01	0.104402	0.040384	2.585	78	0.012
CMT1HZ,	γ 02	-0.25691	0.052482	-4.895	78	0.000
CDOSESQU,	γ 03	-0.03991	0.031548	-1.265	78	0.210
DST1HZ,	γ 04	-0.16329	0.049679	-3.287	78	0.002
DSSQT1HZ,	γ 05	0.124873	0.031678	3.942	78	0.000
For CT1HZ slope, β1						
INTRCPT2,	γ 10	0.096046	0.144447	0.665	78	0.508
CDOSE,	γ 11	0.018007	0.046131	0.39	78	0.697
CMT1HZ,	γ 12	0.035468	0.065986	0.538	78	0.592
CDOSESQU,	γ 13	-0.04779	0.042515	-1.124	78	0.265
DST1HZ,	γ 14	-0.00143	0.034857	-0.041	78	0.968
DSSQT1HZ,	γ 15	-0.00026	0.025063	-0.01	78	0.992

Table 1. HLM results modeling the relationship between average T1 firing rate and average T2 firing rate of individual neurons. CDOSE: centered dose; CMT1HZ, centered mean T1 firing rate; CDOSESQU, centered dose square; DST1HZ: dose* T1 firing rate; DSSQT1HZ: $dose^2$*T1 firing rate. For β0, average T2 firing rate, HLM revealed a significant interaction between dose*mean T1 firing rate and $dose^2$* mean T1 firing rate.

Fixed Effect		Coefficient	Standard Approx. Error	T-ratio	d.f.	P-value
For INTRCPT1, β0						
INTRCPT2,	γ00	0.58794	0.143351	4.101	78	0
CDOSE,	γ 01	0.049865	0.054149	0.921	78	0.36
CMT1HZ,	γ 02	-0.05134	0.09949	-0.516	78	0.607
CDOSESQU,	γ 03	0.037569	0.045891	0.819	78	0.416
DST1HZ,	γ 04	-0.06997	0.082418	-0.849	78	0.399
DSSQT1HZ,	γ 05	0.134731	0.053933	2.498	78	0.015
For CT1HZ slope, β1						
INTRCPT2,	γ 10	0.096046	0.144447	0.665	78	0.508
CDOSE,	γ 11	0.018007	0.046131	0.39	78	0.697
CMT1HZ,	γ 12	0.035468	0.065986	0.538	78	0.592
CDOSESQU,	γ 13	-0.04779	0.042515	-1.124	78	0.265
DST1HZ,	γ 14	-0.00143	0.034857	-0.041	78	0.968
DSSQT1HZ,	γ 15	-0.00026	0.025063	-0.01	78	0.992

Table 2. HLM results modeling the relationship between average T1 firing rate and average T3 firing rate of individual neurons. CDOSE: centered dose; CMT1HZ, centered mean T1 firing rate; CDOSESQU, centered dose square; DST1HZ: dose* T1 firing rate; DSSQT1HZ: $dose^2$*T1 firing rate. For β0, average T2 firing rate, HLM revealed a significant interaction between dose*mean T1 firing rate and $dose^2$* mean T1 firing rate.

