A LONGITUDINAL ANALYSIS OF MEDIUM SPINY NEURON ACTIVITY IN THE DORSOLATERAL STRIATUM DURING CHRONIC COCAINE SELF-ADMINISTRATION

By

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ABSTRACT OF THE THESIS

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Substance abuse is defined by continued consumption of drugs despite their negative consequences, and its treatment is plagued by exceptionally high relapse rates (40-60%). Current behavioral and neurobiological theories of substance abuse predict that with chronic use, drug consumption becomes habitual and neural activity shifts from the nucleus accumbens (NAc) to the dorsolateral Striatum (DLS). In the present study, we sought to test the latter theory: that in the course of chronic cocaine self-administration, DLS neurons acquire phasic patterns of firing in relation to drug-taking behaviors. We recorded from single body part (SBP) neurons in DLS that are specifically related to vertical head movement, (i.e. neck or head sensitive neurons) as well as a control group of non-SBP neurons. Animals self-administered cocaine using a vertical head movement operant and exhibited behavioral evidence of skilled self-administration. To analyze changes in neural firing rate (FR) across cocaine self-administration, we developed a custom generalized mixed model (2x2x12) with 2 levels of *Neuron Type* (*Head Movement and Control*), 2 levels of *Firing Type* (*Phasic and Baseline*) and 12 levels of recording *Session*. *Baseline (non-*

movement) FR decreased in DLS neurons across days, but this decrease was confined to *Head Movement* neurons. *Phasic* (during head movements) FR differed significantly across sessions in both *Head Movement* and *Control* neurons. However, *Phasic* FR was significantly greater than *Baseline* FR only in *Head Movement* neurons, during the first two weeks (*Days* 3-4, 9-10, and 11-12). In the last two weeks, the population of *Head Movement* neurons in DLS contributed less to drug-taking behavior. Inconsistent with the tested theory, DLS does not become globally more active with chronic cocaine SA. Instead, chronic cocaine self-administration is related to a decrease in DLS activity, specifically in neurons that process the skill required for self-administering. Consistent with the tested hypothesis however, a small number of neurons acquired progressively more robust head movement activity after 24+ days of self-administration. During abstinence, these neurons could be responsible for processing or executing relapse behaviors.

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Introduction

Substances have been ingested throughout history for a variety reasons ranging from basic medical needs, to recreation, and religious experience. However, certain classes of substances can lead to abuse disorders. Substances that are most likely to be abused tend to share a common process: they mimic the effect of primary incentives (i.e. food, water, sexual behavior, etc.), and produce motivation to seek and use the substance. They also act directly or indirectly on the brain's reward processing and motivated behavior circuitry [1].

Substance abuse is defined by continued consumption of drugs despite their negative consequences [2], and its treatment is plagued by exceptionally high relapse rates (40-60%) [3]. Resistance to negative consequences is also a characteristic of the operationally defined animal model of habitual behavior [4]. Current behavioral and neurobiological theories of substance abuse have evolved from this notion. Drugs are first thought to be pursued in a goal-directed manner, and consumed specifically for their hedonic properties [5][6][7][14]. During this goal-directed period animals are thought to encode stimulus-action-outcome contingencies. Discrete and diffuse drug-cues become associated with the act of self-administration and the immediate and extended consequences of drug consumption. With chronic use, the act of self-administration becomes skilled and is thought to lose its association with the outcome. Researchers have hypothesized that this transition underlies substance abuse and is responsible for drug consumption which is stimulus bound, effortless, and executed without regard to its consequences [8][9][10][11][12][13].

While substance abuse appears to share qualities with habitual behavior there is some evidence to the contrary. For example, animals self-regulate drug intake based on their current calculated drug level, which they are capable of maintaining within a narrow preferred range [15][16]. By non-contingently maintaining an animal's drug level within its preferred range, the outcome of drug consumption can be experimentally devalued. During this type of manipulation animals reduce responding [15]. Drug consumption can also be overvalued by non-contingently decreasing an animal's drug level below its preferred range, causing a sharp spike in operant responding. During binges, self-administration behavior remains associated to its outcome: the cocaine infusion. However, evidence from studies during abstinence suggests that habitual associations have formed. Because no drug is consumed during abstinence, these associations must have formed during a prior binge.

Evidence for habitual behavior has been observed during reinstatement and relapse testing in animals. For example, in a study from our laboratory [17], after chronic cocaine self-administration animals were non-contingently administered cocaine paired or unpaired with lithium-chloride (to produce sickness). After a period of abstinence, animals that experienced cocaine paired with sickness responded no differently from unpaired controls. This indicates that a habitual association formed during the binge was being expressed during relapse testing [17][18]. It may be that during abstinence previously drug-paired cues produce relapse behaviors before the user processes the outcome of drug intake. This is also supported by ultra-sonic vocalizations studies, which show

that contextual reinstatement does not necessarily involve a hedonic response [60] (a property of goal-directed drug consumption). This could explain why relapse is so pervasive in humans, despite explicit goals of non-consumption. Ironically, the associations between cues and drug consumption behaviors which could underlie habitual relapse behaviors must be learned during prior goal-directed (non-habitual) binges. These associations may manifest as habits during abstinence. Understanding how the brain areas thought to encode skilled and habitual behavior change during chronic drug binges could lead to strategies aimed at fighting relapse.

The dorsolateral striatum (DLS) has been repeatedly implicated in skill learning, [19][20][21][22][23] and in the expression of habitual behavior [24][25][26][27]. DLS is comprised mainly of type IIb, medium spiny projection neurons [28] [29] which receive the appropriate anatomical inputs to facilitate stimulus-action associations necessary for habit formation, and also projects to the appropriate outputs to produce skilled behavior. DLS receives monosynaptic input from sensory-motor cortical regions (S1 and M1)[30][31][32][33][34][35][36][37], as well as multi-synaptic input from the reward centers in ventral striatum (accumbens & ventral pallidum) through their projections to midbrain dopamine neurons (VTA and SNc)[38][39][40][41][42][43]. DLS efferent projections output via globus pallidus (GPi) and substantia nigra reticulata (SNr) to motor thalamus (VA and VL) [44], which then projects to motor (M1) and pre-motor cortex (PMC) [45][46][40]. These anatomical properties allow DLS to integrate sensory and motor processing, and

make it prime target for research relating neural changes to chronic cocaine use. These changes may promote habitual behaviors during abstinence.

Current theory predicts that behavioral control shifts from the nucleus accumbens (NAc) to the dorsolateral striatum (DLS) during chronic cocaine self-administration [47][48][49][59]. Although the NAc and DLS were once thought to belong to separate cortico-subcortical loops, recent anatomical studies have made it clear that the NAc has the capacity to influence the DLS through projections to midbrain dopamine neurons [38][40][41][42][43]. Because of this, many researchers have attempted to determine if the DLS is involved in drug consumption [48][49][50][18]. Currently, there are three types of findings that support the role of DLS in the habitual model of substance abuse. First, manipulations of DLS neurons, such as lesions [18] or dopamine antagonism [50] have been found to cause a reduction in habitual reinstatement and cue controlled drug consumption. Second, extracellular recordings of DLS neurons show that they become progressively more active across days of skill learning [22]. Third, glucose utilization in striatum (a global measure of neural activity) decreases progressively across chronic cocaine self-administration [47]. Unfortunately, manipulations that rely entirely on inactivation of DLS interfere with sensorimotor processing and thus confound habit testing [18][50]. Furthermore, extracellular recording studies have ignored the somatotopic property of DLS neurons [48][22], and contradict Porrino and colleagues' study of glucose utilization. In the present study, we sought to test this same theory: that in the course of chronic cocaine self-administration, DLS neurons will acquire and maintain phasic patterns of

firing in relation to the drug-taking operant. However, we avoided any unnatural manipulations of DLS, and explicitly controlled for the aforementioned somatotopic nature of DLS neurons.

The DLS receives direct topographic inputs from somatomotor regions, each representing discrete body parts [51][52][53][54][55]. Consequently, DLS is segmented into clusters of cells that each respond to passive or active manipulation of a single body part [53][55][56]. In order to appropriately interpret the results of DLS recordings during instrumental behavior, one must control for or eliminate neurons that aren't directly involved in the movement being measured. Analogously, one wouldn't make inferences from M1 leg neurons while studying finger movements. To satisfy this concern, we recorded DLS neurons related to head movement using a new operant device we have developed which precisely measures and reinforces head movements in rats. Using the device, animals learned to perform upward head movements of a minimum distance (~40mm) in order to self-administer cocaine. This vertical head movement was shown to become highly skilled across sessions [15], and unlike an "all or nothing" lever press multiple descriptive analyses of the operant movement were performed: the number of head movements was tracked across days, the velocity of each head movement was tracked, and the efficiency of the movement (accuracy of start and end positions) was assessed across sessions.

We recorded from single body part (SBP) neurons in DLS that are specifically related to vertical head movement, i.e. neck or head sensitive neurons, while animals performed operant head movements for cocaine. Our

recording system enabled tracking the activity of the same neuron(s) across multiple sessions. To ensure that any changes in neuronal firing across sessions are not spuriously related to differences in motor behavior, only neural activity during matched head movements was analyzed. That is, only firing during movements of the same start position, duration, and end position (and thus velocity) were analyzed longitudinally. This control removed most of the possibility that simple differences in sensorimotor processing (different behaviors) could interfere with our analysis. These measures strengthen our interpretations of changes in neuronal activity related to chronic cocaine self-administration and skill learning.

If the SBP neurons in dorsolateral striatum became progressively more engaged throughout training compared to a control set of neurons, it would provide support for the hypothesized shift in neural processing from ventral striatum to DLS during chronic cocaine self-administration. This can also help determine whether the effects of chronic cocaine self-administration are universal, or whether they are specific to the set of neurons which process the operant response.

Methods

Animals and Surgical Procedures

In male Long–Evans rats (Charles River, Wilmington, MA), a catheter was surgically implanted in the right jugular vein and a 16 micro-wire array (Micro-Probes, Gaithsburg Maryland) was implanted in the right DLS. Arrays were constructed from $50_{\mu m}$ stainless steel wires quad coated in Teflon® insulation.

Wires were arranged in a 2x8 comb, with $300_{\mu m}$ between wires and rows (Figure, 1). Arrays were implanted through an angled rectangular craniotomy, with the following corners (X_{mm},Y_{mm}) relative to bregma [(2.8,2.5) (3.4,2.6) (3.4,-0.5) (4.0,-0.4)]. Arrays were lowered using a motorized stereotax [57] at a rate of $200_{\mu m}$ per minute to a depth of 3.9_{mm} below bregma. Recovery took place individual Plexiglas® self-administration chambers, which served as the animals' home cages for the duration of the experiment. Food intake was reduced during the two days preceding the first self-administration session to increase mobility. Animals were provided with food after each self-administration session, to maintain a healthy body weight of roughly $325\text{-}335_{g}$. Cocaine self-administration generally leads to weight loss, so food intake was increased gradually.

Apparatus

The clear Plexiglas® boxes were contained within larger sound attenuating chambers, lighted on a $12{:}12_{h}$ light/dark cycle (dawn at 11:30am). A custom photocell device designed to monitor operant head movements was located on the back left corner of the box, 1.5_{cm} above the floor, outside the Plexiglas®. The photo cell device consists of 6 infrared-emitting diode / receiver pairs, (HOA6299, Honeywell, Morristown NJ). In the device, photocells are stacked on top of one another in a 50^{o} arc over 69_{mm} in order to capture vertical head movements (Figure 2) [20]. All photocell beam breaks were recorded by MED-PC (MED-Associates, Georgia, VT) for offline analysis of different movements, using custom Matlab® scripts. A 5_{cm} x 5_{cm} x10_{cm} white acrylic block was secured in the photocell corner three days prior to the start of self-

administration. The block remained in place whenever the animal was not actively engaged in a self-administration session (i.e. prior to, and after every self-administration session) in order to prevent overnight extinction.

