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

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Abstract

Amphetamine elicits motoric changes by increasing the activity of central neurotransmitters such as dopamine and serotonin, but how these neurochemical signals are transduced into motor commands is unclear. The dorsolateral striatum (DLS), a component of the cortico-subcortical reentrant motor loop, contains abundant neurotransmitter transporters that amphetamine could affect. It has been hypothesized that DLS medium spiny neurons contribute to amphetamine's motor effects. To study striatal activity contributing to amphetamine-induced movements, activity of DLS neurons related to vertical head movement was recorded while tracking head movements before and after acute amphetamine injection. Relative to saline, all amphetamine doses induced head movements above pre-injection levels, revealing an inverted U-shaped dose-response function. Lower doses (1mg/kg and 2 mg/kg, intraperitoneal) induced a greater number of long (distance and duration) movements than the high dose (4 mg/kg), which induced stereotypy. Firing rates (FR) of individual head movement neurons were compared before and after injection during similar head movements, defined by direction, distance, duration, and apex. Changes in FR induced by amphetamine were co-determined by dose and pre-injection FR of the neuron. Specifically, all doses increased the FRs of slower firing neurons but decreased the FRs of faster firing neurons. The magnitudes of elevation or reduction were greater at lower doses, but less pronounced at the high dose, forming an inverted U function. Modulation of DLS firing may interfere with sensorimotor processing. Furthermore, pervasive elevation of slow firing neurons' FRs may feed-forward and increase excitability in thalamocortical premotor areas, contributing to the increased movement initiation rate.

Keywords

Dorsolateral striatum; Amphetamine; Motoric behavior

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1. Introduction

Amphetamine has been widely used medicinally, but is equally prevalent as an abused, addictive drug. Its effects include increased attention, reduced fatigue, and euphoria. Its psychomotor stimulant effects include increased activity in general and repetitive motor activity that lacks variability, i.e. stereotypical behaviors [1]. Low doses produce hyperactivity, but not stereotypical behavior, which is observed at higher doses [2]. More specifically, as theorized by Lyon and Robbins [3], complex behavioral chains and behaviors that require long pauses are stimulated by low doses, but with increasing doses of amphetamine, are replaced by behaviors with shorter duration; with further increases in dose, shorter, repetitive responses predominate.

Amphetamine increases synaptic levels of various neurotransmitters, including dopamine and serotonin, in numerous brain regions [4]. Amphetamine's effects on reward processing, locomotor activity and stereotypies are thought to be mediated primarily by its actions upon dopamine transporters (DAT) and serotonin transporters (SERT) [5,6]. The dorsolateral striatum (DLS) contains regionally co-localized DAT [7] and SERT [8,9], suggesting that both could be involved in modulation of DLS physiology by amphetamine. Indeed, experimental evidence suggests that both striatal dopamine and serotonin are involved in amphetamine induced motoric changes. Lesioning the substantia nigra to deplete striatal dopamine attenuated both locomotion and stereotypical behaviors induced by amphetamine [10]. Also, microinjection of amphetamine into the striatum induced stereotypical behavior [11]. Serotonin appears to potentiate dopamine's induction of stereotypical behavior. Para-chlorophenylalanine, an inhibitor of the rate limiting enzyme of serotonin synthesis, decreased stereotypical behavior induced by high dose amphetamine and increased locomotion [12]. Moreover, microdialysis in the striatum revealed elevation of serotonin in response to amphetamine administration [13, 14], and the change in serotonin level paralleled the emergence of stereotypical behavior [15].

Although interactions of dopamine and serotonin, specifically in striatum, are implicated in amphetamine induced changes in motoric behavior, the mechanism of these behavioral changes, which must involve the activity of single striatal neurons, remains to be clarified. Besides dopamine and serotonin innervation, the DLS, being a major component of the motor loop, also receives topographic afferent inputs from both primary motor (M1) and primary somatosensory cortices (S1). M1 and S1 terminations that correspond to a single body part converge on clusters of DLS neurons. DLS neurons within clusters, which are located in matrisomes, respond during movement or sensory stimulation of the body part that is defined by its cortical inputs [16]. DLS neurons that discharge during active movement, passive manipulation, and tactile stimulation of specific body parts (SBP neurons), including tongue, face, vibrissae, forelimb, hindlimb, and neck are well documented in both primates [17,18] and rodents [19], are medium spiny, projection neurons [20], and comprise the majority of neurons recorded in awake animals [21].

Individual SBP neurons are tuned not only to the movement of a specific body part, but also to parameters of movement. For instance, in rodents, a particular neuron tuned to head movement may fire more intensively when the animal emits a short distance head movement while another head movement neuron may fire more intensively when the animal emits a longer head movement [22, 23]. Moreover, neurons fire preferentially during movement in one direction. Similar results are observed in primates [17].