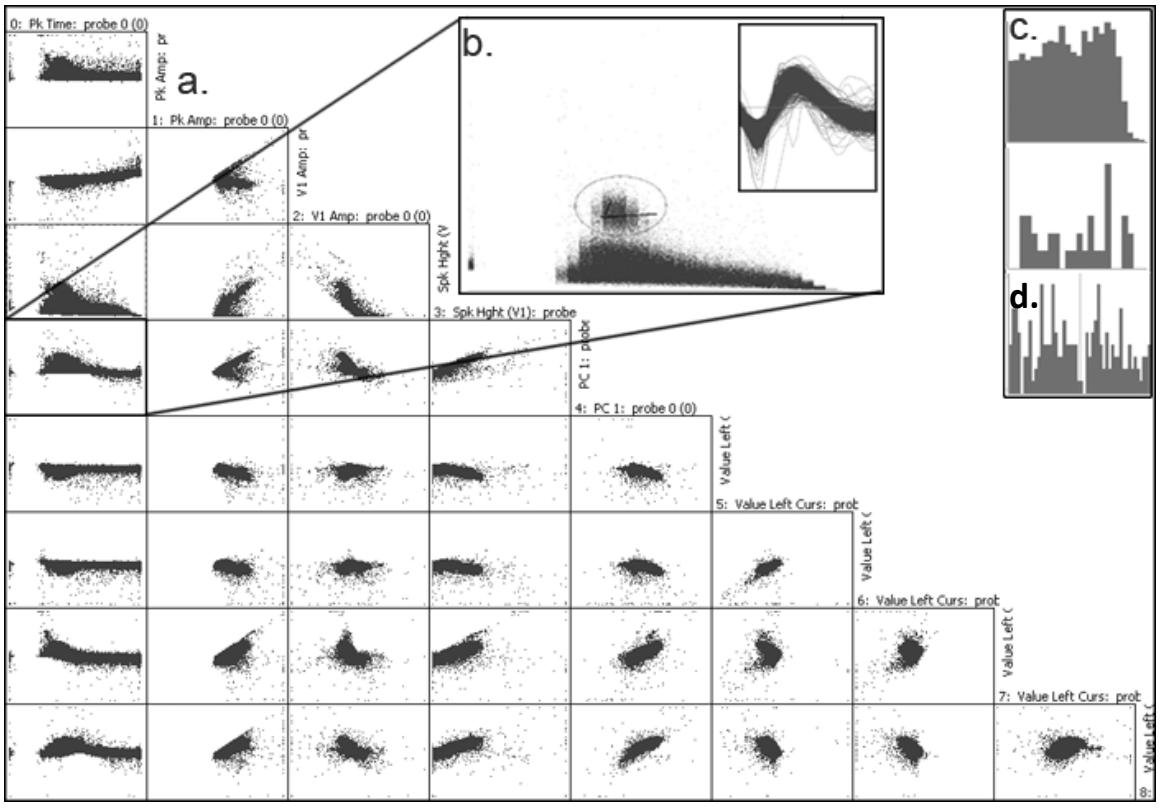

Figure 1. Cluster cutting. (a) Pairs of waveform parameters were used to create scatter plots of electronic signals recorded in a single session. (b) In any parameter space, the experimenter selected naturally clustered signals with similar waveform parameters. The selected clusters of signals were highlighted in other parameter space. The experimenter could modify one selection area in any parameter space if desire. Straight black lines in (b) connected two signals that occurred within a defined interval (1.6 ms in current experiment). (c) Waveforms of selected signals in (b). (d) Auto correlation histogram and cross correlation histogram of two selected clusters of signals. Filling in of the bins around the center vertical line indicates the two selected clusters of signals occur within 1ms of each other at least once, thus violating the refractory period that would be observed if both signals were generated by one neuron.

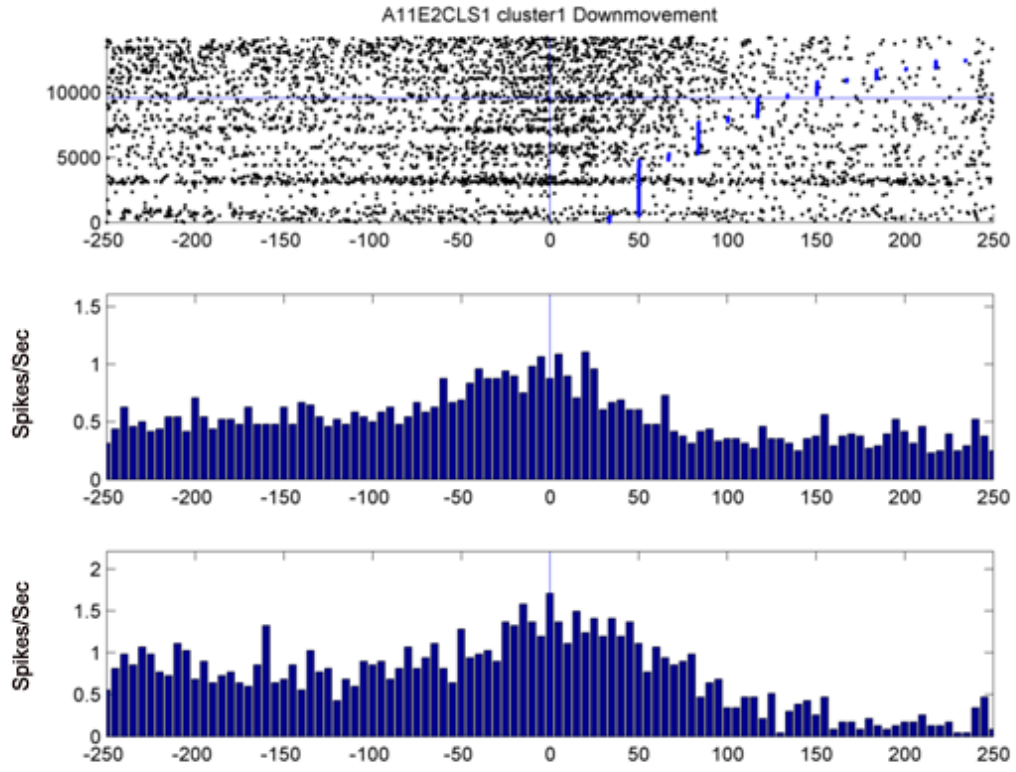

Fig.2, Example neuron correlated with downward head movements. Raster plot centers the neuronal discharges on the initiation of upward head movements (time 0). Head movements were sorted according to their durations (blue dots on raster mark the end of individual head movements, initiated at time 0). Horizontal blue line in raster separates head movements that were shorter than 125 ms from those that were longer in duration. PETHs in second and third rows display firing rates around the initiation of head movements with duration shorter or longer than 125 ms respectively. Bin size=5 ms.

