Temporal correlations among functionally specialized striatal neural ensembles in reward conditioned mice

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Temporal correlations of neural activity in the striatum

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ABSTRACT
As the major input to the basal ganglia, the striatum is innervated by a wide range of other areas. Overlapping input from these regions is speculated to influence temporal correlations among striatal ensembles. However, the network dynamics among behaviorally related neural populations in the striatum has not been extensively studied. We used large-scale neural recordings to monitor activity from striatal ensembles in mice undergoing Pavlovian reward conditioning. A subpopulation of putative medium spiny projection neurons (MSNs) was found to discriminate between cues that predicted the delivery of a reward, and cues that predicted no specific outcome. These cells were preferentially located in lateral subregions of the striatum. Discriminating MSNs were more spontaneously active and more correlated than their non-discriminating counterparts. Furthermore, discriminating fast spiking interneurons (FSIs) represented a highly prevalent group in the recordings, which formed a strongly correlated network with discriminating MSNs. Spike time cross correlation analysis showed the existence of synchronized activity among FSIs, and feedforward inhibitory modulation of MSN spiking by FSIs. These findings suggest that populations of functionally specialized (cue-discriminating) striatal neurons have distinct network dynamics that sets them apart from non-discriminating cells, potentially in order to facilitate accurate behavioral responding during associative reward learning.

INTRODUCTION
Animals must rapidly learn to discriminate environmental cues associated with beneficial outcomes from irrelevant cues. In the vertebrate nervous system, the basal ganglia are a set of interconnected nuclei whose activity has been extensively linked to reward-guided learning and action selection (Graybiel 2000; Kravitz et al. 2012). Within these circuits, the striatum serves as the primary input structure to the basal ganglia as well as an important site of synaptic plasticity (Gerfen and Surmeier 2011; Kreitzer and Malenka 2008). Previous efforts to understand the role
of striatal electrophysiological activity in guiding behavior revealed that this region is strongly
modulated by primary rewards, stimuli that predict rewards, and action (Nicola et al. 2004;  
Roitman et al. 2005; Setlow et al. 2003; Tremblay et al. 1998). Furthermore, a number of 
studies have reported changes in striatal activity that accompany learning (Costa et al. 2004;  
Xiong et al. 2015; Yin et al. 2009). These effects are consistent with a neural circuit that can 
become tuned to select specific behavioral responses in anticipation of an appetitive outcome.
Yet despite a substantial amount of work on neural dynamics in this area, relatively little is 
known about how striatal neurons are functionally organized at the network level.

In contrast to the organization of cortical microcircuits which contain strong local excitation 
(Ko et al. 2011), the striatum is a largely inhibitory structure consisting of GABAergic medium 
spiny projection neurons (MSNs) coupled with a small population of interneurons (Kreitzer and  
Berke 2011; Tepper et al. 2004; Tepper et al. 2008). At millisecond timescales, striatal activity is 
likely to be strongly influenced by shared glutamatergic signaling from cortical, thalamic, and  
limbic inputs (Cowan and Wilson 1994; Kasanetz et al. 2006; Kincaid et al. 1998; Plenz and  
Kitai 1998; Reig and Silberberg 2014; Stern et al. 1998). It is believed that the striatum 
integrates these convergent streams of information, with the resulting activity acting on basal  
ganglia output nuclei connected to association and motor control areas in the cortex (Alexander  
et al. 1986). Computational and experimental studies suggest that the converging input to the  
striatum leads to the formation of functionally specialized subsets of MSNs with temporally  
Ponzi and Wickens 2010; Yim et al. 2011). These findings in the striatum, and a large body of  
work focusing on cortical circuits (Averbeck et al. 2006; Bair et al. 2001; Cohen and Maunsell  
2009; Mitchell et al. 2009; Shadlen and Newsome 1998; Zohary et al. 1994), implicate  
correlated activity in neural computation and behavior. This evidence raises the possibility that  
temporal correlations preferentially occur among task-related groups of neurons in the striatum.
To study this relationship, we used multielectrode probe recordings to simultaneously monitor activity from over 100 units in head-fixed mice undergoing Pavlovian reward conditioning. The large scale of these measurements enabled systematic analysis of correlations among behaviorally and electrophysiologically identified groups of cells. The results of this study reveal a potential organizational principle for the dynamics of striatal neurons that encode similar features during the behavioral task.