Because DLS neurons receive monoaminergic inputs and are primarily involved in sensorimotor function, which is altered by amphetamine's pharmacological actions, it is rational to hypothesize that a key mediator of altered sensorimotor function at the single

neuron level is a modulation of activity of DLS neurons related to movement. The current study focused on amphetamine's acute effects on striatal firing and motoric function. Activity of SBP neurons correlated with vertical head movement was recorded before and after injection of different doses of amphetamine. Each head movement was categorized according to its direction, distance, duration, velocity and apex, and firing before and after administration of amphetamine was compared only during similar head movements, to eliminate variability in firing related to differences in movement parameters to which SBP neurons are sensitive.

2. Methods

2.1 Subjects

A total of 28 male Long-Evans rats were trained and recorded in the treadmill task. Animals were individually housed and kept on a reversed light/dark cycle so that experiments were conducted during their active period. Animals had ad lib water but restricted food access once body weight reached 330 g, at which each animal was maintained prior to head stage implantation surgery. One week after surgery, a sensorimotor exam was followed by a treadmill training session. During treadmill training sessions, animals received an intraperitoneal (i.p.) dose of amphetamine, 0 mg/kg, 1 mg/kg, 2 mg/kg, or 4 mg/kg in 0.5 ml according to their randomly assigned dose group.

Out of the 28 animals used in this study, 7 animals were exposed to 2 different doses, 3 animals were exposed to 3 different doses and the remaining 18 animals to only one dose. There was at least 3 weeks between an individual animal's drug exposure/treadmill sessions and a separate group of neurons were recorded from any animal that had been exposed to drug/treadmill session more than once (i.e., for microwire implanted animals, different wires between sessions).

2.2 Surgery

Animals were initially anesthetized 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. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publications 865–23) and were approved by the Institutional Animal Care and Use Committee, Rutgers University.

Animals in the 1 mg/kg and 2 mg/kg groups and six animals in the 4 mg/kg group were implanted with a microdrive base (Crist Instrument Co., Inc., Hagerstown, MD), 0.2 mm anteroposteriorly and 3.5 mm 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 treadmill training session, a microdrive housing a tungsten microelectrode (1–10 M Ω ; FHC Inc., Bowdoin, ME) was mounted onto the microdrive base. An electrode track profile was created [24] while the microelectrode was lowered to ensure that only striatal neurons were recorded. Once a striatal neuron was encountered, a sensorimotor exam was performed (see below). If the neuron was verified to be related to vertical head movement, the treadmill training session could begin. Throughout this process, a total of one to four hours' time was allowed for the electrode to settle, in order to enhance stability of recordings during the ensuing injections and vigorous motor behavior.

All animals in the 0 mg/kg (saline) group and eight animals in the 4 mg/kg group were implanted with microwire arrays. The microwire array comprised twelve (2 $\times$ 6) Teflon-insulated microwires (California Fine Wire, Grove City, CA, USA, spacing 400 micrometers

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 to -0.4 mm from bregma on the anterior-posterior axis, 3.2 to 4.2 mm from bregma on the medial-lateral axis, and between -3.5 to -3.7 mm on the dorsal-ventral axis from skull level, where clusters of head movement neurons are densely located [22, 19]. The connector strip was mounted on the center of the skull with dental cement. The top of the connector strip was 1.0 cm above skull level in every animal. 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), band pass 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 kHz sampling frequency.

Parameters of waveforms of striatal neurons were similar whether recorded by stainless steel microwires or tungsten microelectrodes using the microdrive. In addition, in the drug free period, the average firing rates during head movement of neurons recorded by the two types of electrodes (0.65 ± 0.19 (Mean $\pm$ SEM) spikes/sec, and 0.97 ± 0.1 spikes/sec, for tungsten microelectrodes and microwires respectively) were not statistically different ($t = -1.618$, $p > 0.05$). 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 ($7' \times 12' \times 5'$ Plexiglas walls) and connected to the harness. Amplified, filtered signals recorded from the animal were monitored both on oscilloscopes and with headphones. A complete sensorimotor exam was performed on the signal recorded from the microelectrode or from every microwire that exhibited neuronal activity 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 [24]. Correlation was determined if substantial modulation of firing was observed (primarily by audio output from the headphones) during the three situations. Head movement correlated neurons were documented and these alone would be recorded during treadmill training.

2.3.2 Treadmill training—Animals were connected to the harness and placed in the training chamber with the silicon-coated treadmill belt as the floor. Two LEDs facing forward on the headstage of the harness were separated by 9 mm dorsoventrally. A camera was positioned in front of the treadmill facing the animal, tracking the position of each LED (the position of each LED was digitized to a pair of numbers representing x, y coordinates, sampled and stored every 16.7 ms). The bottom LED had blinders on both left and right sides, so that if the animal exhibited a horizontal deviation of more than 20° away from the camera on either side, the bottom LED was not visible. Direction, distance, duration, and apex of head movements were derived offline by the position record of the LEDs (see 2.4.1 for more details). A custom designed computer program (based on software provided by Datawave Technology, Loveland, 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 cycle allowed periods of rest and yet achieved the goal of maximizing the time spent facing forward during treadmill-on to enable diode tracking of head movement. Nonetheless, animals varied in time spent facing away from the camera (circling, head bobbing, etc.), particularly after amphetamine injection. Therefore, time

spent facing the camera was determined for each subject and used as the denominator for determining rate of head movement (see below).