Body Exam

Prior to self-administration, all animals underwent a full body exam. Neuronal signals were amplified, and played through a pair of headphones, to confirm spiking. Neurons were categorized while each body part was passively manipulated, or while the body part was actively moving. For a neuron to be considered body part sensitive, a noticeable burst in firing had to occur during the active or passive manipulation of that body part alone (Sup. Video, 1). These categorizations were used as confirmation of each neuron's single body part specificity outside the self-administration task. Only neurons that were previously verified to process vertical head movement were analyzed in the head movement data set. Neurons that were not classified as body part sensitive during the body exam were entered into a control data set, to determine if changes in DLS firing were specific to the neurons involved in the head movement operant.

Self-Administration Training

Animals underwent 25 days of self-administration training. Sessions lasted 6 hours, or until 80 infusions were earned, whichever occurred first. The first ten infusions were considered a 'loading' period, and were marked by a shortened inter stimulus interval (10_s). A criterion head movement during the load period produced a CS tone, and a 7.5_s ($0.71_{mg/kg}$) infusion of cocaine. Criterion head movements made during the ITI produced the CS tone and were

recorded with no programmed consequence. After the first ten infusions, the load period was considered complete, and the inter-stimulus interval was extended to a pseudorandom variable interval 30 second schedule. The cocaine infusion was also shortened to 3.75_{s} ($.355_{mg/kg}$) for the next 10 infusions. Finally, the infusions were shortened to 1.875_{s} ($.1775_{mg/kg}$). This is considered the 'maintenance' phase of training. The maintenance phase was designed so that animals can produce the maximum number of head movements while maintaining a stable drug level. This allowed them to repeat the same movements hundreds of time per session, providing the best conditions for skill learning and habit development, as well as generating adequate numbers of movements for neural analysis.

Behavioral Analysis

Behavioral variables (criterion head movements, velocity, etc.) were analyzed as a function of training day, using repeated measures ANOVAs (PASW 18, Chicago IL). The alpha criterion for all tests was 0.05. For any repeated measures ANOVA where sphericity could not be assumed, a Huynh-Felt correction was applied. Corrected contrast tests (Holm-Bonferroni) were used to determine where behavior stabilized, by comparing each day in the first two weeks to the last week of self-administration.

Electrophysiological Recordings

Animals were recorded approximately every other day for 25 days. Neural signals were stored digitally for offline analysis. During each session, electrophysiological recordings began concurrent with the start of self-

administration, and terminated at the end of the session. Isolation of individual neural waveforms from background noise was performed offline using SciWorks spike sorting and separation software (DataWave Technologies; Longmont, CO). All waveforms of the putative individual neuron during the entire session (6_h) are displayed in temporal order on a computer-simulated oscilloscope to assess the stability of neural waveforms within session. Waveforms whose parameters did not remain stable were discarded. An inter-spike interval (ISI) histogram was also constructed. If discharges occurred within the first 2_{ms} in the ISI, corresponding to a neuron's natural refractory period, the recording was not considered that of a single neuron and was discarded. Neurons exhibiting signal-to-noise (SN) ratios less than 2:1 were also discarded.

Calculation of Baseline Firing Rate

Baseline FR was defined as the average FR during all non-movement instances on a given day. Video tracking analysis in each session isolated 1 second periods of non-movement in which the animal's tracked position (head position) did not deviate (±3 pixels; Figure 3). To ensure that differences in inclusion and exclusion during spike sorting did not affect FR measures, neurons were excluded from analysis if the neuron's change in SN ratio was significantly and positively correlated with *Baseline* FR change (n=2). If a neuron's waveforms (i.e., signal) became more difficult to separate from noise, forcing exclusion of signal and *Baseline* FR to decrease, it was removed from analysis. For the remaining neurons used in this analysis, changes in *Baseline* FR could not be explained by spike sorting differences across time. The *Baseline* FR for

each neuron was entered into the model of FR change, to determine if changes in *Phasic* and *Baseline* FR are related.

Construction of Peri-Event Time Histograms

Because of the somatotopic nature of DLS, only firing within the operant head movement was analyzed with respect to drug-taking. *Phasic* firing that occurs during head movements was visualized by constructing rasters and peri-event time histograms (PETHs) that displayed neuronal discharges within $\pm 1_s$ of the end of each head movement. The offsets of head movements were aligned at time zero, and the onsets of head movements were sorted by length and indicated with a colored dot on the raster. The histograms represent the average FR during 10_{ms} bins surrounding the head movement and are color coded by day.

Calculation of Phasic Firing Rate

Movements were sorted and blocked into 50 discrete categories of start position, end position and duration. Start position is broken into 4 bins starting at photocell (PC) 1 and incrementing by 1 until PC 4, while end position is broken into 4 bins starting at PC 3 and increment by 1 until PC 6. Duration consists of 5 bins starting at 100_{ms} and incrementing by 180_{ms} until 1000_{ms}; note that only movements longer than 100_{ms} that contain at least 2 whole PC breaks were accepted and many categories at the extremes contain no observations (Figure, 4). Movements from different sessions that fall into the same category of start position, distance, and duration were considered “matched sets”. In accordance with our previous work, only categories containing at least 5 movements per session were considered for analysis [20]. A neuron’s *Phasic* FR was calculated

from the average FR within categories that could be matched across a minimum of 4 sessions. All movement categories that did not meet these criteria were discarded. Each session's *Phasic* FR was calculated from the same movement categories selected from all other sessions, thus creating matched sets that controlled for sensory-motor differences across sessions.

Analysis of Firing Rate Change

Modeling of behaviorally matched firing rates was accomplished using a custom generalized linear mixed model. The model is 2x2x12, with 2 levels of *Neuron Type* (*Head Movement & Control*), 2 levels of *Firing Type* (*Phasic & Baseline*), and 12 levels of recording *Session* (Figure, 5). Using this model we asked a series of explicit questions about the effect of chronic cocaine self-administration on DLS neurons:

1) Is there a difference in the relationship between *Baseline & Phasic* FR for *Head Movement* and *Control* neurons? (*Neuron Type* x *Firing Type* Interaction)

2) Is there a difference in the change across *Sessions* for *Head Movement* and *Control* neurons? (*Neuron Type* x *Session* Interaction)

3) Is there a difference in change across *Sessions* for *Baseline* and *Phasic* FR? (*Firing Type* x *Session* Interaction)

Significant interactions would highlight the need to differentiate among body part sensitive neurons and to determine the particular body parts being moved during any recording of DLS neurons. Under that same model, we used simple effects

interactions and post-hoc tests to ask questions which clarify the specific nature of changes in DLS neurons:

1) Does the relationship between *Baseline* and *Phasic* FR change across sessions for either *Head Movement* or *Control* neurons? (*Session* x *Firing Type* Interaction – sliced by *Neuron Type*)

2) Which of the four categories of FR (see Figure, 5) change significantly across sessions? (*Neuron Type* x *Firing Type* x *Session* - sliced by *Neuron Type* x *Firing Type*)

3) On which days does each *Neuron Type* contribute most to the drug-taking behavior? (Holm-Bonferroni corrected post-hoc comparisons of *Phasic* and *Baseline* FR – sliced by *Neuron Type*)

Notably, interpreting the main effects of this model is inappropriate. For example, interpreting the main effect of session would collapse across neurons with different somatomotor sensitivities during both movement and non-movement. This failure would be similar to that of most other studies of DLS firing, which do not address the fundamental somatotopic property of DLS neurons.

Results

Behavioral Indices of Skill Learning

Animals in this task exhibited behavioral evidence of skilled self-administration (Figure, 6). Animals significantly increased drug-taking head movements [$F(20,360)=5.83$, $p<.001$; Figure, 6a] and total drug consumption [$F(20,360)=8.78$, $p<.001$; Figure, 6b] across sessions. Animals also significantly

increased their movement velocity across sessions [$F(20,360)=1.68$, $p=.034$; Figure, 6d], providing evidence that the head movement behavior became skilled. Finally, animals learn to begin their head movements closer to the required start position across sessions [$F(20,360)=4.35$, $p<.001$; Figure, 6c], providing evidence that the animals' movements became more efficient across sessions. Interestingly, animals did not learn to stop their head movement at the maximum required photocell. There were no consequences for making a movement that was too long, so animals may have been employing a strategy that minimized failed movements, as opposed to making the maximally efficient movement.

Histology

Microwire arrays (2x8) were implanted into 18 animals (Figure, 1). Of those 288 implanted wires, 265 were localized to the DLS using immunohistochemical staining for calbindin 28-k. From those 265 wires, 235 single units were isolated during offline spike sorting. Of those 235 units, 27 (11.4%) were deemed head or neck movement sensitive during the body exam. Of the remaining single units, 30 (12%) were randomly selected from neurons not categorized as being body part sensitive during the body exam, to serve as a *Control* group. The locations of all neurons in the final analysis are shown on representative histological slices (Figure, 7). While some neurons appear to be located in the calbindin positive region of DMS on these representative slices, they were in fact localized to calbindin negative regions on their actual histological slices. A small number of neurons ($n=7$) were recorded before the

introduction of immumohistochemistry to this study, and were localized to DLS using atlas overlays with the definitions provided in our previous DLS studies [53].

Preliminary Graphical Analysis of Head Movement Neurons

Preliminary exploration of the data showed that there is heterogeneity in the longitudinal trends of *Head Movement* neurons. For some neurons, *Phasic* firing rate tends to decrease across training (Figure, 8a), while for others *Phasic* FR tends to increase (Figure, 9a), and for still others FR remains relatively unchanged (Figure, 10a). These raster-PETHs were not intended to be directly analyzed, but they facilitate visualization of the specificity with which DLS neurons process particular movements. Firing in these neurons is time locked to the beginning and end of head movements. The spikes used for modeling of *Phasic* FR were extracted from recorded head movements (between the colored dots and dashed line; Figure, 8a; 9a; 10a) and were divided by each movement's duration to yield firing rate. Modeling of *Phasic* FR was behaviorally controlled through matched sets. Interestingly, individual matched sets exhibited their own trajectories across sessions (Figure, 8b; 9b; 10b). Not only did individual neurons' *Phasic* and *Baseline* FR appear to show different trends across sessions (Figure, 8b; 9b; 10b), but the FR for discrete movements within neurons (i.e., individual matched sets) changed differentially across sessions. Importantly, trends were not related to any changes in the SN ratio of our recordings, likely because of the stability of recordings. We observed relatively little change in recorded waveforms across sessions (Figure, 8a; 9a; 10a). There is also no indication that any of the changes in these neurons are linear. Accordingly, our

model of population FR includes *Session* as a categorical variable. *Baseline* FR was also included in the population model, to control for spike sorting differences across days. If *Baseline* and *Phasic* FR change differently across *Sessions*, the changes cannot be explained by differences in spike sorting, which should affect *Baseline* and *Phasic* firing equally.