MATERIALS AND METHODS

Animals and surgical procedures
All procedures were approved by the University of California, Los Angeles Chancellor’s Animal Research Committee. Singly housed male C57Bl/6J mice (n=9, 12-16 weeks old, The Jackson Laboratory) were used in the experiments. Animals underwent an initial surgery under isoflurane anesthesia in a stereotaxic apparatus to bilaterally fix stainless steel head restraint bars (10 mm x 7.5 mm, 0.6 g) on the skull. Animals were anesthetized with isoflurane for a second surgery on the recording session day to make a craniotomy for acute microprobe insertion. Rectangular craniotomies (0.5 mm AP x 2 mm ML) were centered on the following striatal coordinates relative to bregma: AP, 1.25-1.3 mm, ML, 0.95 mm. An additional craniotomy was made over the posterior cerebellum for placement of an electrical reference wire.

Behavioral task
After recovery from the first surgery, animals were food restricted and fed daily after each training session to maintain ~90% of their baseline weight. They received water *ad libitum*. During daily training sessions, animals were mounted on the head bar bracket on the recording rig and stood on a polystyrene spherical treadmill (200 mm diameter, Graham Sweet Studios) that rotated along a single axis during forward/backward ambulation. The treadmill velocity was
monitored with an optical mouse. Delivery of the reward solution (5 μL, 10% sweetened condensed milk) was from a tube positioned between an infrared lick meter (Island Motion), and was controlled by an audible solenoid valve actuation (Neptune Research). We studied the behavioral and electrophysiological profiles of previously inexperienced mice trained with odors for the first time. Before conditioning, animals were habituated to head fixation by receiving rewards alone (maximum 100 rewards per daily session, 13-21 s inter-trial interval, ITI), and exposed to a constant flow of odorless air (1.5 L/min) through a tube. After animals successfully consumed 90% of delivered rewards for two consecutive days, they underwent surgery for recording and began conditioning with olfactory cues using an olfactometer. Odorants were introduced by bubbling air (0.15 L/min) through aromatic liquids diluted 1:10 in mineral oil (Sigma-Aldrich), and mixing this product with the 1.5 L/min stream of air. The task involved two stimulus conditions consisting of either a 1 s olfactory cue (CS+) followed by a temporal delay of 1.5 s and subsequent delivery of a reward solution, or a different 1 s olfactory cue (CS-) that was not followed by reward (Fig. 1A). Odors were presented in pseudorandom order (1 s duration, 17-29 s ITI). The CS+ consisted of amyl acetate and the CS- consisted of citral. Correct CS+ hit trials were defined as those containing anticipatory licking activity detected between t=0-2.5 s from the cue onset, i.e., prior to reward delivery. Correct CS- withholding trials were defined as the absence of any licking activity between t=0-5 s from the cue onset. Mice underwent recording on the first day of odor conditioning. During the recording animals received 100 CS+ trials paired with reward delivered at 2.5 s after cue onset and 100 CS- trials with no reward.

Electrophysiological recordings

Silicon microprobes (Shobe et al. 2015) were fabricated in a silicon microelectromechanical systems foundry (Innovative Micro Technology). Each silicon microprobe contained a total of 256 electrodes (10 μm x 10 μm electrode dimensions, ~30 μm electrode spacing) distributed on
multiple silicon prongs. Recordings were performed using one of two device designs. The prongs of each device type were arranged to provide high-density electrophysiological measurements across a large area of the striatum. Type I probes had 4 prongs positioned at fixed depths, with 64 electrodes per prong distributed along 1 mm at the tapered tip of each shaft. Type II probes had 5 prongs distributed at different depths, with 50 or 52 electrodes per prong. Recording and spike sorting procedures are described in (Shobe et al. 2015).