The treadmill training session lasted for four hours. At the start of the first hour, which served as the pre-drug baseline, referred to as T1, animals received a saline injection, to control for any possible behavioral effects induced by the injection. One hour into the session, 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 into 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 session was referred to as T2) and to allow for examination of reversal during the last hour (typically hour 4, referred to as T3).

2.4 Data Analysis

2.4.1 Head Movement Profile—X and Y coordinates of LED positions were analyzed off-line with respect to their timestamps (60 Hz sampling frequency). Movements were derived only when the position records from both LEDs were available. The camera was positioned in front of the treadmill, facing the animal. During vertical head movements, as a result of the pivoting of the rat's head on its neck, the LEDs on the harness arced away from the camera, which the camera could not directly observe. Therefore, to prevent distortion of the observed vertical y-component of each movement, y-coordinates were adjusted post hoc by the addition of constant values that were calculated based on trigonometric ratios. These constants progressively increased for higher y-coordinates and allowed for a more accurate representation of position along the entire arc of the movement. Also, the animal's position on the treadmill determined its distance from the camera, which in turn could affect the observed measured movement length. Nonetheless, when animals were walking forward AND facing the camera (the latter being the only instance when both LEDs could be monitored and thus when head movements could be assessed), animals tended to stay at a similar location near the front, thereby avoiding being pushed into the back wall. When at this location or depth, each pixel corresponded to 1.0 mm.

The first stage of movement reconstruction involved movement segmentation. Since the interest of this study was vertical movements, the movement segmentation was conducted according to the Y coordinates. Upward head movements were defined by continuously increasing Y coordinates, whereas downward head movements were defined by continuously decreasing Y coordinates. A pause in vertical movement was defined by non-changing Y coordinates between two consecutive 16.7 msec samples. The start or end of an individual movement was defined by a directional change (up or down), or a pause in movement (pause offset or onset, respectively). Movements preceding or following a period of time where the LEDs were not detectable were discarded, due to their undefined end or start times, respectively.

Movement parameters including duration, distance, valley, apex, velocity, slant, curvature and tilt were then calculated for every individual head movement. Any curved, slanted, horizontal, or crooked movements were then filtered out based on movement parameters, leaving only straight, vertical head movements. Specifically, each movement had to be longer than 4 mm in distance, 30 ms in duration, less than 18.4 degree slant from vertical (slant was derived as the following: the arctangent of the maximal horizontal displacement divided by the maximal vertical displacement), and a curvature of less than 0.15 (curvature was defined as a ratio, whose numerator was the furthest point along a line perpendicular to the line connecting the movement start and end position, and whose denominator was the length of the line connecting the movement start and end position, i.e. distance of the

movement). Movement processing was performed by a custom-designed program written by Martin B. Wolske [22, 23].

2.4.2 Neuronal Signals—Neuronal data were played back offline from the entire session and sorted according to the shape of the waveform (Datawave Technology). Sorting criteria involved several waveform parameters: peak time, peak amplitude, spike time, spike height, principle components and four custom cursors and inter-spike interval, described in detail previously [23].

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, which was determined from the position record of the LEDs. 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; (2) neuron showed a more than 2-fold modulation of firing in a PETH generated around active head movement; and (3) neuron was differentially modulated around upward head movement versus downward head movement. If the above criteria were not met, the neuron was excluded from further analysis.

2.4.4 Amphetamine's effect on DLS neuron firing—Once a DLS neuron was determined to be correlated with directional head movement, the drug effect on firing was compared during similar movements before and after drug administration. The rationale for assessing neural firing only during similar movements was to provide “behavioral equivalence” [25]. It is well established that psychostimulants, including amphetamine, change motor profiles. Comparing firing rate (FR) across similar movements eliminates the possibility that any FR changes might be due, for example, to feedback from altered movement parameters. Our strategy was to eliminate or minimize pre- vs. post-injection differences in all known variables to which each neuron was sensitive, in order to strip away all differences except those related to amphetamine's actions.

Motor behavioral equivalence was achieved by constructing matched pairs, as follows. The apex, duration, and distance were used to categorize each individual movement. Each parameter was divided into 5 levels. Apex: 125 mm–144 mm, 145 mm–164 mm, 165–184 mm, 185–204 mm, 205 mm–255 mm. 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. Distance: 4 mm–15 mm (designated as 15), 16 mm–27 mm (27), 28 mm–39 mm (39), 40 mm–51 mm (51), 51 mm and longer. The apex, duration, and distance levels define 125 (5×5×5) movement categories (a three-dimensional matrix), where each movement fits into only one category. Movements that fell into the same category were considered similar movements. Note that velocity, i.e., distance/duration, also was similar within each category. The FRs of all movements within one category were computed (# spikes/duration of that movement) and averaged, resulting in one average FR for each category. Each movement category had to contain more than five movements or the data were discarded due to inadequate sampling of firing for the specific movement category. Three-dimensional matrices of FRs were constructed with apex, duration, and distance levels comprising the three dimensions. One matrix was constructed for each time period (i.e. T1, T2 and T3), yielding three matrices for each session (for each neuron). Movements in the same category before (T1) and after (T2) drug administration were designated as matched pairs. Comparison of the average FRs between the two members of a matched pair was the basis for all assessments of drug effects on firing. Similarly, reversal was assessed by comparing the FRs 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 to allow analysis of FR.