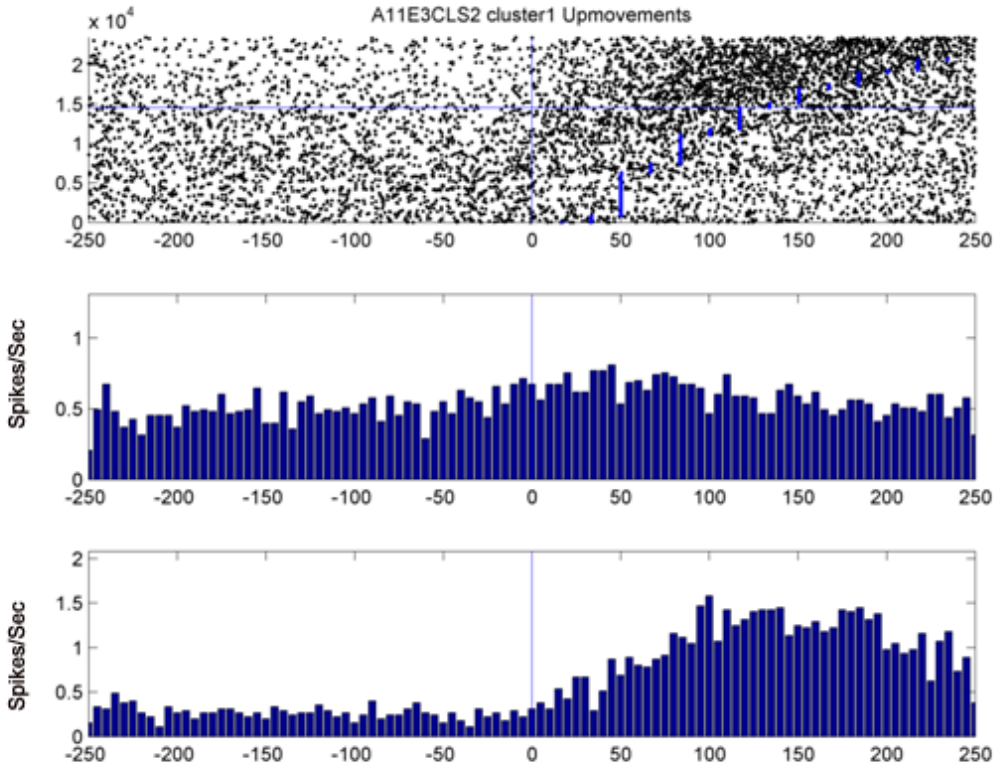

Fig.3, Example neuron correlated with upward head movements. Raster and PETHs were constructed similarly as in Fig.2, except neuronal discharges were displayed around the initiation of downward movement. Notice that this neuron preferentially increased its firing rate during longer head movements.

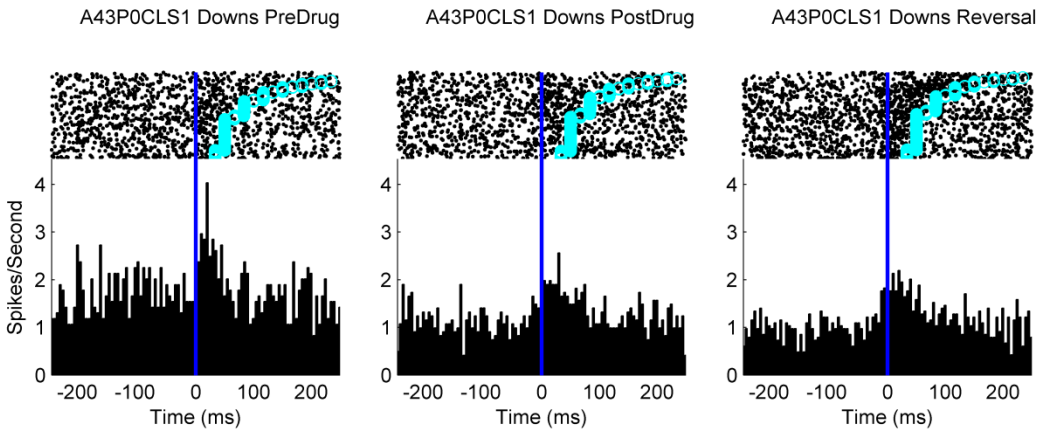

Figure 4. Head movement neuron firing pattern before and after saline injection. Rasters (top row) center neuronal discharges on the initiation of head movements. Blue dots on the right side of time zero indicate the completion of each head movement. Head movements were sorted by duration. PETHs (bottom row) display firing rates around the initiation of head movements. The three columns represent pre-saline administration (T1), post-saline administration (T2), and reversal (T3).