**Striatal unit classification**

All analysis was carried out with custom Matlab scripts. We used spike waveform trough-to-peak (t_tr-pk) duration and coefficient of variation (CV) of baseline firing rate to classify units into putative MSNs, fast spiking interneurons (FSIs), and tonically active interneurons (TANs) (Aosaki et al. 1994; Bennett and Wilson 1999; Gage et al. 2010; Mallet et al. 2005). FSIs were separated from non-FSIs by their narrow waveform (maximum FSI t_tr-pk = 0.475 ms, minimum non-FSI t_tr-pk = 0.55 ms, and maximum non-FSI t_tr-pk = 1.25 ms). TANs were separated from other non-FSI units by the regularity of their baseline firing (maximum TAN CV = 1.5), leaving units that exceeded this CV as putative MSNs. Around 9% of all units were not classified in any of these three categories and were excluded from further analysis.

**Histology**

After recording, animals were overdosed with sodium pentobarbital and perfused with 10% formalin solution. Brains were extracted and fixed overnight at 4°C. To confirm the correct targeting of the microprobe, tissue was stained for tyrosine hydroxylase (TH) using sheep anti-TH primary (Millipore, 1:500) and TRITC-conjugated donkey anti-sheep secondary antibodies (Jackson ImmunoResearch, 1:100). Microprobe tracks were determined by locations of DiD fluorescence in images of TH-stained sections. We confirmed that recordings were located at approximately the same coronal section of the striatum (range of AP positions relative to
bregma: 1.1 to 1.4 mm). We could subsequently determine the approximate mediolateral and dorsoventral silicon prong positions, and thus cell position.

**Discriminating cell identification**

Cue-triggered firing rate, $R(t)$, was calculated from the average firing rate of CS+ trials with anticipatory responding (correct hits) and CS- trials without licking (correct withholding). The time bin size was 50 ms. The baseline period was defined as the 5 s interval preceding cue presentation, and the average firing in this period was used to calculate the baseline-subtracted change in firing, $\Delta R(t)$. The mean baseline subtracted and normalized firing rate was obtained with the expression $\frac{\Delta R(t)}{\Delta R_{\text{max}}}$. Discriminating units were determined by comparing the distributions of firing rate during correct CS+ hit, $R_{CS+}(t)$, and correct CS- withholding, $R_{CS-}(t)$, trials using a permutation test on individual time bins (10,000 iterations). For each time bin, we shuffled the labels of firing activity for each trial to create two distributions of firing rates, $R_{CS+,\text{shuffled}}(t)$ and $R_{CS-\text{,shuffled}}(t)$ shuffled, that could be expected by chance for each trial type. We defined a unit as being discriminating if the absolute value of the difference between $R_{CS+}(t)$ and $R_{CS-}(t)$ for at least two consecutive time bins was higher than the 99th percentile of the distribution of differences between $R_{CS+,\text{shuffled}}(t)$ and $R_{CS-\text{,shuffled}}(t)$.

**Behavior correlations**

Pearson correlations between spiking activity of individual units and lick rate or treadmill velocity were calculated over the entire recording, in time bins of 50 ms.

**Signal and resting state correlations**

For signal correlations, we calculated the Pearson correlation coefficient on the $R_{CS+}(t)$ signal of simultaneously recorded units, using 50 ms time bins from $t=0$ to 2.5 s after cue onset on
correct CS+ hit trials. We defined resting periods as intervals of at least 2 s during which animals did not make any detected movements (running, licking) and were not presented with any external stimuli (cues and reward). To find the resting state spike count correlations, we serially concatenated spiking activity occurring within these epochs to create a continuous time series vector (500 s, 10 ms bins) representing the resting state firing rate, $R_{\text{rest}}(t)$. We calculated resting state activity for each individual unit, and then obtained the Pearson correlation coefficient using these vectors. To detect significant correlations, we used the permutation test for correlations on each unit pair containing spiking activity in the resting state. This test involved shuffling $R_{\text{rest}}(t)$ for one unit in the pair and recalculating the correlation coefficient (1000 iterations). This resulted in a distribution of possible correlation coefficients that could be expected by chance. We determined significance if the absolute value of the observed correlation exceeded the 99 percent confidence interval of the absolute value of shuffled values. The correlation probability is the fraction of significant pairs detected out of all possible simultaneously recorded pairs separated by 0.025 to 1 mm. We excluded pairs of units closer than 0.025 mm from all temporal correlation analysis, to minimize any effect of possible spike sorting errors.