Change in DLS Activity across Chronic Cocaine Self-Administration

Modeling of FR was accomplished using a custom generalized mixed model with 2 levels of *Neuron Type* (*Head Movement* & *Control*), 2 levels of *Firing Type* (*Phasic & Baseline*), and 12 levels of recording *Session*. While there was a significant main effect of *Firing Type, Neuron Type*, and recording *Session*, these results are not interpretable. The main effect of *Firing Type* ignores whether differences between *Baseline* and *Phasic* firing are genuine, or due to the inclusion of neurons with different somatomotor sensitivities. The main effect of *Neuron Type* does not determine whether differences between *Head Movement and Control* neurons are genuine, or due to differences in behavior. And the main effect of *Session* does not determine whether differences across *Sessions* are genuine, or due to differences in behavior or somatomotor sensitivities. Thus, we attempt to clarify these questions by analyzing the interactions in the model.

First, we explored the relationship between *Baseline* and *Phasic* firing rates for *Head Movement* and *Control* neurons. We found a significant *Neuron Type x Firing Type* interaction [$F(11,966)=20.02$, $p<.0001$], indicating that the relationship between *Baseline* and *Phasic* FR differed between *Head Movement*

and Control neurons (Figure, 11). This was expected, as the *Control* neurons do not unconditionally process the head movement, and therefore do not show increased *Phasic* FR compared to non-movement (*Baseline*) FR. However, this interaction collapses session, so it does not help determine how DLS changes longitudinally. Next, we determined if there was a difference in the change across sessions for *Head Movement* and *Control* neurons. We found a significant *Neuron Type x Session* interaction [$F(11,966)=2.37$, $p=.0068$], indicating that the *Head Movement* and *Control* neurons change differentially across chronic cocaine self-administration (Figure, 12). While this test collapses *Baseline* and *Phasic* firing, it highlights the need to differentiate between body part sensitive neurons in DLS, as these neurons are constrained to behave differently based on their somatomotor sensitivities, or lack thereof. Next, we examined whether there was a difference in the change across sessions for *Baseline* and *Phasic* FR. We found a significant *Firing Type* x *Session* interaction [$F(11,966)=2.40$, $p=0.0061$] indicating that *Phasic* and *Baseline* FRs change differentially across sessions (Figure, 13). While this test collapsed *Head Movement* and *Control* neurons, it highlights the need for strong behavioral matches in longitudinal designs. It also provides a control for spike sorting differences across days, which would affect *Baseline* and *Phasic* FR equally.

Still, in order to determine the exact nature of these changes, we analyzed simple effect interactions and corrected post-hoc comparisons within groups.

Baseline and *Phasic* FR changed differentially across sessions for *Head Movement* and *Control* Neurons (*Session x Firing Type* interaction - sliced by

Neuron Type) for both *Head Movement* [*F(*23,966)=9.33, *p*<.0001; Figure, 15] and *Control* neurons [*F(*23,966)=6.88, *p*<.0001; Figure, 14]. Although it is clear that *Baseline* and *Phasic* FR underwent more change in *Head Movement* neurons than *Control* neurons, these results reinforce the need for precise behavioral matching when comparing firing rates in DLS. Because of the direct glutamatergic input from M1 and S1, neurons in DLS exhibit *Phasic* FR modulations during movement. Even when comparing firing rates between successive trials, it is imperative to ensure that differences in movement are not confounding results. We also found that changes across *Sessions* were confined to particular *Neuron Type* x *Firing Type* groups. We found a significant effect of *Session* for *Head Movement* neurons in both *Phasic* [*F(*11,966)=5.02,*p*<.0001; Figure 15] and *Baseline* [*F(*11,966)=3.57,*p*<.0001; Figure 14] FR. Both *Baseline* and *Phasic* FR tended to decrease across sessions for *Head Movement* neurons, although they changed at different rates. Both sets of firing rates also tended to increase slightly near the end of training, which may be due to a small population of neurons which increased in firing rate late in training. We also found a significant difference across *Sessions* in the *Control* neurons for *Phasic* FR [*F(*11,966)=2.96,*p*=.007; Figure, 13], but not for their *Baseline* FR [*F(*11,966)=1.51, *p*=.121; Figure, 13]. Although significant, it is clear that the change across *Sessions* of *Control* neurons was much smaller than the changes of *Head Movement* neurons.

Finally, we wanted to determine on which days *Phasic* firing rates were different from *Baseline* firing rates. That is, on which days were neurons

contributing most to the operant head movement? The leading hypothesis suggests that neurons in DLS are most influential late in chronic cocaine self-administration. However, we found the opposite to be true: *Head Movement* neurons spike significantly more during head movements than *Baseline* early in training. Specifically they fire more during movement on *Sessions* $2^{p=.007}$, $5^{p=.032}$, and $6^{p=.037}$ (Figure, 15). *Control* neurons did not have significantly differently *Phasic* and *Baseline* FR on any individual *Session*. These results are in accordance with our previous studies of DLS activity, showing that the influence of body part sensitive neurons wanes across skill learning [19][20][21].

Discussion

Animals in this task learned to self-administer cocaine in a skilled fashion, similar to animals with only a catheter leash but without a recording harness attached to the skull [15]. This is important, because for drug-taking behaviors to be expressed habitually at relapse, it is essential for skilled self-administration to be repeatedly associated with drug-related cues. Even if behavior appears goal-directed during binge behavior, all of the stimulus-response associations that could form and underlie a habit are occurring during active self-administration. That is, if animals express habits during relapse testing, they must have learned those associations during self-administration, i.e., during prior binges. That is precisely why we chose to study the effects of chronic cocaine SA on a brain region that has been repeatedly implicated in skill learning, and the acquisition of habitual behavior. It has been hypothesized that dorsolateral striatum undergoes changes during cocaine SA that make it more influential after extended training,

and is presumed to be responsible for the expression of habitual relapse. However, while most modern theories of substance abuse assume this progression, it is inconsistently supported by disparate physiological evidence [18][22][47]. To our knowledge, the present study is the first to systematically examine changes in DLS activity across chronic cocaine self-administration, while explicitly controlling for the somatotopic nature of these neurons.

Out of a total of 288 wires implanted, 235 units were recorded and isolated. Of those 235 units, 27 (11.4%) were classified as head movement sensitive, while 30 (12%) others were selected as neurons not sensitive to activity of body parts, to serve as controls. While this is a relatively low number of units, each neuron was recorded 6 hours a day for 4-12 days. In all, 541 neuron sessions were sorted offline for a total of 3246 hours of single unit activity. This is the most data to ever enter one of our electrophysiological models. Recent advances in robotic surgery and immumohistochemistry have allowed us to track microwires accurately (Sup. Video, 2) and localize the tips using the chemical signature of the brain region. In this case, DLS stains negative for calbindin-d28k as compared to the surrounding striatum, and wire tips were localized to the calbindin deficient area (Figure, 7).

Dorsolateral striatum is often cited as becoming progressively more involved after chronic cocaine self-administration. However, one of the strongest pieces of evidence cited somewhat inappropriately in support of this claim actually describes a progressive decrease in glucose utilization in the DLS of monkeys self-administering cocaine [47]. Measuring 2-deoxyglucose allows one

to infer a brain area's activity level is from its metabolic needs [58]. The technique has relatively low temporal resolution, ostensibly making it a measure of baseline activity. Interestingly our measure of *Baseline* FR agreed with, and further clarified the results of Porrino and colleagues [47]. *Baseline* FR in our animals decreased in DLS neurons across days, but this decrease was confined to the *Head Movement* neurons. Only neurons directly processing the operant response exhibited decreases in *Baseline* FR (Figure, 15). This finding suggests that cocaine may selectively affect the behaviorally relevant neurons; changes in DLS are selective to neurons involved in learning the SA skill.

DLS activity during the operant response (*Phasic* FR) also changed significantly across *Sessions*. This trend was primarily driven by a decrease in *Head Movement* neuron activity during the first two weeks of training, followed by a small increase in the remaining weeks. Inconsistent with the original hypothesis, DLS neurons were found to contribute most to drug-taking movements early, rather than late in training. *Phasic* FR was significantly greater than *Baseline* FR only during the first two weeks (*Session* 2, 5 and 6), meaning that in the last two weeks, the population of *Head Movement* neurons in DLS was contributing less to the drug-taking behavior. Consistent with the tested hypothesis, heterogeneity was observed in the way individual neurons changed across time. Individual neurons' FR increased, decreased, or remained relatively unchanged, and there was no indication that these changes were linear. A small number of neurons even acquired progressively more robust head movement activity after 23+ days of self-administration (Figure, 9a). This heterogeneity may

involve the anatomical properties of DLS. Somatotopically organized neurons in DLS exist in 3-dimensional clusters (~300$_{\mu m}$ across) which all process the same body part (e.g., head movement / neck musculature). These clusters may work together in a process that does not require the same neuron to perform precisely the same job every day or even during every movement. Still, our population model gives us the clearest picture to date of the changes that occur in DLS with chronic cocaine SA. It shows that the theorized shift in activity toward DLS is not occurring on a population level, and at best is an oversimplification of a specific upward trend in a minority of neurons explicitly processing drug-taking behaviors.

The complexity inherent in large scale data analysis often poses more questions than it answers. The first and foremost being, what do the opposite (decreasing and increasing) trends in different *Head Movement* neurons represent? The population level decrease in activity may represent a decrease in the role of DLS in executing or processing the movement. This processing could have shifted throughout training to efferent structures such as motor thalamus, or pre-motor cortex. Alternatively, DLS could become more efficient at processing or executing movements with training. It may be that many neurons initially needed for a behavior are replaced by a select, efficient ensemble. The neurons in our study which became progressively more involved in processing the head movement may be analogous to proposed striatal “expert” neurons [61][62]. These neurons become sharply tuned to particular behavioral events during t-maze learning, and after extinction, they regain their phasic activity during reinstatement. Our data appear to be concordant with the notion that early in

training many candidate neurons spike during a behavior, but with extended training and competitive selection, neurons with sharply tuned responses appear [62], although sharply tuned responses to body part movements exist unconditionally in DLS neurons [53]. Still, the “expertly” tuned neurons in our task remain active after chronic cocaine SA, and could be the DLS neurons responsible for expressing habitual behaviors in abstinence, in response to drug cues. However, medium spiny projection neurons are unresponsive to the antecedent cues responsible for triggering habits [70]. For this hypothesis to be true, another cell type, e.g., parvalbumin interneurons would have to process the drug related cues and gate the expert MSNs responsible for executing habits.

There is some controversial anatomical evidence surrounding the hypothesis that distinct striato-fugal projections arise from separate neuronal populations in the striatum named the direct and indirect pathways. These subpopulations are thought to have exclusively D1 or D2 dopamine receptors, project to separate segments of the globuls pallidus, and have ostensibly opposing effects [63]. However, tract tracing studies have revealed an abundance of striatal projection neurons with highly collateralized axons that provide branches to two or three of the striatal recipient structures [64]. Furthermore, some anatomical studies have shown that 78-100% of striatal neurons contain D1 and D2 receptors [65][66]. Still, there is a mounting body of evidence that shows functional differences between striatal neuron subtypes [67][68][69]. It would be of great interest to determine whether decreasing or increasing neurons belong to the DRD1 or DRD2 genetic subtype. If the direction of change were specific to

pathways with opposite effects the trends could be synergistic, which would affect the way we interpret our current results.