The matched pair data were considered hierarchical in nature because the different matched pairs were nested within neurons, and in turn nested within subjects. 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 (see [22] for a more detailed exposition of the application of HLM to matched pair neural data).

The matched pair data conformed to 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.

Overall, the HLM tested whether a relationship exists between the average T2 FR (FR after drug administration) of a neuron and its average T1FR (FR before drug administration), the dose being administered, and their interaction. The within-neuron level of the HLM quantifies the linear relationship between the FRs during T1 of individual matched pairs and FRs during their T2 counterparts for individual neurons. The between neuron level of the HLM utilized average FR of all matched pairs from a given neuron, the dose of amphetamine, and their interaction to predict the empirical Bayesian estimated average T2 FR from all matched pairs of the neuron in question (obtained from the within-neuron model, corresponds to within-neuron intercept, β_0) and the linear relationship between the FRs of T1-T2 matched pairs (obtained from the within-neuron model, corresponds to within-neuron slope, β_1). More specifically, the level two predictors were: drug dose administered to the animal, dose² (in order to characterize a quadratic dose response curve), average FR during T1 of each neuron, the interaction of dose by average T1 FR of each neuron, and the interaction of dose² by average T1 FR of each neuron. A similar HLM was implemented to examine reversal from putative drug effects, i.e. predicting average FR in T3. Detailed implementation of the model can be found in the appendix.

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 effects 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, thus providing adequate numbers for analysis of movement rate. Specifically, head 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 collapsed (i.e., simplified) into four quadrants, short distance-short duration (15 mm – 67 ms, 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.

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

2.4.6 Video analysis of amphetamine's effects on stereotypic head

movements—The video signal from the camera was also sent to a videocassette recorder (Sony Super Beta SL-HF750 or Sony VHS SLV-789HF; Sony Corp. of America, New York, NY). The Datawave computer time-stamped each frame of the video recording at a resolution of 30 frames/s (33 ms between frames) by way of a video frame counter (model VC-436; Thalner Electronics Laboratories, Ann Arbor, MI). Video recordings were used post hoc, to supplement diode tracker data in assessing whether higher doses of amphetamine induced stereotypic head movements.

3 Results

3.1 Amphetamine's effect on dorsolateral striatum neurons

3.1.1 Amphetamine-induced changes in firing rate—Twenty-eight Long-Evans rats were successfully 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 from the 4 mg/kg group. All neurons reported here were histologically verified to be located in the dorsolateral striatum (Figure 1).

Table 1 lists the HLM results modeling the relationship between average T1 FR and average T2 FR of individual neurons. For the within neuron intercept β_0 , i.e. average T2 FR, the HLM revealed a significant interaction between dose and average T1 FR, $\gamma_{04} = -0.163286$, $t(78) = -3.287$, $p < 0.01$, and a significant interaction between dose² and average T1 FR, $\gamma_{05} = 0.124873$, $t(78) = 3.942$, $p < 0.001$. These results indicate that T2 FR of individual neurons depended on the dose of drug administered and the average pre-drug (i.e. T1) FR of the neuron. Specifically, amphetamine elevated average FRs of slow firing neurons but reduced average FRs of faster firing neurons. Further, the magnitude of elevation and reduction depend on the dose of amphetamine administered (Fig. 2). For the within neuron slope β_1 , HLM failed to reveal significant effects of dose, dose², T1 FR, or their interactions. That is, relationships among the FRs of the T1 T2 matched pairs of individual neurons were not reliably modified by dose, dose², average T1 FR or their interactions. The absence of significance indicates a lack of predictable FR dependence at a within neuron level (Figure 5).

Figure 3 shows the OLS regression of each neuron's empirical Bayesian estimated (BE) average T2 FR on its average T1 FR. These regressions elucidate interaction effect between dose² and average T1 FR on average T2 FR. 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 lower BE average T2 FR than its average T1 FR. On the other hand, a dot above the reference line indicates that the neuron represented by the dot exhibited a higher BE average T2 FR than its average T1 FR. The slope and intercept with 95% confidence interval of the OLS regression for individual doses were: dose 0, 0.357 (0.204, 0.510) and 0.502 (0.318, 0.685); dose 1, -0.102 (-0.248, 0.044) and 1.033 (0.807, 1.259);

dose 2, -0.280 ($-0.469, -0.091$) and 1.395 ($1.175, 1.614$); dose 4, 0.046 ($-0.190, 0.281$) and 1.067 ($0.808, 1.326$). Both the regression graph and the slope coefficients showed a dose-dependent and FR-dependent T1-T2 FR relationship. In Dose 0, the slope was positive yet smaller than 1, indicating that average FRs related to head movement decreased with the passage of time in the treadmill task. In contrast, doses 1, 2, and 4 displayed slopes significantly smaller (rotated clockwise) than that of dose 0, indicating that the average FRs of neurons exhibiting slower T1 FRs were enhanced by amphetamine, whereas the average FRs of neurons exhibiting faster T1 FRs were reduced by amphetamine. Further, relative to dose 0, at doses 1 and 2, the slopes exhibited a progressively greater clockwise turn whereas the dose 4 slope exhibited a lesser clockwise turn. This curvilinear relationship observed in the graphs was verified by the significant interaction between dose² and average T1 FR.