**Spike time cross correlations**

We calculated the spike time cross correlogram (CCG) between pairs of units using 1 ms time bins and a lag of ±25 ms. We used spike trains from the entire recording session for this analysis. To identify significant cross correlations we recalculated the CCG after adding a random 0-5 ms jitter to the spike train (500 iterations), and determining the confidence interval from this distribution (Fujisawa et al. 2008).
**Statistical tests**

We performed nonparametric permutation tests to determine the significance of linear correlation analysis (Shobe et al. 2015). We used 1,000 to 10,000 shuffles per test. Paired t-test analysis was performed using standard Matlab functions. ANOVAs were performed using GraphPad Prism software.

**RESULTS**

**Mice learn a stimulus discrimination task**

To combine large-scale neural recordings with a behavioral assay of stimulus discrimination, we implemented a Pavlovian reward-based odor discrimination task in head-restrained mice (Shobe et al. 2015). Animals typically learned the association between odor presentation and reward delivery within one recording session (Figs. 1B). To examine how responding to the two trial types changed over time we divided the training session into blocks of 25 trials (Fig. 1C).

On average, hit rate increased relative to false alarm rate during training. A two-way repeated-measures ANOVA revealed a significant interaction between time and trial type (p=0.002, F$_{3,48}$=5.94). Moreover, mice were more likely to respond to CS+ trials than CS- trials in the last block (p<0.05, Sidak’s test for multiple comparisons). The difference between hit rate and false alarm rate was used to quantify discriminatory performance on the task. This measure steadily increased across blocked trials (p=0.005, F$_{3,24}$=8.4, one-way, repeated measures ANOVA; Fig. 1D). Together, these results indicate that on average mice were able to develop selective anticipatory responding to CS+ and withholding of licking to CS- in a single training session.

**Large-scale recordings reveal a population of discriminating MSNs**

To examine the dynamics of large populations of striatal neurons during the stimulus discrimination task, we employed silicon microprobes with 256 recording sites. We used two
customized designs that allowed recordings from either dorsal or ventral (nucleus accumbens) striatal subregions, or simultaneously from both. In all cases the recording sites spanned a large extent of the striatum along the mediolateral axis (Figs. 2A, 2B). Recordings captured spiking activity distributed across the microprobe (Fig. 2C), with each session yielding an average of 115 (range: 51-188) simultaneously measured units. The spike waveforms had a median signal-to-noise ratio of 9 (Fig. 2D). We used spike waveform width and the CV of baseline firing rate to classify units as putative MSNs, FSIs, and TANs (Figs. 2E, 2F). We found that 52% of TANs showed burst/pause responses to rewards (Fig. 2F, bottom), which are characteristic firing properties of this cell type (Aosaki et al. 1994). MSNs and TANs displayed wider spike waveforms than FSIs, and MSNs showed lower firing rates (mean±SD: 1.1±1.4 Hz) than both FSIs (mean±SD: 8.7±12.4 Hz) and TANs (mean±SD: 4.0±1.8 Hz, p<0.05, unpaired t-test, Bonferroni corrected; Fig. 2G). MSNs and FSIs represented the highest proportion of recorded units (Fig. 2H), and we therefore focused the majority of our analysis on these two subpopulations.