In all, it is clear from the present findings that the striatal shift hypothesis under-represents the complexity of DLS changes. It ignores the somatotopic nature of MSNs, and ignores the contribution of interneurons entirely. The spatiotemporal resolution of the present recordings provide the clearest picture to date regarding the nature of dorsolateral striatal changes during cocaine self-administration. Interestingly these changes were confined primarily to the medium spiny neurons which directly process the head movement skill. *Baseline* activity in these neurons decreased across *Sessions* in accordance with previous measures of global DLS activity during chronic cocaine SA [47]. And, contrary to the current hypothesis, the majority of DLS *Head Movement* neurons also decrease their contribution to drug-taking throughout self-administration. Nonetheless, there exists a subset of DLS neurons which become progressively more involved in the head movement operant across SA. These neurons could represent the proposed “expert” neurons that, through competitive selection, become primarily responsible for processing or executing operant behaviors. These neurons also may be responsible for the expression of habitual behaviors during abstinence. Future research is needed to address the remaining questions surrounding these changes: can we determine the neuronal sub-type specificity of these trends, and do they fit with our current understanding of striatal function? Answering these questions could have broad impacts on the treatment of chronically relapsing substance abuse.

References

[1] Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology Reviews.35*, 217-238.

[2] American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC.

[3] McLellan A.T., Lewis D.C., O'Brien C.P., &Kleber, H.D.. (2000). Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *JAMA*, 284,1689–1695

[4] Dickinson, A. (1985). Actions and habits: The development of behavioural autonomy. *Philosophical Transactions of the Royal Society B*, *308*(1135), 67-78.

[5] Koob, G. (1996). Drug addiction: The yin and yang of hedonic homeostasis. *Neuron*, *16*(5), 893–896

[6] Ahmed, S.H., & Koob, G.F. (1998). Transition from moderate to excessive drug intake: Change in hedonic set point. *Science*, *282*(5387),298-300.

[7] Deroche, V., Le Moal, M., & Piazza, P. (1999). Cocaine self-administration increases the incentive motivational properties of the drug in rats. *European Journal of Neuroscience*. *11*(8), 2731-2736.

[8] Tiffany, S.T. (1990). A cognitive model of drug urges and drug-use behavior: Role of automatic and non-automatic processes. *Psychological Reviews.97*(2), 147–168.

[9] Leshner, A. (1999). Science is revolutionizing our view of addiction, and what to do about it. *American Journal of Psychiatry*. *156*(1),1-3.

[10] Wise, R. (2002). Brain reward circuitry: Insights from unsensed incentives. *Neuron,36*, 229-240.

[11] Volkow, N., Li, T.. (2005). The neuroscience of addiction. *Nature Neuroscience*,*8*(11),1429-1430.

[12] Hyman, S., Malenka, R., &Nestler, E.. (2006). Neural mechanisms of addiction: The role of reward-related learning and memory. *Annual Review Neuroscience*,*29*, 565-598.

[13] Feltenstein, M., See, R.. (2008). The neurocircuitry of addiction: An overview. *British Journal of Pharmacology, 154*(2), 261-274.

[14] Robinson, T.& Berridge, K. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews*, *18*(3), 247-291.

[15] Root, D., Barker, D., Ma, S., Coffey, K., Fabbricatore, A., & West, M. (2011). Evidence for learned skill during cocaine self-administration in rats. *Psychopharmacology*, *217*, 91-100.

[16] Zimmer, B., Dobrin, C., & Roberts, D. (2013). Examination of behavioral strategies regulating cocaine intake in rats. *Psychopharmacology*, *225*(4), 935-944.

[17] Root, D., Fabbricatore, A., Barker, D., Ma, S., Pawlak, A., & West, M. (2009). Evidence for habitual and goal-directed behavior following devaluation of cocaine: A multifaceted interpretation of relapse*. PLoS One*, *4*(9).

[18] Zapata, A., Minney, V., &Shippenberg, T. (2010). Shift from goal-directed to habitual cocaine seeking after prolonged experience in rats. *The Journal of Neuroscience*, *30*(46), 15457-15463.

[19] Carelli, R., Wolske, M., West, M. (1997). Loss of lever press-related firing of rat striatal forelimb neurons after repeated sessions in a lever pressing task. *The Journal of Neuroscience,17*, 1804–1814.

[20] Tang, C., Pawlak, A., Prokopenko, V., West, M.. (2007). Changes in activity of the striatum during formation of a motor habit. *European Journal of Neuroscience*, *25*, 1212-1227,.

[21] Tang, C., Root, D., Duke, D., Zhu, Y., Teixeria, K., Ma, S., Barker, D., &West, M.. (2009). Decreased firing of striatal neurons related to licking during acquisition and overtraining of a licking task. *Journal of Neuroscience*, *29*(44), 13952-13961

[22] Yin, H. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nature Neuroscience*, *12*(3), 333-341.

[23] Turner, R.& Desmurget, M. (2010). Basal ganglia contributions to motor control: A vigorous tutor. *Current Opinion in Neurobiology*, *20*, 704-716.

[24] Yin, H., Knowlton, B., &Balleine, B.. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *European Journal of Neuroscience.19*(1),181-189.

[25] Yin, H., Mulcare, S., Hilário, M., Clouse, E., Holloway, T., Davis, M.,& Costa, R. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nature Neuroscience*, *12*(3), 333-341.

[26] McDonald, R.& Hong, N. (2004). A dissociation of dorsolateral striatum and amygdala function on the same stimulus–response habit task. *Neuroscience,124*(3), 507-513.

[27] Devan, B., Hong, N., & McDonald, R. (2011). Parallel associative processing in the dorsal striatum: Segregation of stimulus–response and cognitive control subregions. *Neurobiology of Learning and Memory*, *96*(2), 95-120.

[28] Wilson, C.& Groves, P. (1980). Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: A study employing intracellular inject of horseradish peroxidase*. Journal of Comparative Neurology*, *194*(3), 599-615.

[29] Kimura, M., Kato, M.,& Shimazaki, H. (1990). Physiological properties of projection neurons in the monkey striatum to the globuspallidus. *Experimental Brain Research*, *82*, 672-676.

[30] Künzle, H. (1975). Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia: An autoradiographic study in Macacafascicularis. *Brain Research*, *188*(2),195-209.

[31] Künzle, H. (1977). Projections from the primary somatosensory cortex to basal ganglia and thalamus in the monkey. *Experimental Brain Research*, *30*(4), 481-492.

[32] Selemon, L. & Goldman-Rakic, P.(1985). Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. *The Journal of Neuroscience*, *5*(3), 776-794.

[33] McGeorge, A. & Faull, R. (1989). The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience*, *29*(3), 503-37.

[34] Ebrahimi, A., Pochet, R.,& Roger, M. (1992). Topographical organization of the projections from physiologically identified areas of the motor cortex to the striatum in the rat. *Neuroscience Research*, *14*(1), 39-60.

[35] Flaherty, A.& Graybiel, A. (1994). Input-output organization of the sensorimotor striatum in the squirrel monkey. *Journal of Neuroscience*, *14*, 599-610.

[36] Haber, S., Lynd-Balta, E., & Spooren, W. (1994). Integrative aspects of basal ganglia circuitry. In: The Basal Ganglia IV, 71-80. New York: Plenum Press.

[37] Parent, A. & Hazrati, L. (1995) Functional anatomy of the basal ganglia. I. The cortico-basal gangliathalamo-cortical loop. *Brain Research Reviews*, *20*, 91-127.

[38] Berendse, H., Groenewegen, H., & Lohman, A. (1992). Compartmental distribution of ventral striatal neurons projecting to the mesencephalon in the rat. *The Journal of Neuroscience*, *12*(6), 2079-2103.

[39] Brog, J., Salyapongse, A., Deutch, A., & Zahm, D. (1993). The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. *Journal of Comparative Neurology*, *338*, 255-278.

[40] Haber, S., Fudge J.,& McFarland, N. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *The Journal of Neuroscience*, *20*(6), 2369-2382.

[41] Otake, K.& Nakamura, Y. (2000). Possible pathways through which neurons of the shell of the nucleus accumbens influence the outflow of the core of the nucleus accumbens. *Brain Development*, *22*, S17-S26.

[42] Zahm, D. (2000). An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neuroscience Biobehavioral Reviews*, *24*, 85-105.

[43] Haber, S. (2003).The primate basal ganglia: Parallel and integrative networks. *Journal of Chemical Neuroanatomy*,*26*, 317-330.

[44] Parent, A. & Hazrati, L. (1995b).Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. *Brain Research Reviews*, *20*, 128-154.

[45] Alexander, G., DeLong, M., & Strick, P. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Reviews Neuroscience,9*, 357-381.

[46] Parent, A. & Hazrati, L. (1993). Anatomical aspects of information processing in primate basal ganglia. *Trends in Neuroscience*, *16*, 111-116.

[47] Porrino, L., Lyons, D., Smith, H., Daunais, J., & Nader, M. (2004). Cocaine self-administration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. *The Journal of Neuroscience*, *24*(14), 3554-3562.

[48] Takahashi, Y., Roesch, M., Stalnker, T., & Schoenbaum, G. (2007). Cocaine exposure shifts the balance of associative encoding from ventral to dorsolateral striatum. *Frontiers in Integrative Neuroscience*,*1*(11), 1-10.

[49] Everitt, B., Robbins, T. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion*. Nature Neuroscience*, *8*(11),1481-1489.

[50] Vanderschuren, L., Di Ciano, P.,& Everitt, B. (2005). Involvement of the dorsal striatum in cue-controlled cocaine seeking. *The Journal of Neuroscience,25*,8665-8770

[51] Liles, S. & Updyke, B. (1985). Projection of the digit and wrist area of precentral gyrus to the putamen: Relation between topography and physiological properties of neurons in the putamen. *Brain Research*, *339*(2), 245-255.

[52] Alexander, G. & Delong, M. (1985). Microstimulation of the primate neostriatum. II. Somatotopic organization of striatal microexcitable zones and their relation to neuronal response properties. *Journal of Neurophysiology*, *53*, 1417-1430.

[53] Carelli, R. & West, M. (1991). Representation of the body by single neurons in the dorsolateral striatum of the awake, unrestrained rat. *Journal of Comparative Neurology*, *309*(1), 231-349.

[54] Mittler, T., Cho, J., Peoples, L.,& West, M. (1994). Representation of the body in the lateral striatum of the freely moving rat: Single neurons related to licking. *Experimental Brain Research,98*(1), 163-167.

[55] Cho, J. & West, M. (1997). Distribution of single neurons related to body parts in the lateral striatum of the rat. *Brain Research*, *756*(1), 241-246.

[56] West, M. (1998) Anesthetics eliminate somatosensory-evoked discharges of neurons in the somatotopically organized sensorimotor striatum of the rat. *The Journal of Neuroscience,18*(1), 9055-9068.

[57] Coffey, K., Barker, D., Ma, S., & West, M. (2013) Building an open-source robotic stereotaxic instrument. *The Journal of Visualized Experiments. 80*(e51006).

[58] Sokoloff, L. (1981). Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *Journal of Cerebral Blood Flow & Metabolism*, *1*(1), 7-36.

[59] Belin, D., & Everit, B. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Cell*, *57*(3), 432-441

[60] Barker, D., Bercovicz, D., Servilio, L., Simmons, S., Ma, S., Root, D., & West, M. (2013). Rat ultrasonic vocalizations demonstrate that the motivation to contextually reinstate cocaine-seeking behavior does not necessarily involve a hedonic response. *Addiction Biology*.

[61] Graybiel, A., Aosaki, T., Flaherty, A., & Kimura, M. (1994). The basal ganglia and adaptive motor control. *Science*, *265*(5180), 1826-1831.