3.1.2 Reversal—A separate HLM was utilized to model the relationship between average T1 FR and average T3 FR of individual neurons to assess reversal of drug effects. For the within neuron intercept β_0 , i.e. average T3FR, the HLM failed to reveal a significant interaction between dose and average T1 FR, $\gamma_{04} = -0.069972$, $t(78) = -0.849$, $p = 0.399$, but revealed a significant interaction between dose² and average T1 FR, $\gamma_{05} = 0.134731$, $t(78) = 2.498$, $p < 0.015$. Figure 4 illustrates the results, for comparison to Figure 3. The T1- T3 slope (Fig. 4) and T1-T2 slope (Fig. 3) for dose 0 are nearly identical, whereas all three amphetamine groups exhibited a counter-clock wise turn in the T1-T3 slope compared with the T1-T2 slope, indicating a reversal of drug effects on firing. The slope and intercept with 95% confidence interval of the OLS regression for individual doses were: dose 0, 0.446 ($0.271, 0.621$) and 0.242 ($0.033, 0.452$); dose 1, 0.013 ($-0.121, 0.142$) and 0.422 ($0.213, 0.630$); dose 2, 0.016 ($-0.329, 0.361$) and 0.714 ($0.314, 1.114$); dose 4, 0.518 ($0.146, 0.890$) and 0.461 ($0.053, 0.869$).

3.2 Amphetamine's effects on behavior

3.2.1 Drug induced head movement during treadmill off—The treadmill off period was examined across T1 and T2 to assess whether amphetamine itself (in the absence of treadmill influences) induced head movements at the doses used in the present study. 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 of 25 behavioral categories. The GLIMMIX model uncovered a 4 way interaction between dose, time, distance and duration, $F(48,4655) = 2.36$, $p < 0.0001$. The 25 movement categories defined by distance and duration were blocked into 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 the interaction of dose, time, and quadrant. For doses 0 versus 1, $t(4655) = 2.18$, $p < 0.05$; for doses 0 versus 2, $t(4655) = 3.94$, $p < 0.0001$; for doses 0 versus 4, $t(4655) = 2.31$, $p < 0.0001$ indicating 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.

Video analysis during treadmill off corroborated these results. In T1, animals exhibited little or no movement, typically resting near the front of the chamber. In marked contrast, animals rarely or never stopped moving after amphetamine injection. Doses 1 and 2 induced head movements and locomotion in the form of circling and rearing within the confines of the chamber, but no repetitive head bobbing. Selectively and consistently at the high dose, locomotion was absent and was replaced by repetitive head bobbing at rates of 1 to 1.5 per sec.

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 movements 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 dose 0 and all other dose groups: for doses 0 versus 1, $t(4754)=2.2$, $p<0.05$; for doses 0 versus 2, $t(4754)=4.72$, $p<0.0001$; for doses 0 versus 4, $t(4754)=6.61$, $p<0.0001$. Figure 6 displays head movement rates in each category. Compared to the dose 0 group, which exhibited a rather stable response pattern in all movement categories across T1 and T2 (and T3), all amphetamine groups in T2 showed increased numbers of head movements in general. The clear shift downward and to the left in T2 relative to T1 indicates that all doses induced movements of greater velocity. The 1mg/kg and 2 mg/kg groups showed an intensive induction of longer distance and duration movements after drug administration. The dose 4 group, while exhibiting significantly more head movements than dose 0, showed fewer long movements compared to the lower dose groups (Figure 6). Corroborated by the induction of high frequency head bobbing selectively at the high dose during treadmill off, these data indicate that the high dose induced repetitive head movements of a shorter length. Thus amphetamine's induction of head movements exhibited an inverted U-shaped dose-response function.

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, $t(4754)=0.1$, $p=0.9213$. 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, $t(4754)=3.53$, $p<0.001$. This relationship is shown in Figure 9. Graphically, both dose 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 recovered (dose 1) or reversed (dose 2) by the last hour, T3, except at the highest dose.