When visualized at the population level, striatal MSNs exhibited distinct activity patterns during correct CS+ hit and correct CS- withholding trials (pooled data from 9 animals, n=841 MSNs; Fig. 3A). Since the network appeared to distinguish between the two stimulus conditions, we quantified the fractions of MSNs whose firing rate significantly differed between correct CS+ hit trials and correct CS- withholding trials between the cue onset (t=0 s) and reward delivery time (t=2.5 s; Fig. 3B). These units are referred to as discriminating cells. On average, 23% of MSNs were found to have firing rates that discriminated between these trial types (Fig. 3C). All discriminating MSNs showed higher firing to CS+ relative to CS- cues. In addition to showing different firing rates to the two cues, discriminating MSNs showed overall higher firing rate responses during correct CS+ hit trials as compared to their non-discriminating MSN counterparts (p=0.0004, paired t-test; Fig. 3D). This indicates that non-discriminating MSNs tend to be a less active population during CS+ trials. We next inquired whether these
populations differentially respond to movements that were concurrently measured in the experiment (licking and running). We correlated spiking activity of discriminating and non-discriminating MSNs with lick rate and treadmill velocity. We found that discriminating MSN firing rate was more correlated to lick rate than non-discriminating MSNs ($p=0.0002$, paired t-test; Fig. 3E). On the other hand, discriminating and non-discriminating MSNs were equally correlated to treadmill velocity ($p=0.9$, paired t-test). These observations show that discriminating MSNs were more selective for licking, and were equally selective for running speed relative to non-discriminating MSNs. Thus, it appears that some discriminating MSNs are modulated by both licking and running, which is consistent with a study showing that MSNs can multiplex multiple aspects of behavior (Rueda-Orozco and Robbe 2015). Furthermore, our definition of discriminating cells does not preclude that these neurons encode other behaviors, including non-motor aspects of the task.

Taking advantage of the high throughput recording capabilities of silicon microprobes, we mapped neural activity across a large extent of one section of the anterior striatum. Using their estimated position, neurons were assigned to one of twelve subregions forming a 4 x 3 compartment grid (Fig. 4A). The activity of the pooled population in each compartment was then averaged. Mean cue-triggered firing was found to be heterogeneous across the different grid compartments (Fig. 4B). Most strikingly, the responses to CS+ and CS- trials were most segregated in the lateral portions of the striatum. We also found that discriminating MSNs were more likely to be found on laterally positioned electrodes (discriminating MSN mediolateral position: 1.74 mm, 1 SD=0.15 mm, non-discriminating MSN mediolateral position: 1.5 mm, 1 SD=0.15 mm, $p=0.02$, $n=9$, paired t-test; Fig. 4C). In agreement with these two observations, the mediolateral position of individual MSNs was correlated with greater differences between correct CS+ and CS- firing ($r=0.2$, $p<0.0001$, permutation test for correlations; Fig. 4D, left). We did not detect a corresponding correlation between CS+ and CS- firing and dorsoventral unit position ($r=-0.05$, $p=0.18$, permutation test for correlations; Fig. 4D, right). Altogether, MSN
population firing activity in the lateral striatum was more likely to show discriminatory firing, and
that discriminating MSNs are more selective for licking than non-discriminating MSNs.

Previous work has shown that striatal neuron firing is altered as training progresses
(Tremblay et al. 1998), suggesting that similar effects may be present in our recordings. We
therefore investigated how discriminating and non-discriminating MSN firing changed over time
by measuring the difference between mean CS+ and CS- evoked firing in blocks of 25 trials
(Figs. 5A, 5B). We observed a steady change in discriminating MSN firing activity across trial
blocks, but did not see this pattern in non-discriminating MSNs. A two-way, repeated-measures
ANOVA revealed a significant effect of trial block (p<0.0001, F_{3,48}=11.3), and MSN population
(p=0.0003, F_{3,48}=21.83) and showed an interaction between trial block and MSN population
(p<0.0001, F_{3,48}=10.76). These results suggest that discriminating MSNs underwent a significant
divergence from the remaining MSN population in encoding correct hit and withholding trials
over time (Fig. 5C).