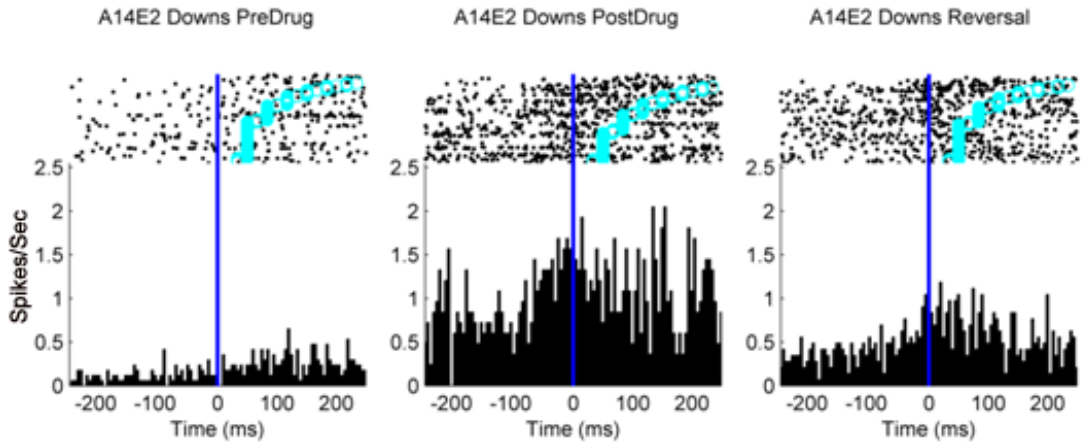

Figure 5, Head movement neuron firing pattern before and after 1mg/kg amphetamine administration. Rasters and PETHs were constructed similarly as Fig. 4. The three columns represent pre-amphetamine administration (T1), post-amphetamine administration (T2), and reversal (T3). This neuron's firing rate increased after amphetamine injection.

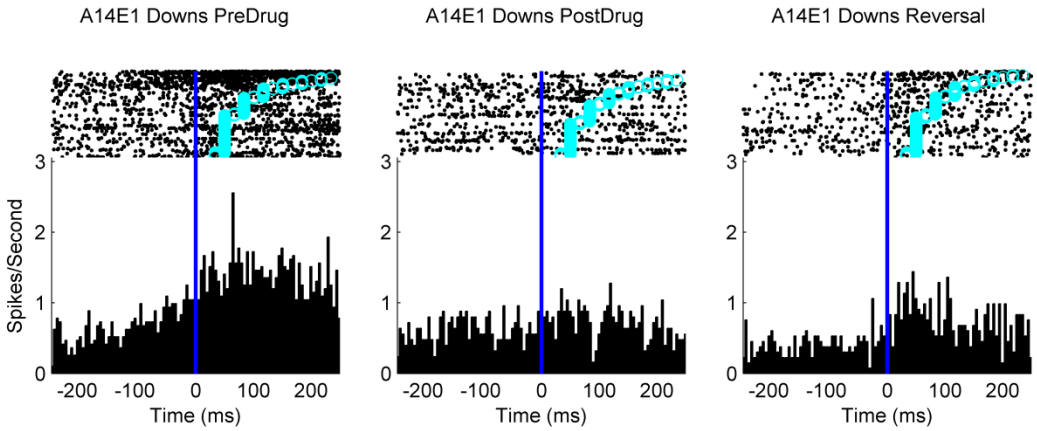

Figure 6, Head movement neuron firing pattern before and after 2mg/kg amphetamine administration. Rasters and PETHs were constructed similarly as Fig. 4. The three columns represent pre-amphetamine administration (T1), post-amphetamine administration (T2), and reversal (T3). This faster firing neuron's firing rate was reduced after amphetamine injection.

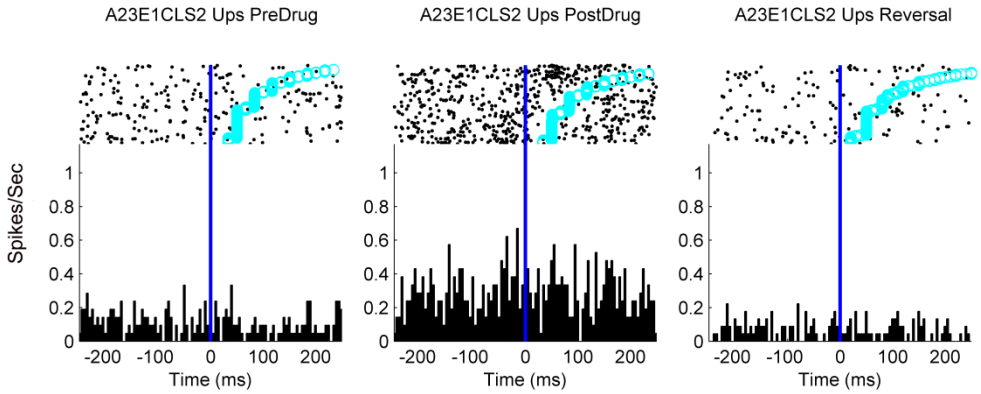

Figure 7. Head movement neuron firing pattern before and after 2 mg/kg amphetamine administration. Rasters and PETHs were constructed similarly as Fig. 4. The three columns represent pre-amphetamine administration (T1), post-amphetamine administration (T2), and reversal (T3). This slower firing neuron's firing rate increased after amphetamine injection (T2). Figures 6 and 7 together indicate that the change in firing rate of DLS neurons does not depend exclusively on dose of drug administered, but also on pre-drug firing rate.