4 Discussion

Amphetamine-induced changes in spontaneous vertical head movements were assessed simultaneously with changes in FR of DLS neurons related to vertical head movement. Longer movements were induced by lower doses but were less prevalent at the high dose, consistent with earlier research [2, 15]. Given the high dose's induction of significantly more head movements than dose 0, but fewer long head movements compared to lower doses, and its induction of stereotypic head bobbing, the results are consistent with Lyon and Robbins [3] synthesis, i.e., with increasing dose, the frequency of movement initiation increases while the frequency of longer duration behaviors decreases. Demonstrating dose dependent induction of movements attributed to amphetamine's actions in the striatum [10, 11], we asked: what are striatal neurons doing during these movements?

Early studies of striatal mechanisms underlying amphetamine-induced behavioral changes were not conclusive. Amphetamine iontophoresis inhibited activity [26, 27]. Systemic injection in anesthetized [28, 29] or awake animals [30, 31], decreased FR at lower doses and increased FR at higher doses. However, consideration of ongoing motoric activity

yielded a different mixture of responses: movement-sensitive firing exhibited inhibition or excitation following low or high doses of amphetamine [32, 33, 34].

Changes in DLS firing observed after amphetamine injection may reflect pharmacological effects in striatum. In addition, motor differences induced by the drug's widespread actions would alter sensorimotor signals projected to the striatum, contributing to altered DLS firing. We sought to eliminate pre- versus post-injection differences in sensorimotor inputs to striatum by comparing FRs during similar movements before and after amphetamine administration (cf. [34, 35]). This was particularly important, given the sensitivity of individual SBP neurons to movement parameters. Thus, our interpretations of differences in FR as pharmacological effects were free of potential influences of altered sensorimotor input accompanying amphetamine-induced motoric changes. Matching of movements was applied post hoc, so that movements during the experiment were not constrained, enabling verification of amphetamine's induction of unconditioned head movements and thus enabling a different perspective from other studies of stimulant effects on striatal FR during conditioned head movements [22, 36].

The significant interactions between dose by T1 FR and dose² by T1 FR indicate that T2 FR is co-determined by dose and the striatal neuron's pre-drug (T1) FR. This FR dependence was first recognized by Pederson et al [37], and helps explain the typical mixture of increasing and decreasing FRs noted above. At the "between neuron", or population level, amphetamine altered the pattern of FR change with time (Fig. 3, Dose 0). At dose 0, T2 average FRs were diminished compared to T1, indicating a natural decline in FRs during treadmill walking for two hours. This differs slightly from the T1-T2 stability of DLS FR in our previous study, which may involve the emission of thousands of reinforced head movements continuously and with little variation [22], compared with free variation in movement without extrinsic reinforcement, but more monotonous behavior during forced treadmill locomotion here. Regardless, similar to those studies of cocaine's effects [22, 36], our main finding is that DLS FRs were modified by doses of amphetamine in a firing rate dependent manner.

Specifically, as dose increased from 0 to 2 mg/kg, the relationship between average T1 and T2 FRs showed a systematic clockwise turn. As amphetamine dose increased, we observed more enhancement of FR for neurons exhibiting a slower average T1 FR, but more reduction in FR for neurons exhibiting a faster average T1 FR. This clockwise trend is the same as that observed with increasing doses of cocaine [22, 37]. Thus, the pattern of stimulant elevation of FRs of slow firing neurons has been consistently observed across all doses of both amphetamine and cocaine and across different paradigms, i.e., unconditioned treadmill walking as well as conditioning tasks (conditioned head movement and conditioned licking). Thus, stimulants likely have similar effects on the population of striatal neurons phasically related to movements. Due to physiological differences [23], these results may not extend to DLS neurons that are not sensitive to movements). Amidst numerous similar results across these studies, one difference is that reduced FRs of faster firing neurons, observed only at the high dose of cocaine, were observed at all doses of amphetamine. This yielded a strong clockwise trend across low and moderate amphetamine doses. The escalating clockwise trend did not hold at the high dose (Fig. 3, Dose 4) of amphetamine, which nonetheless increased average FRs of slower firing neurons and decreased FRs of faster firing neurons. Possible explanations of this difference include the difference between unconditioned versus conditioned head movements, given that the latter were associated with higher FR [22], or different pharmacological effects of cocaine versus amphetamine.

Cocaine blocks DAT, SERT, and NET, whereas amphetamine's major effect is reversing transport through these transporters [38, 39]. Administration of cocaine or amphetamine

increases striatal dopamine and serotonin levels [14, 40], which have been quantified in relation to amphetamine's dose-dependent induction of behavioral changes [15, 41]. With respect to dose-dependent profiles of DA versus serotonin, the suppression of faster firing striatal neurons observed here at all doses of amphetamine but only at the high dose of cocaine (20 mg/kg; [22, 36]) corresponds more closely with the strong elevation of striatal dopamine by low or high doses of amphetamine or a high dose of cocaine [15, 42] than with serotonin's elevation by low or high doses of cocaine [43] and by higher doses of amphetamine [15]. The uniform increase of low striatal FR by all doses of amphetamine or cocaine may involve elevation of either serotonin or dopamine [44, 45], or both.