Discriminating MSNs form a temporally correlated ensemble
The large scale of our silicon microprobe measurements provided a unique opportunity to
analyze correlated dynamics among hundreds of simultaneously recorded striatal cell pairs in
order to study how these populations interact at the network level. Numerous studies suggest
that correlated spontaneous neural activity reflects the underlying connectivity of the network
(Ko et al. 2011; Ringach 2009; Stern et al. 1998). As such, the resting state has become an
important measurement modality in neuroscience (Cole et al. 2014; Raichle 2010; Reimer et al.
2014). To focus on spontaneous activity we calculated pairwise spike count Pearson
correlations of units recorded in the same session during intermittent periods when animals
were at rest. This corresponded to times when mice were neither moving on the treadmill, nor
licking or receiving explicit olfactory or reward stimuli (Fig. 6A).
We found that the strength of resting correlations decreased as a function of MSN pair separation ($p<0.0001$, $F_{(9,24695)}=63.75$, one-way ANOVA; Fig. 6B). This spatial clustering suggests that neighboring cells are more likely to share information, possibly via overlapping input. We next tested whether correlated resting state dynamics show any resemblance to correlated activity during the behavioral task. Indeed, we identified a significant relationship between resting and task-evoked signal correlations during correct CS+ hit trials ($p<0.0001$, $r=0.104$, permutation test for correlations; Fig. 6C). Since the small magnitudes of resting state correlation coefficients are difficult to interpret (Cohen and Kohn 2011), we identified correlations that could not occur by chance ($p<0.01$, permutation test for correlations, see Materials and Methods). We found that MSNs with significant resting correlations had a higher signal correlation coefficient than uncorrelated MSNs ($p=0.0008$, paired t-test; Fig. 6D). Together, these results demonstrate that the resting state of the striatal network contains an intrinsic organization that is related to behaviorally modulated dynamics. This relationship is consistent with a model in which common input drives correlated spontaneous as well as task-evoked striatal activity.

Using resting state correlation analysis, we asked if discriminating cells were distinct from the remaining population in terms of their likelihood of showing significant resting correlations. Indeed, we found that discriminating MSNs were more likely to exhibit significant correlations with one another than to non-discriminating MSNs ($p=0.0096$, paired t-test; Fig. 6E). In addition to being more correlated in their spontaneous activity, discriminating MSNs were more excitable as revealed by their higher spontaneous firing rate ($p=0.0109$, paired t-test; Fig. 6F). We next investigated whether discriminating MSNs changed their correlation strengths over time during the course of the recording session. The recording was divided into four blocks corresponding to 25 CS+ trials, and network connectivity was analyzed separately during resting periods occurring within each block of trials. We found that there was no effect of time in either the probability of significant correlation among discriminating MSNs or between discriminating and
non-discriminating MSNs \((p=0.143, F_{(3,48)}=1.85\), two-way, repeated measures ANOVA; Fig. 6G).

Similarly, there was no significant effect of time in the resting state firing rates \((p=0.8, F_{(3,48)}=0.34\), two-way, repeated measures ANOVA; Fig. 6H). These findings suggest that resting state correlations among discriminating MSNs remains relatively stable over the course of the first training session. However, we cannot rule out the possibility that resting correlations or firing change over more extended periods of training.

Firing rate is known to influence neural correlations between cells (Cohen and Kohn 2011; de la Rocha et al. 2007). On one hand, there may be a biological basis for this relationship: for example, experiments in cortical circuits show that highly active cells are more likely to be coupled (Yassin et al. 2010). But to examine whether temporal correlations are purely explained by higher firing, we looked at the relationship between resting firing rate and correlation probability. As expected, the probability of finding significant resting state correlations was greatest when both MSNs had relatively high (>1 Hz) firing rate (Fig. 6I). Next, to account for the effects of firing rate we examined the correlation probability for pairs of cells whose resting rate did not exceed 1 Hz. Discriminating MSNs remained more likely to be correlated to one another than to non-discriminating MSNs \((p=0.042\), paired t-test; Fig 6J) despite their no longer having significantly higher resting state firing \((p=0.3\), paired t-test; Fig. 6K). Together, these results show that with respect to non-discriminating MSNs, discriminating neurons represent a functionally specialized network in the striatum that is more tightly correlated during both spontaneous and task-evoked activity.