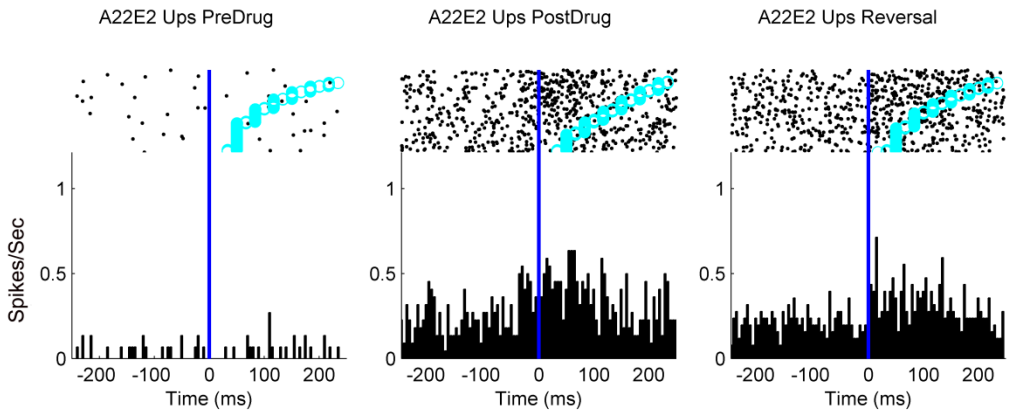

Figure 8. Head movement neuron firing pattern before and after 4 mg/kg amphetamine administration. Rasters and PETHs were constructed similarly as Fig. 4. The three columns represent pre-amphetamine administration (T1), post-amphetamine administration (T2), and reversal (T3). This slow firing neuron increased firing rate after amphetamine injection.

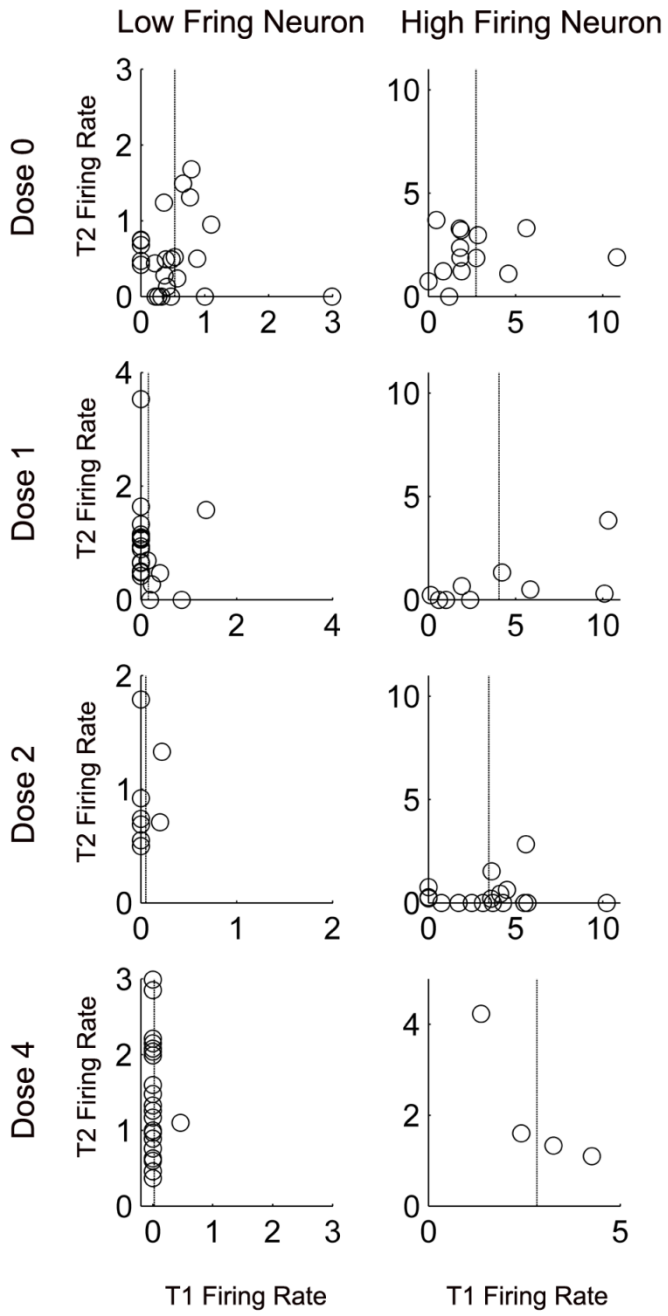

Figure 9. Changes in firing rates (spikes/sec) of matched pairs from T1 to T2 within individual neurons. For individual dose groups (in rows), two representative neurons are presented, one with lower average T1 firing rate, the other with higher average T1 firing rate. Each scatterplot represents one neuron, and each dot represents one matched pair. The vertical line in individual scatter plots indicates mean T1firing rate.