[62] Barnes, T., Kubota, Y., Hu, D., Jin, D., & Graybiel, A. (2005). Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. *Nature*, *437*(7062), 1158-1161.

[63] Smith, Y., Beyan, M., Shink, E., & Bolam, J. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. *Neuroscience Oxford*, *86*, 353-388.

[64] Parent, A., Sato, F., Wu, Y., Gauthier, J., Lévesque, M., & Parent, M. (2000). Organization of the basal ganglia: The importance of axonal collateralization. *Trends in Neurosciences*, *23*, S20-S27.

[65] Aizman, O., Brismar, H., Uhlén, P., Zettergren, E., Levey, A., Forssberg, H., ... & Aperia, A. (2000). Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. *Nature Neuroscience*, *3*(3), 226-230.

[66] Hersch, S., Ciliax, B., Gutekunst, C., Rees, H., Heilman, C., Yung, K., ... & Levey, A. (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *The Journal of Neuroscience*, *15*(7), 5222-5237.

[67] Lobo, M., Covington, H., Chaudhury, D., Friedman, A., Sun, H., Damez-Werno, D., ... & Nestler, E. (2010). Cell type–specific loss of BDNF

signaling mimics optogenetic control of cocaine reward. *Science*, *330*(6002), 385-390.

[68] Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Hervé, D., Valjent, E., & Girault, J. (2008). Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *The Journal of neuroscience*, *28*(22), 5671-5685.

[69 Luo, Z., Volkow, N., Heintz, N., Pan, Y., & Du, C. (2011). Acute cocaine induces fast activation of D1 receptor and progressive deactivation of D2 receptor striatal neurons: In vivo optical microprobe [Ca2+] imaging. *The Journal of Neuroscience*, *31*(37), 13180-13190.

[70] Root, D., Tang, C., Ma, S., Pawlak, A. P., & West, M. O. (2010). Absence of cue-evoked firing in rat dorsolateral striatum neurons. *Behavioural Brain Research*, *211*(1), 23-32.

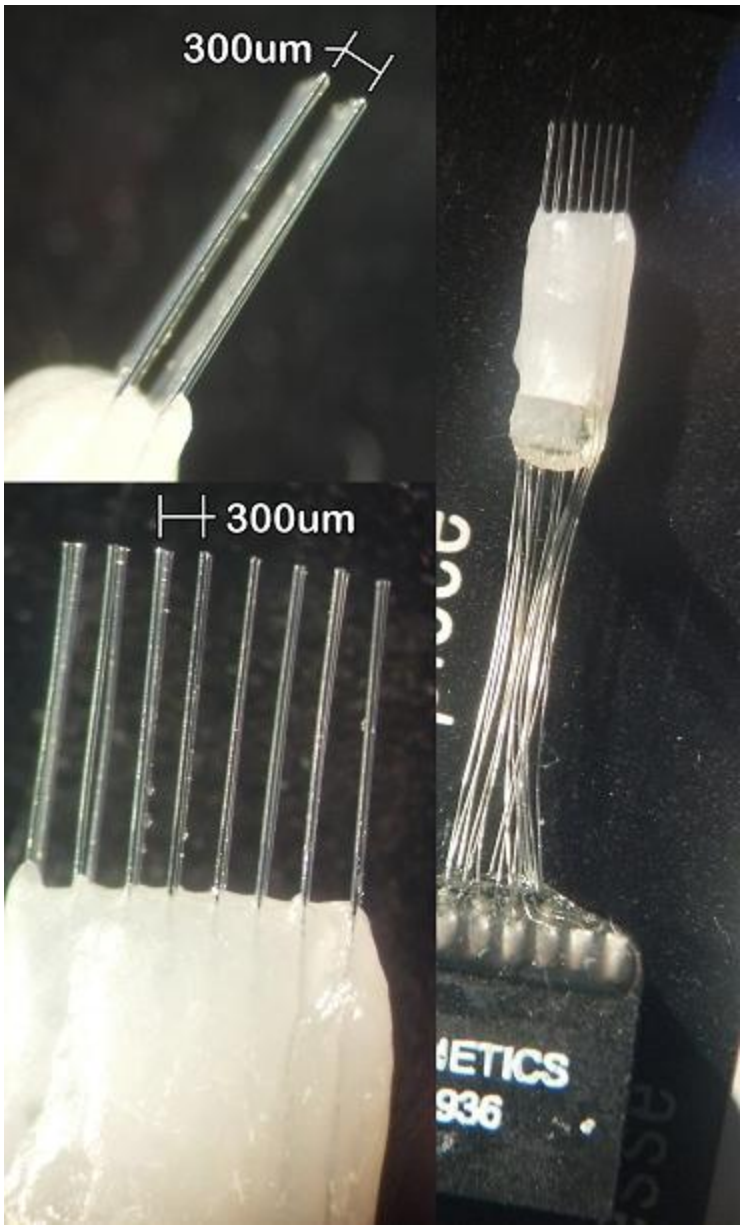

Figure 1. Low magnification images of the microwire arrays. Arrays were constructed from $50_{\mu m}$ stainless steel wires quad coated in Teflon® insulation, and were arranged in a 2x8 comb, with $300_{\mu m}$ between wires and rows.

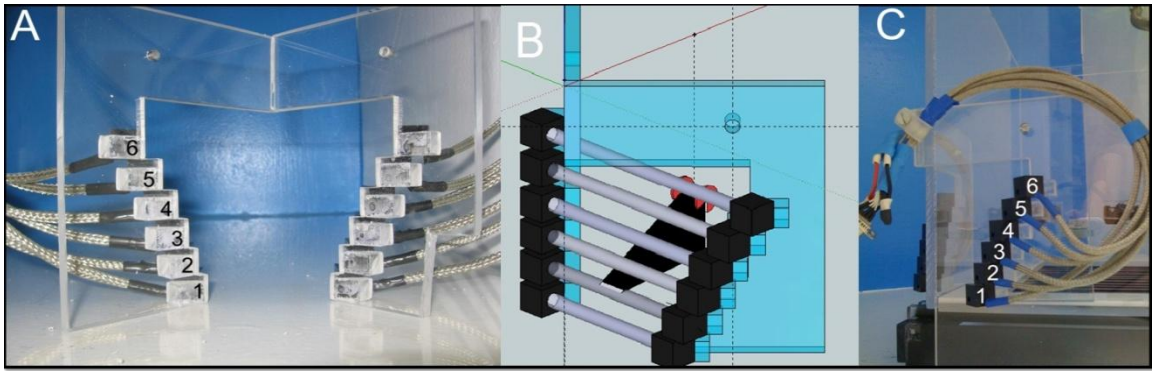

Figure 2. Operant photocell device, designed to monitor and reinforce head movements in rats. A) An inside view of the device showing the order of the photocells. B) A 3D rendering of the device and photo cell beams, showing a rat's position immediately prior to emitting a head movement. C) A view of the device connected to the back corner of the self-administration chamber. The device is entirely outside the chamber, and is not subject to perturbations by the animal.

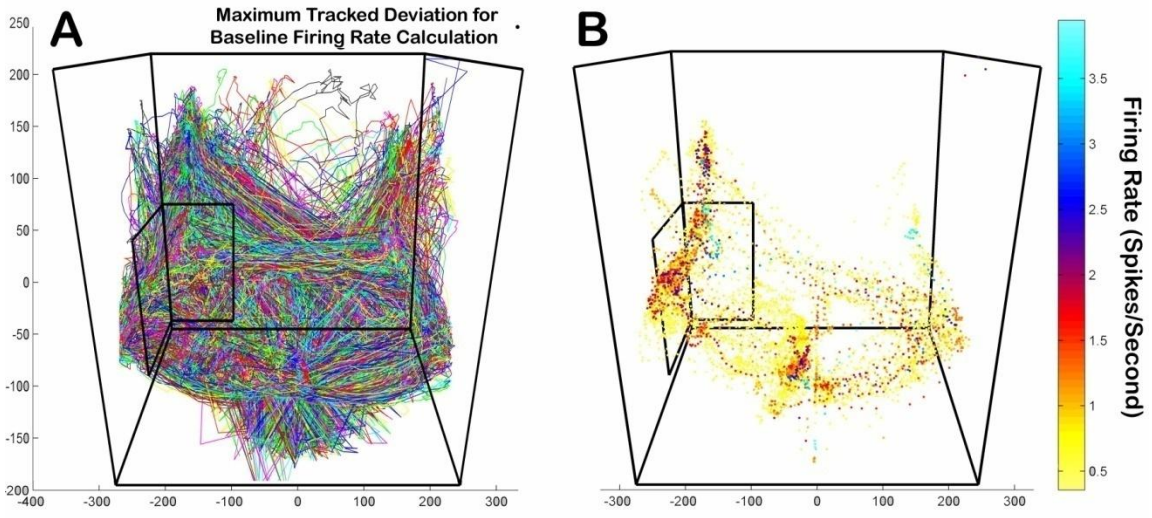

Figure 3. A) An animal's tracked position for a single *Session* (6_h), plotted with varying color for clarity. The photocells are represented by the box in the back left corner. B) The firing rate at each point in the chamber. For clarity, only firing rates greater than 1.56 standard deviations from the mean (top 6%) are plotted. Notice that this neuron fires during the upward head movement in the corner, but also during smaller head movements made at the front of the chamber, the locus of stereotypy for this particular animal. Non-movement is defined as periods of 1 second with no more than 3 pixel deviation in any direction (shown in scale).

Average Neural Firing Rates in Spikes/Second (Early: e.g. Day 1)

Duration →	100 -280ms	280-460ms	460-640ms	640-820ms	820-1000ms
Start : End Photocell ↓					
1:3	2.6	NA	NA	NA	NA
1:4	3.2	2.3	2.8	2.6	NA
1:5	NA	2.6	2.6	NA	1.9
1:6	NA	2.2	2.6	2.6	2.6
2:4	3.6	2.6	2.3	1.6	NA
2:5	3.2	2.9	2.3	1.4	NA
2:6	NA	NA	2.9	2.6	1.5
3:4	4.6	2.8	2.6	2.2	NA
3:5	2.1	1.5	NA	NA	NA
3:6	2.2	2.6	NA	NA	NA

Average Neural Firing Rates in Spikes/Second (Middle: e.g. Day 12)

Duration →	100 -280ms	280-460ms	460-640ms	640-820ms	820-1000ms
Start : End Photocell ↓					
1:3	1.6	2.6	NA	NA	NA
1:4	3.1	2.7	NA	2.3	2.2
1:5	NA	2.6	2.3	3.2	1
1:6	NA	6.3	2.6	2.6	2.7
2:4	3.6	2.6	2.5	NA	NA
2:5	NA	1.4	2.3	1.4	NA
2:6	NA	NA	1.3	2.6	3.2
3:4	4.6	2.6	2.6	1.5	NA
3:5	2.1	2.1	2.7	NA	2.7
3:6	2.2	3.8	2.2	NA	NA

Average Neural Firing Rates in Spikes/Second (Late: e.g. Day 24)

Duration →	100 -280ms	280-460ms	460-640ms	640-820ms	820-1000ms
Start : End Photocell ↓					
1:3	2.6	NA	NA	NA	NA
1:4	3.2	2.3	NA	2.6	NA
1:5	NA	2.62	2.4	1.5	1.4
1:6	NA	2.2	NA	1.8	2.2
2:4	3.6	2.6	2.6	1.6	NA
2:5	3.2	1.9	2.3	1.4	NA
2:6	1.7	NA	1.5	2.62	4.2
3:4	4.6	1.4	2.63	2.9	NA
3:5	2.1	2.2	2.6	NA	1.6
3:6	NA	3.6	NA	NA	NA

Figure 4. Example matched set tables for a single neuron. Values represent the average firing rate of a neuron during movements that fall into each category. Categories with fewer than 5 movements are excluded, indicated by an NA. Cells that are underlined have matches across all *Sessions*. Only these firing rates are entered into our statistical model, controlling for motor behavioral differences across *Sessions*.