**FSIs contribute to striatal microcircuit synchrony**

The striatal microcircuit contains a population of fast spiking interneurons which are thought to be involved in regulating striatal output signals (Berke 2011; Koos and Tepper 1999). As observed for MSNs, FSIs activity was modulated during the task, forming trajectories that qualitatively resembled the MSN population in their responses to CS+ and CS- trials (Figs. 7A).
We found that FSIs were even more likely to be classified as discriminating than MSNs (p=0.011, paired t-test; Fig. 7B) and were more correlated to licking rate (p=0.016, paired t-test; Fig. 7C) and running velocity (p=0.012, paired t-test) than MSNs. Approximately 15% of the recorded units were putative FSIs, providing a sufficiently large population for resting state correlation analysis. Resting state firing between FSIs was also more likely to be correlated than between MSNs (p<0.0001, paired t-test; Fig. 7D). We found that discriminating FSIs were more likely to be correlated in their resting state activity to discriminating MSNs than to non-discriminating MSNs (p=0.04, paired t-test, Fig. 7E). We also compared the distributions of resting correlations among discriminating MSN pairs and pairs of discriminating MSNs and FSIs as a function of pairwise distance. We found that there were significant effects of both population (p<0.0001, F(9,4446)=31.66, two-way ANOVA; Fig. 7F) and pairwise distance (p<0.0001, F(9,4446)=4.96), suggesting that discriminating FSIs are more likely to be coupled with discriminating MSNs at greater distances than discriminating MSNs are coupled to each other. Together, these results show that discriminating FSIs and MSNs form a temporally correlated ensemble.

We next searched for potential microcircuit mechanisms for how striatal cell types might interact locally. We analyzed spike time cross correlations to assess the temporal relationship between cells on a millisecond timescale. We used a spike time jitter test (Fujisawa et al. 2008) to identify pairs with significant low latency cross correlations, consistent with the occurrence of direct synaptic coupling between these cells. To characterize average significant cross correlation trends we performed an analysis on the entire recording session and pooled results from all animals in the study (n=9 mice). Only 0.1% (38/28452 pairs) of MSN pairs showed evidence of low latency cross correlations typical of monosynaptic coupling (Fig. 8A). FSIs may also contribute to MSN activity through local interactions. Because of gap junction coupling FSIs form a highly interconnected network (Hjorth et al. 2009; Koos and Tepper 1999; Lau et al. 2010; Russo et al. 2013). In agreement with their predicted synchrony, we found that FSIs had a
higher interaction rate than MSNs (5.2%, 63/1221 pairs; Fig. 8B), and their cross correlation showed positive and symmetric coupling. Furthermore, we found that a subset of FSIs are coupled with MSNs (0.75%, 77/10323 pairs; Fig. 8C) in a manner consistent with feedforward inhibition (Koos and Tepper 1999). Our results show that the likelihood of finding significant FSI-MSN interactions with this analysis is relatively small, and another study failed to find these interactions altogether (Gage et al. 2010). We also detected a small population (~4%) of tonically active neurons in our recordings, and found that some FSIs were coupled to TANs (2.4%, 9/381 pairs; Fig. 8D). We detected very sparse TAN-MSN (0.05%, 1/2085 pairs) and zero TAN-TAN connections (0/88 pairs). Taken together the cross correlation analysis suggests that FSIs form a highly synchronized subnetwork of cells that can alter the timing of MSN activity, although the impact of these interactions on circuit activity during behavior is not yet fully understood.

**DISCUSSION**

This study used large-scale neural recordings to characterize striatal network activity in mice learning to associate specific odor cues with rewards. We initially focused our analysis on single-unit activity during the task and identified a subpopulation of putative MSNs whose firing responses discriminated between correctly performed CS+ and CS- trials. Similar responses in individual striatal units have been previously reported during cue discrimination tasks (Nicola et al. 2004; Setlow et al. 2003; Tremblay et al. 1998). When compared to non-discriminating units, discriminating MSNs displayed higher firing rate change during CS+ trials. The entire population of discriminating MSNs also responded with higher activity to CS+ trials than to CS- trials. These discriminatory responses were not uniformly distributed in the striatum. By mapping the recorded neural activity, we showed that discriminating MSNs were more likely to be found in the lateral side of the striatum. This appears to be consistent with the known anatomical organization of this structure; specifically, that the dorsolateral striatum receives significant input
from the sensorimotor areas of the overlying cortex (Alexander et al. 1986; McGeorge and Faull 1989). Furthermore, single-unit measurements in the lateral striatum of rodents have been previously found to represent sensory and motor activity of different body parts, including those of the orofacial area and forelimbs (Cho and West 1997). In our study, discriminating MSNs were significantly more correlated with lick rate than non-discriminating MSNs. Hence, this discriminating population could be involved in generating striatal output signals that mediate stimulus-specific anticipatory licking. We also showed that discriminating MSNs were modulated by running speed. However, running speed was equally correlated to both discriminating and non-discriminating neuron firing. This may also be evidence that some discriminatory neurons are modulated by both types of behaviors, supporting a report on the multiplexed coding properties of striatal neurons (Rueda-Orozco and Robbe 2015). These results do not rule out that other aspects of the task also contribute to discriminating MSN coding.