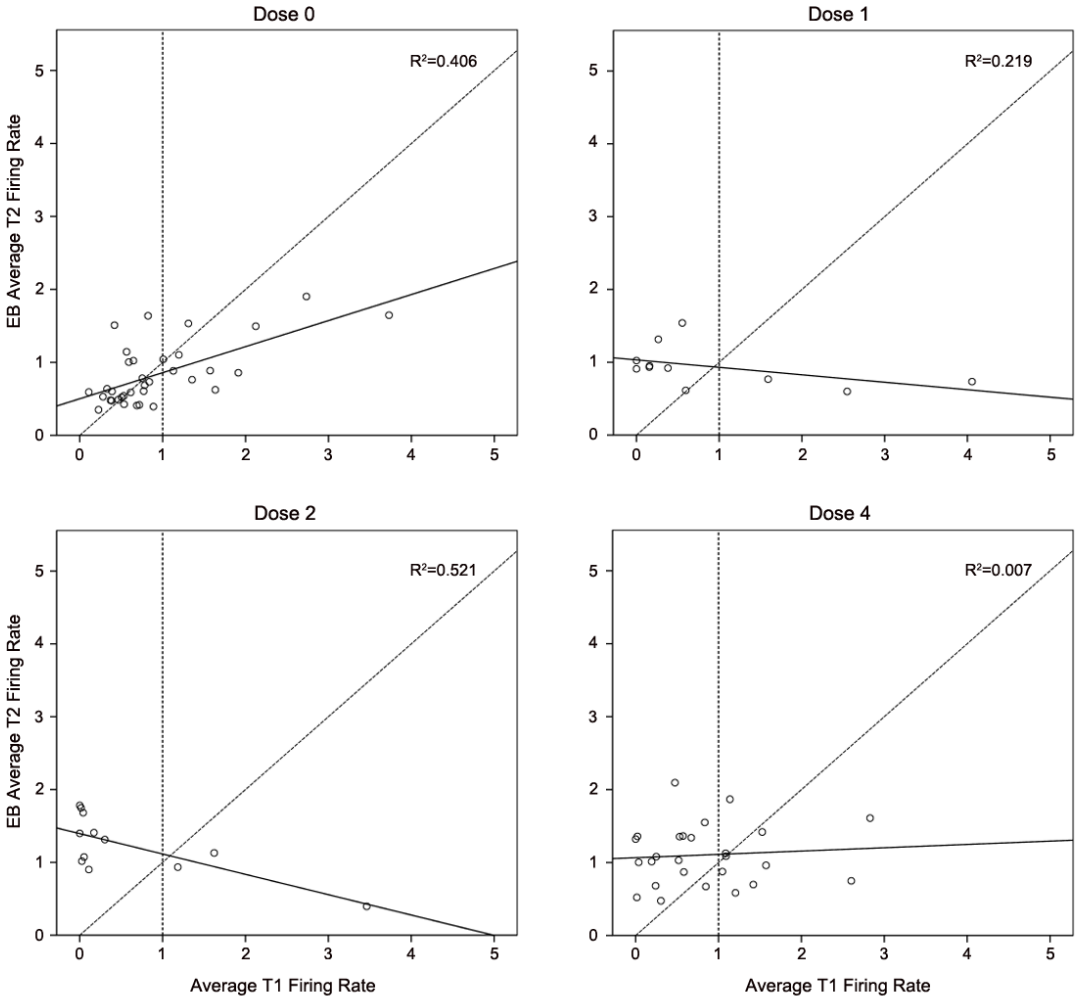

Figure 10. Graphic representation of results of the hierarchical linear model on T1-T2 firing rate (spikes/sec). Empirical Bayesian estimates derived from HLM of the average T2FR and raw average T1FR plotted for each dose of amphetamine using scatter plots. Dashed vertical line at average T1 firing rate = 1.0 spikes/sec designates cut-off between slower versus faster firing neurons.