Neuron Type →	Head Movement	Control
Firing Type ↓		
Phasic	**Head - Phasic**	**Control - Phasic**
Baseline	**Head - Baseline**	**Control - Baseline**
		x12 Sessions

Figure 5. Custom generalized mixed model. This model was developed to determine the nature of changes in DLS across cocaine self-administration. The model contains 2 levels of *Neuron Type* (*Head Movement & Control*), 2 levels of *Firing Type* (*Phasic* & *Baseline*), and 12 levels of recording *Session* (2x2x12).

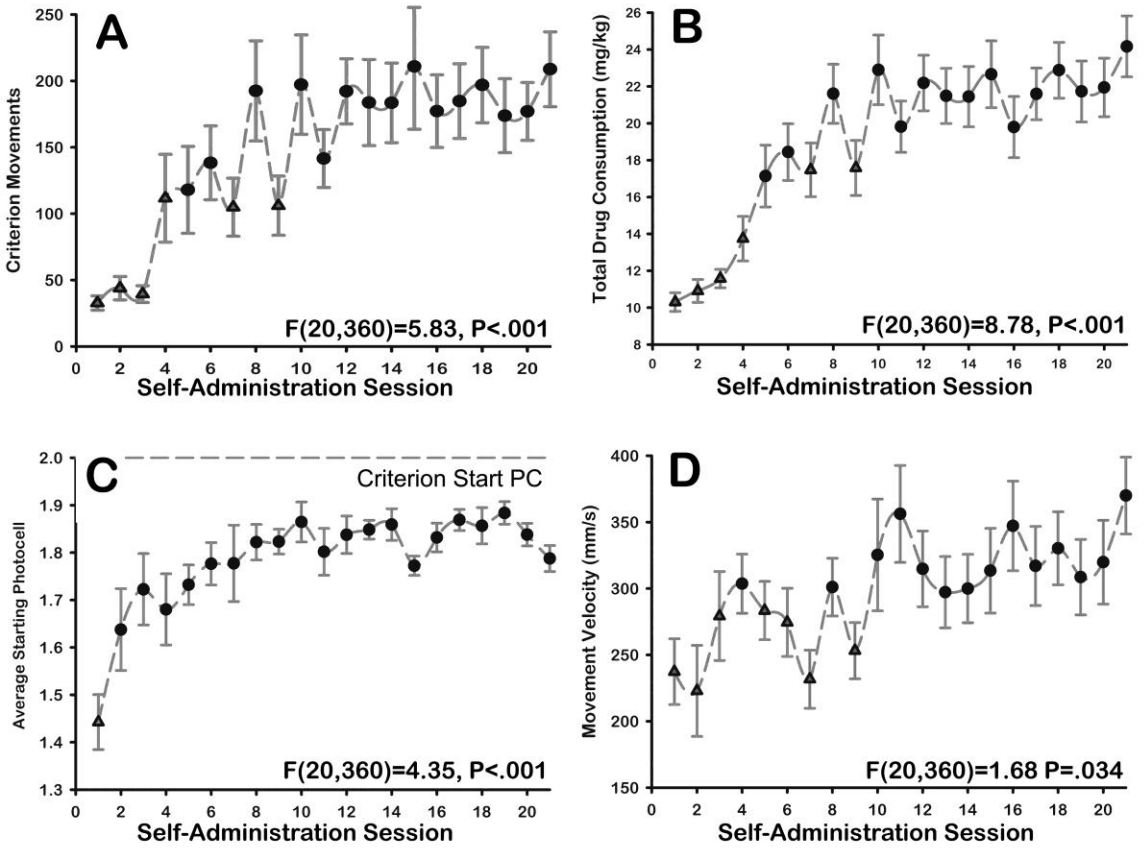

Figure 6. We found behavioral evidence of skilled self-administration. A) Animals significantly increased head movements [$F(20,360)=5.83$, $p<.001$] and B) total drug consumption [$F(20,360)=8.78$, $p<.001$] across *days*. C) Animals also learned to begin their head movements closer to the required start position [$F(20,360)=4.35$, $p<.001$] D) and they significantly increased their movement velocity across days [$F(20,360)=1.68$, $p=.034$].

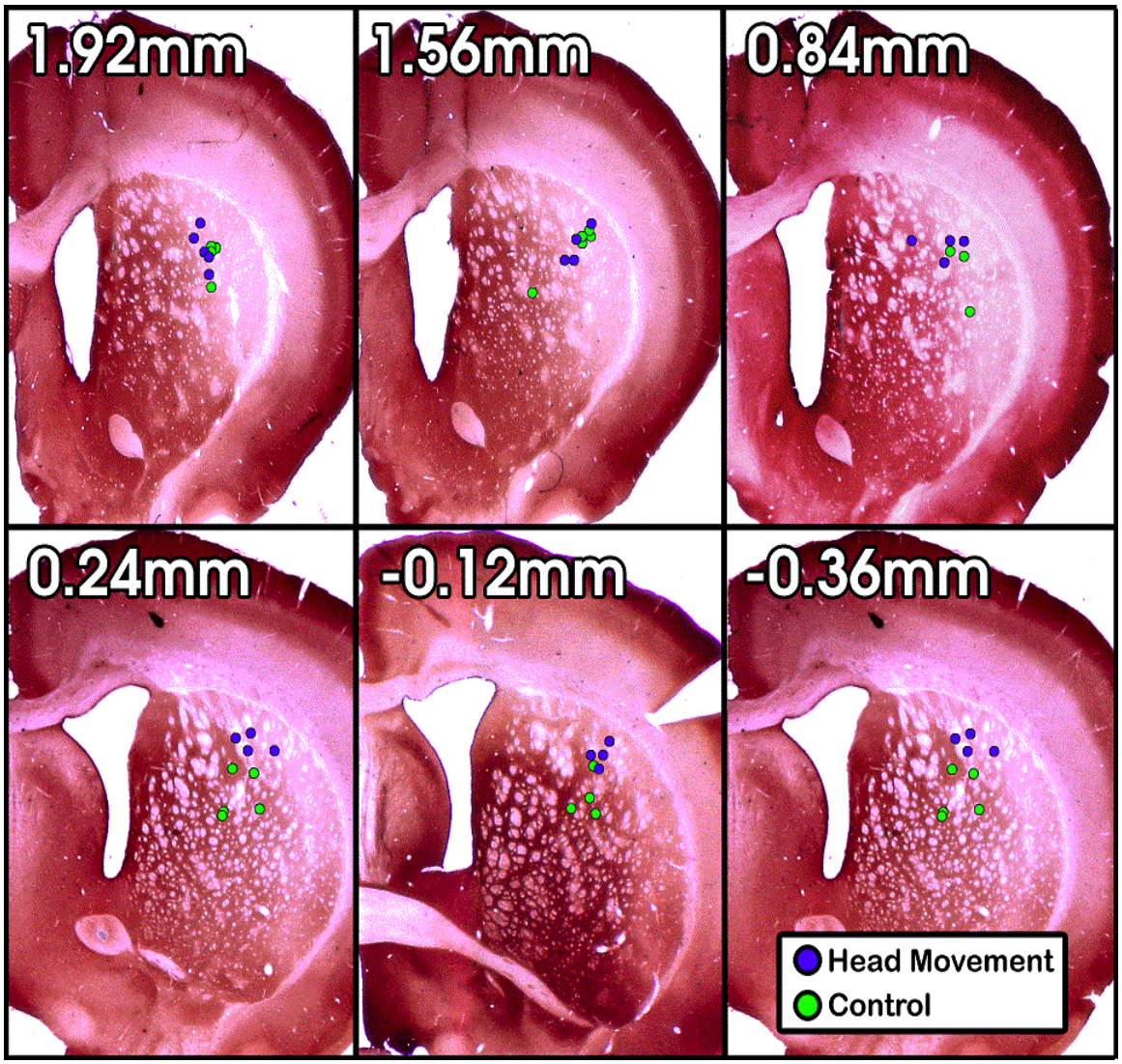

Figure 7. Representative histological slices containing the approximate location of every wire included in this study. Wires recording *Head Movement* neurons are shown in blue, while *Control* neurons are shown in green.

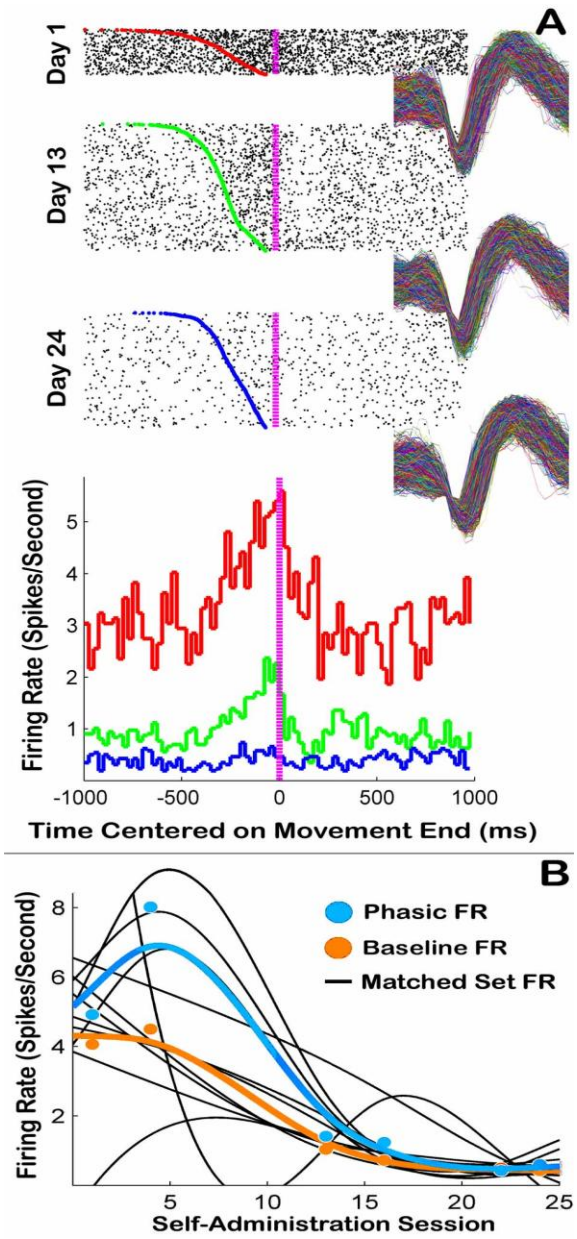

Figure 8. A) Example Raster-PETHs of the *Phasic* activity of a single neuron, held stable across 24 days. The 3 stacked rasters represent an early, middle, and late day, as well as 3 corresponding histograms below. Each horizontal line in the rasters represents a trial containing 1 movement, and each black dot represents an action potential. Movements are sorted by length, and the onset of each movement is plotted with a colored dot. The end of each movement is aligned to time zero. The histograms are a summation of these action potentials with 10ms time bins, and the colors correspond to the day. B) This neuron's *Phasic* FR decreases across *Sessions*, until it reaches *Baseline* firing levels. The trends for individual matched sets that make up the *Phasic* FR are plotted as a white line.