As evidenced by the present study, amphetamine's effects on motor behavior are mediated partially through striatal SBP neurons, which are medium spiny projection neurons (MSNs), based on electrophysiological criteria [46], and the fact that MSNs constitute 95% of the striatal neuron population. Amphetamine-induced FR changes in SBP neurons would alter the signals sent through the pallidothalamocortical motor loop, contributing to changes in motoric activity. MSNs receive excitatory glutamatergic inputs from cortical areas, and modulatory signals from striatal interneurons and from monoaminergic neurons [47]. Cortical inputs from M1 and S1 define the receptive field of a given striatal neuron. Integration of signals from several MSNs by striatal interneurons might coordinate MSN activity and may be involved in stimulant-induced stereotypy [48]. Inputs from monoaminergic neurons might contain global information regarding the psychophysiological state of the organism. Intralaminar thalamic projections are situated to modulate both the cortical and striatal components of the corticosubcortical motor loop [49, 50]. All these signals may simultaneously modulate the activity of MSNs. The particular neurochemical balance influencing a given striatal neuron likely is reflected in its FR and by extrapolation, the differential FRs of individual neurons. Amphetamine likely shifted the DLS neurochemical balance by affecting these and other mechanisms, resulting in a differential modulation of FR which remained linked to each neuron's neurochemical balance in T1, in that it depended on the neuron's T1 FR. Although we did not study mechanisms, one possibility is that, given that excitatory input is necessary for MSNs to reach the up-state in order to fire [51, 52, 53], slow firing DLS neurons may receive input from cortical neurons that are less active than those innervating faster firing DLS neurons; S1 neurons exhibit firing rate dependent responses to stimulants [54] that could contribute to the same in DLS neurons.

The parallel, inverted U functions of dose exhibited simultaneously by changes in behavior and DLS activity are consistent with a pivotal (but not exclusive) role of the DLS in mediating amphetamine induced behavioral changes. Previous studies showed intra-striatal microinjection of amphetamine induced motoric changes [11], indicating the involvement of striatum in amphetamine's systemically induced motoric changes. However, the fact that we observed differential neural activity before and after drug administration when the animal was performing the same movements suggests that the DLS influences the movement profile in an indirect fashion. That is, the firing rate of an SBP neuron might contribute to but not exclusively determine the parameters of ongoing movements. For example, the altered FRs of head movement neurons during the same movement after amphetamine injection might function as abnormal feedback signals to premotor areas affecting real-time movement modification. As a result, the observed matched movements during T2 might not be intended by the animal in the same way as in T1, but rather reflect the disrupted DLS activity. At all doses of amphetamine or cocaine [22, 36], strong elevations in movement-related FRs of the large population of striatal slower firing neurons, during essentially non-stop movement of nearly all body parts, are projected to premotor areas, generating a loop of elevated drive to continue moving, while open-loop output activates lower motor neurons. Moreover, elevations of slow, together with reductions of fast firing DLS neurons' FRs would likely

compromise sensorimotor processing. The FR dependent effect of stimulants that we have consistently observed suggests that a narrower range in the DLS FR distribution may play a significant role in stimulant-induced behavioral dysfunction, and suggests that the natural variety of FRs of DLS neurons is important for normal motor function [55]. Further clarification is needed of differences between slow versus fast firing DLS neurons, a key distinction identified in several recent studies [22, 23, 36, 56].

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Nonstandard abbreviations

DLS	dorsolateral striatum
FR	firing rate
DAT	dopamine transporter
SERT	serotonin transporter
NET	norepinephrine transporter
M1	primary motor cortex
S1	primary somatosensory cortex
SBP neuron	single body part neuron
PETH	peri-event time histogram
HLM	hierarchical linear model
GLIMMIX	generalized linear mixed models

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Appendix

HLM

The within-neuron model consisted of the FRs of the individual matched pairs. For the within-neuron model, T2 FRs were regressed on centered T1 FRs, with a separate regression model computed for each individual neuron. Centered T1 FRs were calculated by subtracting the average pre drug FR of all matched pairs for a given individual neuron from the pre drug FRs of all the matched pairs of that neuron. This transformation rendered the intercept of the regression as the average T2 FR, which was advantageous because it allowed the level two portion of the HLM to model the average T2 FR as a function of average T1 FR 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 FR of the i th matched pair for the j th neuron, $T1FR$ is the T1 FR 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 utilized 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, dose², average T1FR of each neuron, the interaction of dose by average T1FR of each neuron, and the interaction of dose² by average T1FR of each neuron. The dose, dose², 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:

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

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

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.

Appendix Figure 1.