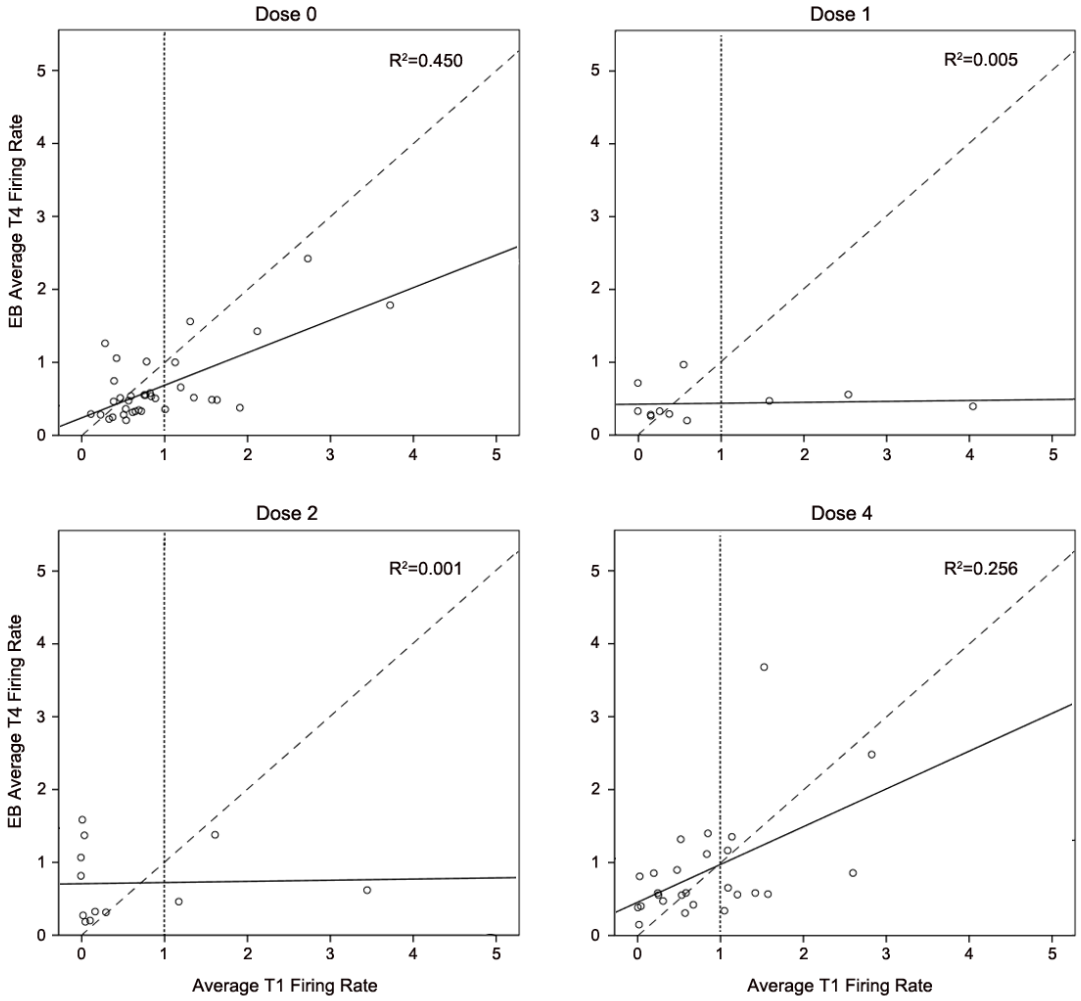

Figure 11. Graphic representation of results of the hierarchical linear model on T1-T4 firing rate (spikes/sec). Empirical Bayesian estimates derived from HLM of the average T4FR and raw average T1FR plotted for each dose of amphetamine using scatter plots. Dashed vertical line at 1.0 average T1 firing rate designates cut-off between slower versus faster firing neurons