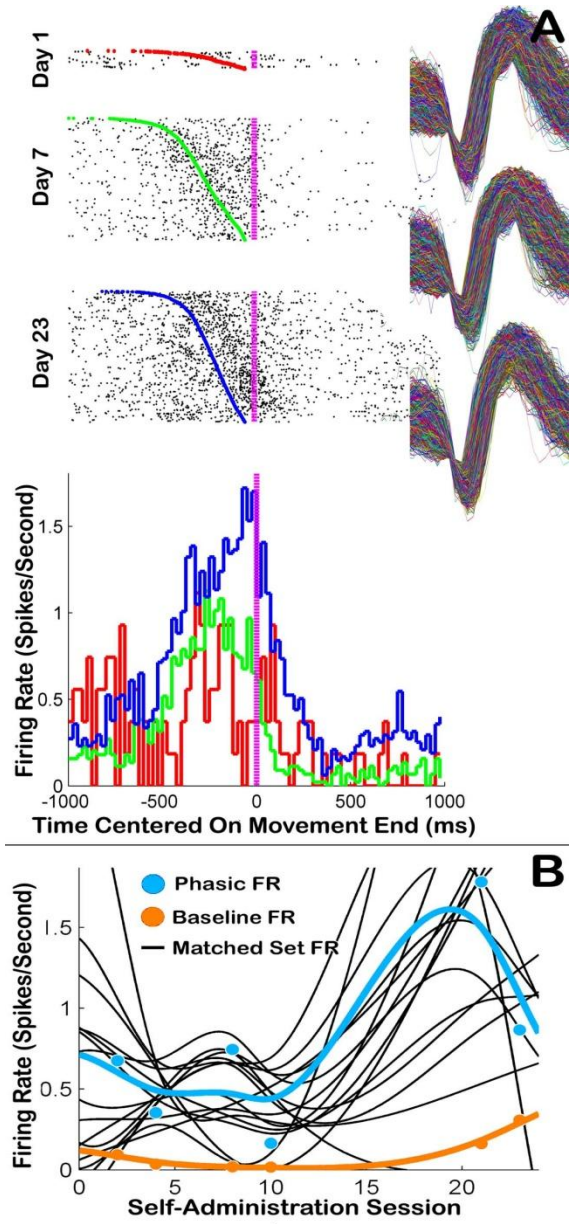

Figure 9. A) Example Raster-PETHs of the *Phasic* activity of a single neuron, held stable across 23 days. The 3 stacked rasters represent an early, middle, and late day, as well as 3 corresponding histograms below. Each horizontal line in the rasters represents a trial containing 1 movement, and each black dot represents an action potential. Movements are sorted by length, and the onset of each movement is plotted with a colored dot. The end of each movement is aligned to time zero. The histograms are a summation of these action potentials with 10ms time bins, and the colors correspond to the day. B) This neuron's *Phasic* FR increases across *Sessions*, especially in relation to *Baseline* firing levels. The trends for individual matched sets that make up the *Phasic* FR are plotted as a white line.

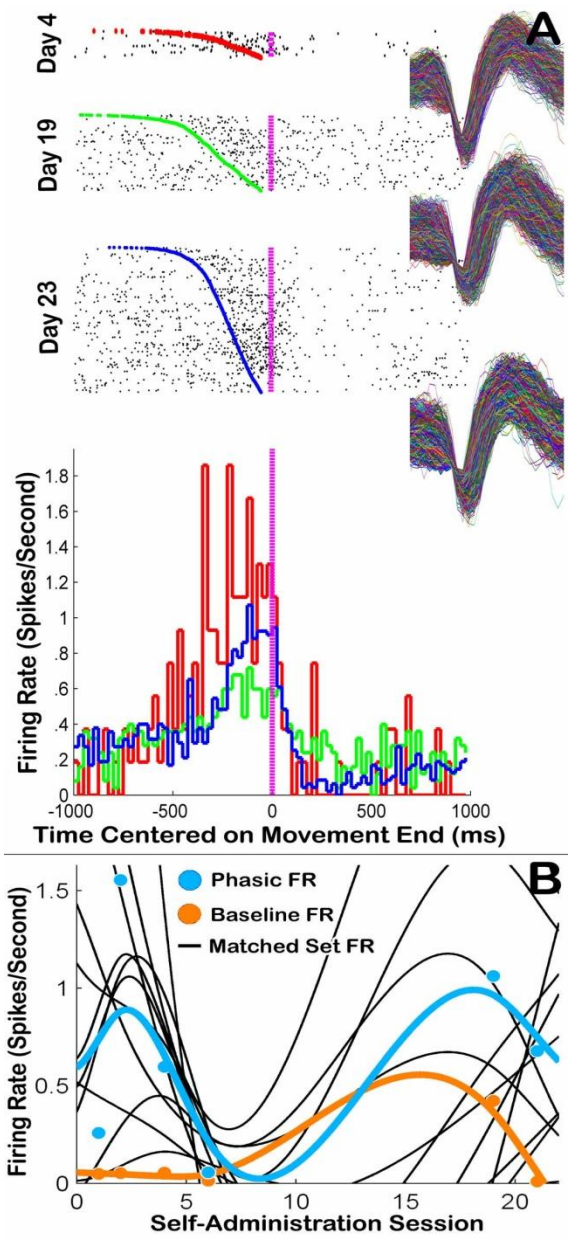

Figure 10. A) Example Raster-PETHs of the *Phasic* activity of a single neuron, held stable across 23 days. The 3 stacked rasters represent an early, middle, and late day, as well as 3 corresponding histograms below. Each horizontal line in the rasters represents a trial containing 1 movement, and each white dot represents an action potential. Movements are sorted by length, and the onset of each movement is plotted with a colored dot. The end of each movement is aligned to time zero. The histograms are a summation of these action potentials with 10ms time bins, and the colors correspond to the day. B) This neuron's *Phasic* FR fluctuates across *Sessions*, but shows similar head movement processing on early and late days. The trends for individual matched sets that make up the *Phasic* FR are plotted as white lines.

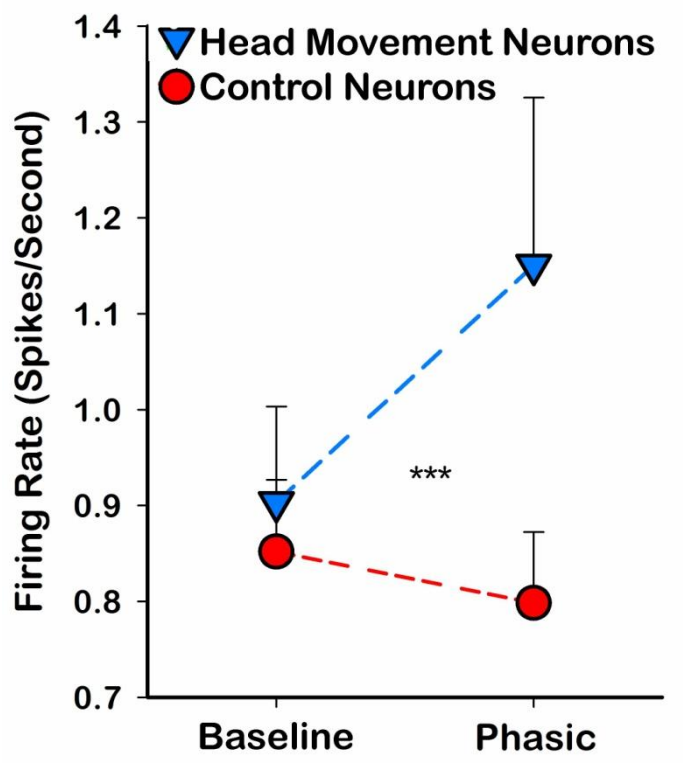

Figure 11. The relationship between *Baseline* and *Phasic* FR differed between *Head Movement and Control* neurons [*Neuron Type x Firing Type* Interaction; *F(*11,966)=20.02, *p*<.0001***]. This was expected, as the *Control* neurons do not unconditionally process the head movement, and therefore do not show increased *Phasic* FR compared to non-movement (*Baseline*) FR.

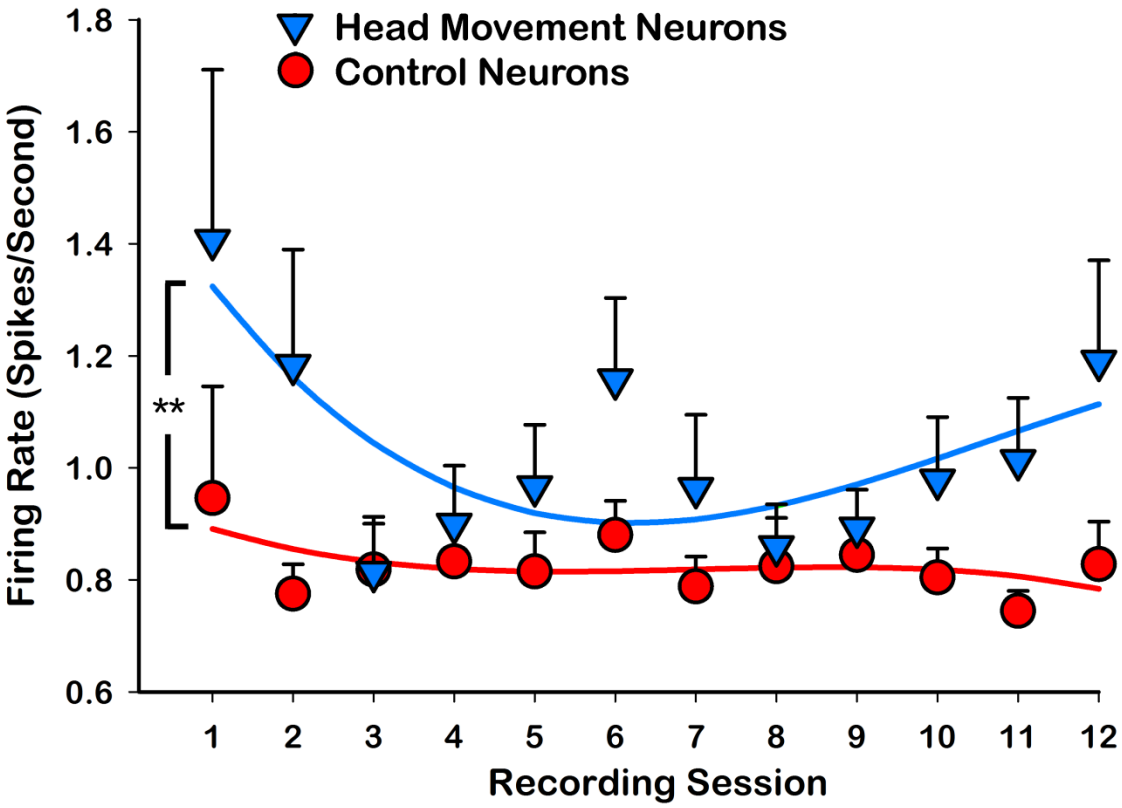

Figure 12. Head Movement and *Control* neurons change differentially across chronic cocaine self-administration *[Neuron Type x Session* Interaction; *F(*11,966)=2.37, *p*=.0068**]. While this test collapses *Baseline* and *Phasic* firing, it highlights the need to differentiate between body part sensitive neurons in DLS. *Session* is comprised of data from 2 self-administration days (e.g. days 1 & 2= *Session* 1… days 23 & 24= *Session* 12).