A path diagram representation of the HLM of neuronal firing rates (Eqs. (1)–(3)). The path diagram combines standard path analytic conventions (Hayduk, 1987; Kline, 1998) with those suggested by Curran and Bauer (2007) and Muthén and Muthén (1998–2010) for representing HLM results. The lines with one way arrows represent the regression of a dependent variable on independent variable(s), in which the dependent variable is on the end of the line with the arrow. The level 1 (within-neuron, Equ. (1)) component of the HLM model is inside the box and the level 2 (between-neuron, Equ. (2)–(3)) component of the HLM model is represented by the variables outside the box. The observed variables are represented by the rectangles. The triangles represent the level 1 (β_{0j}) and level 2 (γ_{00}) intercepts. The circles on the lines with the level 1 parameters (β_{0j} , β_{1j}) indicate that those parameters are random, i.e., they vary across different neurons. The lines with the arrows from the level 2 parameters that connect to the level 1 random effects indicate that the level 2 variables predict the level 1 random effects. The level 1 and 2 errors (e_{ij} , μ_{0j} & μ_{1j}) are

represented in the diagram as another set of predictors of the dependent variable, although without boxes. Both level 2 errors are assumed to be correlated as represented by the line with the double-headed arrows.

Highlights

- Rats were required to locomote on a treadmill as a means of inducing head movements
- Striatal head movement neurons were recorded along with head movement profiles
- Amphetamine altered head movement profiles in the task dose-dependently
- Activity of head movement neurons was affected by amphetamine dose-dependently
- Both head movement and firing exhibited inverted-U shaped dose-response functions

Fig. 1.

Location 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 at that location. Six coronal sections of striatum are shown with the anterior posterior distance from bregma labeled [57].

Fig. 2.

Representative head movement neurons' firing pattern before and after injection of different doses of amphetamine (five rows). Rasters center neuronal discharges on initiation of head movements (time zero). Cyan dots on right side of time zero indicate completion of each head movement. Head movements were sorted by duration. PETHs display firing rates around the initiation of head movements. The three columns represent pre-administration (T1), post-administration (T2), and reversal (T3). Row one: Firing rate of the neuron did not show substantial modulation after saline injection. Row two: This neuron's firing rate increased after 1 mg/kg amphetamine injection and decreased during last hour of experiment, indicating reversal. Row three: This neuron's firing rate was reduced after 2 mg/kg amphetamine injection and increased during last hour, indicating reversal. Row four:

This neuron's firing rate was enhanced after 2 mg/kg amphetamine injection and reduced during last hour, indicating reversal. Row five: This neuron increased firing rate after 4 mg/kg amphetamine injection; firing rate did not further increase but partially reversed during last hour. Firing rate changes after amphetamine injection depended on pre-drug (T1) firing rate, increasing for slow firing neurons but decreasing for faster firing neurons.

Fig. 3.

Firing rate dependent effect of amphetamine. 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. Each dot represents one neuron. Change in firing rate after drug injection was co-determined by dose and pre-drug (T1) firing rate of the neuron.

Fig. 4.

Reversal during last hour. Graphic representation of results of the hierarchical linear model on T1-T3 firing rate (spikes/sec). Empirical Bayesian estimates derived from HLM of the average T3FR 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. Each dot represents one neuron.

Fig. 5.

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 (left), the other with higher average T1 firing rate (right). Each scatter plot represents one neuron, and each dot represents one matched pair. The vertical line in individual scatter plots indicates mean T1 firing rate.

Fig. 6.

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

Table 1

HLM results modeling the relationship between average T1 firing rate and average T2 firing rate of individual neurons.

Standard Approx.					
Fixed Effect	Coefficient	Error	T-ratio	d.f.	P-value
For INTRCPT1, β_0					
INTRCPT2, γ_{00}	1.083036	0.094126	11.506	78	*** $p<0.001$
CDOSE, γ_{01}	0.104402	0.040384	2.585	78	* $p<0.05$
CMT1HZ, γ_{02}	-0.25691	0.052482	-4.895	78	*** $p<0.001$
CDOSESQU, γ_{03}	-0.03991	0.031548	-1.265	78	0.210
DST1HZ, γ_{04}	-0.16329	0.049679	-3.287	78	** $p<0.01$
DSSQT1HZ, γ_{05}	0.124873	0.031678	3.942	78	*** $p<0.001$
For CT1HZ slope, β_1					
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

CDOSE: centered dose; CMT1HZ, centered mean T1 firing rate; CDOSESQU, centered dose squared;

DST1HZ: dose* T1 firing rate; DSSQT1HZ: dose²*T1 firing rate.

For β_0 , average T2 firing rate, HLM revealed a significant interaction between dose*mean T1 firing rate and dose²* mean T1 firing rate.

Table 2

HLM results modeling the relationship between average T1 firing rate and average T3 firing rate of individual neurons.

Standard Approx.					
Fixed Effect	Coefficient	Error	T-ratio	d.f.	P-value
For INTRCPT1, β_0					
INTRCPT2, γ_{00}	0.58794	0.143351	4.101	78	*** $P < 0.001$
CDOSE, γ_{01}	0.049865	0.054149	0.921	78	0.360
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	* $P < 0.05$
For CT1HZ slope, β_1					
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

CDOSE: centered dose; CMT1HZ, centered mean T1 firing rate; CDOSESQU, centered dose squared;

DST1HZ: dose* T1 firing rate; DSSQT1HZ: dose²*T1 firing rate.

For β_0 , average T2 firing rate, HLM revealed a significant interaction between dose*mean T1 firing rate and dose²* mean T1 firing rate.