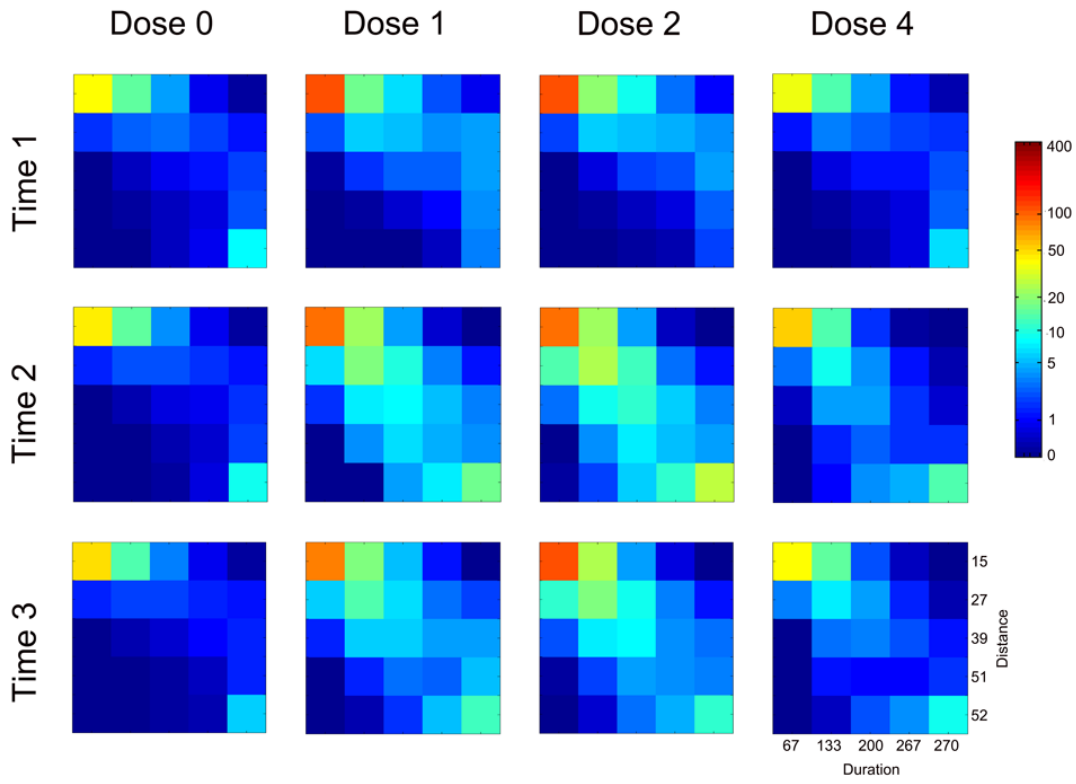

Figure 12. Heat maps of head movement behavior during treadmill on for individual dose groups (columns) during three time periods. Individual heat map contains 25 cells, representing categories of head movements defined by distance and duration (see bottom right heat map for specification). Color in individual cells represents the observed median head movement rate of all animals in the specified dose group, time period and movement category. Head movement rate is converted to a log scale for visualization purposes.

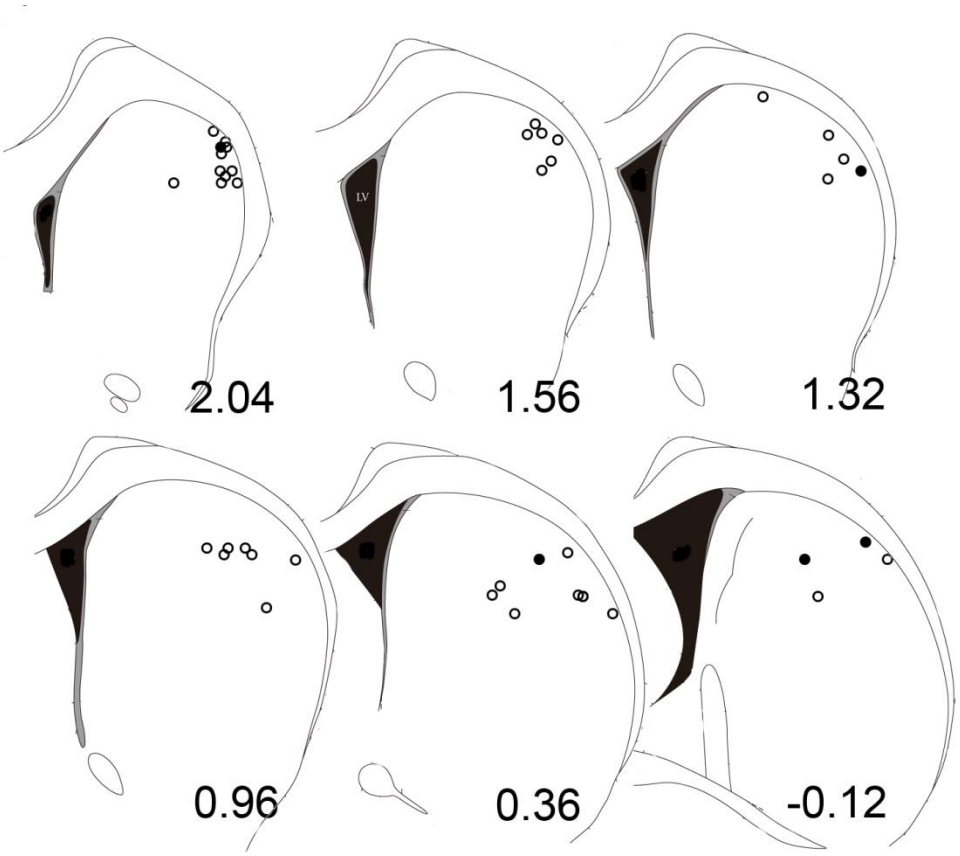

Fig.13, Location of the 84 head movement neurons recorded in this study, histologically verified to be in dorsolateral striatum. Open circles represent one single neuron recorded; black dots represent two or more single neurons recorded. Six coronal sections of striatum are shown with their anteriorposterior distance from bregma labeled (Paxinos & Watson, 2005).

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