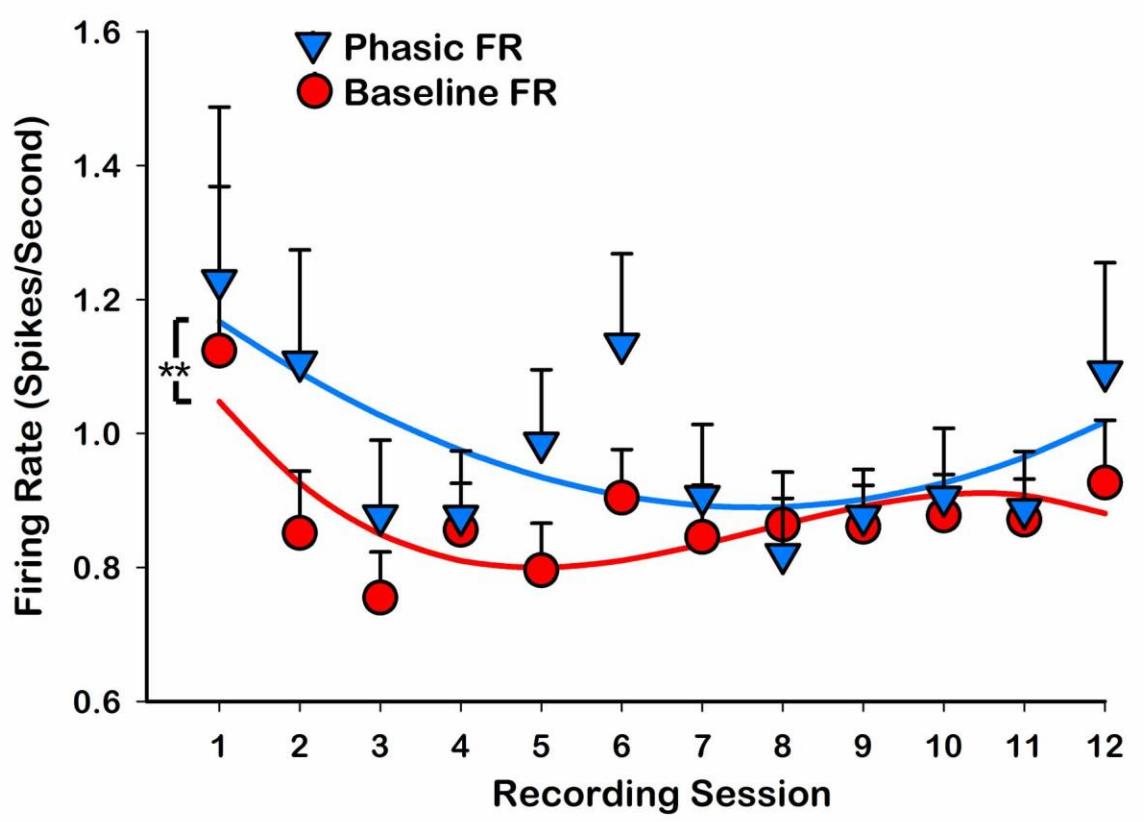

Figure 13 . Phasic and *Baseline* FRs change differentially across sessions [*Firing Type* x *Session* interaction; *F(*11,966)=2.40, *p*=0.0061**]. While this test collapsed *Head Movement* and *Control* neurons, it highlights the need for strong behavior matches in longitudinal designs. It also provides a control for spike sorting differences across days, which would affect *Baseline* and *Phasic* FR equally. *Session* is comprised of data from 2 self-administration days (e.g. days 1 & 2= *Session* 1… days 23 & 24= *Session* 12).

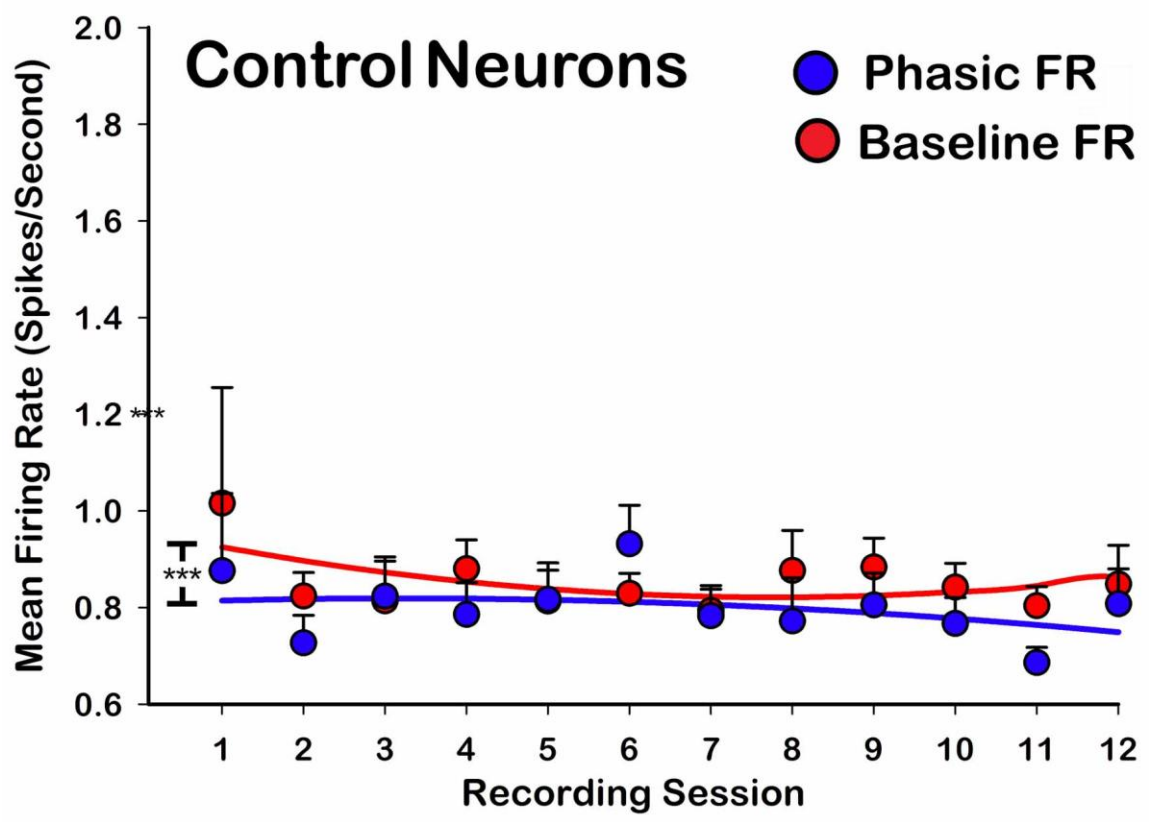

Figure 14. Significant *Session x Firing Type* interaction for *Control* neurons [*F(*23,966)=6.88, *p*<.0001***]. Although it is clear that neither *Baseline* nor *Phasic* FR underwent much change for *Control* neurons, they do change differentially. These results reinforce the need for precise behavioral matching when comparing firing rates in DLS. We also found a significant effect of *Session* for *Phasic* FR in *Control* neurons [*F(*11,966)=2.96, *p*=.007]. Although significant, it is clear that the change across sessions for *Control* neurons is much smaller than that of *Head Movement* neurons. There were no days on which Phasic FR was significantly different from *Baseline* FR for *Control* neurons (Holm-Bonferroni corrected post-hocs). *Session* is comprised of data from 2 self-administration days (e.g. days 1 & 2= *Session* 1… days 23 & 24= *Session* 12).

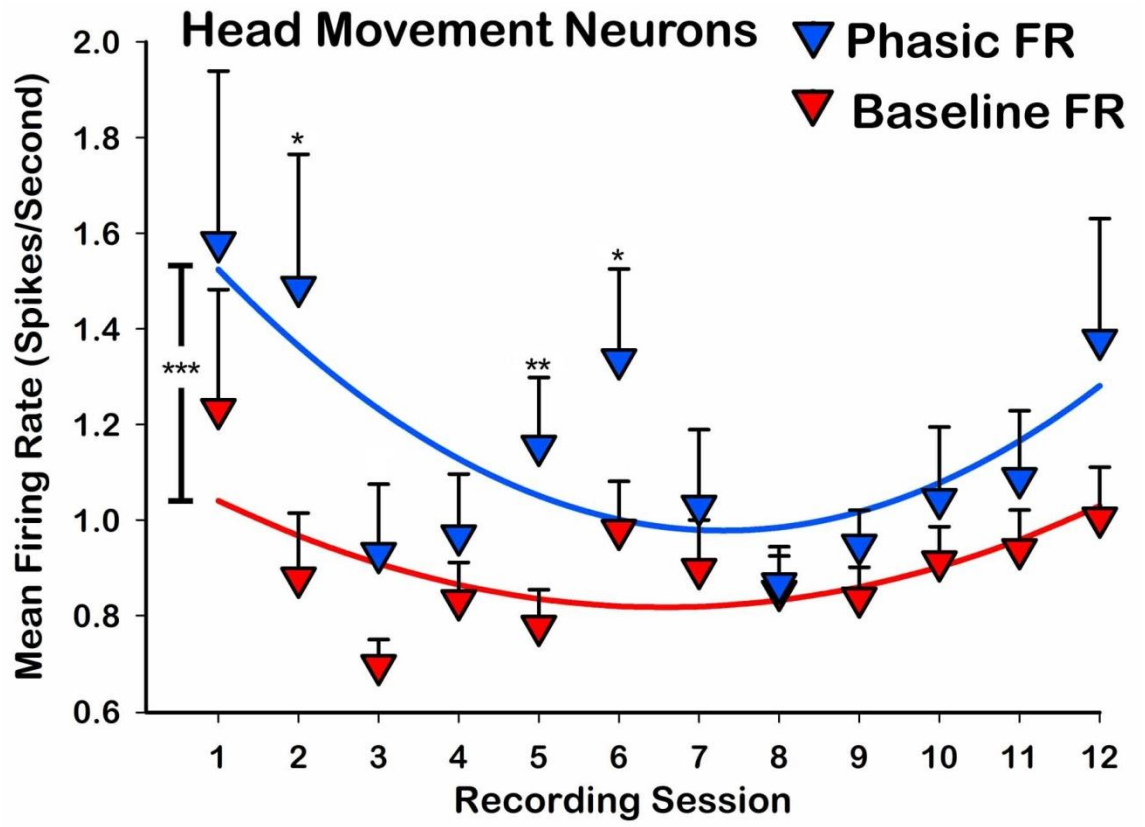

Figure 15. We found a significant effect of session for *Head Movement* neurons in both *Phasic* [$F(11,966)=5.02$, $p<.0001$] and *Baseline* [$F(11,966)=3.57$, $p<.0001$] FR. Both *Baseline* and *Phasic* FR tend to decrease across sessions for *Head Movement* neurons, although a significant *Session x Firing Type* interaction (sliced by *Neuron Type*) for *Head Movement* neurons [$F(23,966)=9.33$, $p<.0001$***] indicates that they changed at different rates. *Phasic* FR was found to be significantly different from *Baseline* FR (and thus contributing to operant responding) early in training (Sessions $2^{p=.007**}$, $5^{p=.032*}$, and $6^{p=.037*}$). This is in contrast to the striatal shift hypothesis, which predicts that DLS will contribute most late in training. *Session* is comprised of data from 2 self-administration days (e.g. days 1 & 2= *Session* 1… days 23 & 24= *Session* 12).