Since there have been reports that the dorsomedial striatum is engaged during early stages of procedural learning (Thorn et al. 2010; Yin et al. 2009; Yin et al. 2005), it was slightly unexpected that we observed little change in activity in the medial striatal subregions (Fig. 4B). This might reflect differences in learning or behavioral strategies between our study and other work. The dorsolateral striatum has been implicated in mediating stimulus-response associations that appear in well-trained animals (Corbit and Janak 2007). However, some studies have reported that lesions to the dorsolateral striatum also impair acquisition of stimulus-response associations as well (Featherstone and McDonald 2004). Our results show that at least in the case of the Pavlovian reward association task employed here, the lateral striatum appears to be more active than the medial regions in the early stage of training. Further work needs to elucidate the role of specific microcircuits in the lateral striatum in acquiring and expressing this behavior. In addition to evidence that the medial and lateral striatal subregions have different functional contributions to behavior, the ventral striatum (nucleus accumbens) is also known to have unique reward processing functions in comparison to the dorsal striatum.
This is thought to be partially based on the limbic inputs that this area receives (Voorn et al. 2004). We did not detect a strong relationship between MSN position along the dorsoventral axis and the degree of differential coding between CS+ and CS- trials. This was evidence that the dorsolateral and ventrolateral striatum both contained discriminating units. Thus as an approximation, we assumed no difference in temporal correlation properties among pairs of discriminating neurons throughout the striatum. Although this is likely to be an oversimplification, our recordings lacked the single-unit throughput to reliably examine differences in correlations between the dorsal and ventral striatum. Furthermore, our assumption does not imply that these subregions encode identical information, and indeed, the ability to map activity across a section of the striatum revealed marked differences in cue-evoked neural dynamics.

We took our analysis of discriminating units in a new direction by investigating temporal correlations among this population. In order to avoid the potentially confounding effects of behavior and stimuli on neural activity and correlations, we examined spontaneous activity, which coincided with periods when animals were at rest. An extensive body of literature has shown that spontaneous neural activity is related to behaviorally evoked activity (Arieli et al. 1996; Raichle 2010; Ringach 2009). We extended this principle to striatal microcircuits by demonstrating that resting state MSN correlations are correlated to signal correlations. We speculate that experience-dependent plasticity in the striatum may establish neural ensembles whose synaptic connectivity predisposes them to fire together both during behavior (leading to high signal correlation), and thus also during rest (leading to high resting correlation) (Fregnac 2003). A novel finding of this study was that discriminating units are more likely to exhibit significant resting state correlations to each other than to non-discriminating units. Correlated activity among neurons is attributed to common sources of input (Cohen and Kohn 2011). We therefore postulate that an important factor that mediates the observed pattern of correlations between discriminating MSNs is shared glutamatergic connections (Stern et al. 1998; Wilson 2013). Glutamatergic axons that innervate the striatum are widely distributed (Kincaid and Wilson 2004).
1996), which may enable multiple MSNs to receive the same excitatory signals. But since individual axons form only a few synapses with any individual MSN, eliciting action potentials may require coordinated glutamatergic activity from many axon terminals representing a diverse range of input (Kincaid and Wilson 1996). This may explain why the activity of the recorded MSNs was correlated with more than one type of behavior (e.g., licking and running).

We did not find that discriminating neurons became more correlated in their spontaneous activity during learning, nor did they show a change in their excitability. This observation could be explained either because our analysis methods did not have the temporal resolution to observe the changes, or that the discriminating MSN ensemble was already established prior to the experiment. In the latter scenario, discriminating neurons may already have been established by an animal’s past experiences in its home cage prior to training, which would have involved some form of licking and running behaviors.

In addition to shared glutamatergic inputs, temporal correlations among MSNs are likely to be mediated by striatal interneurons such as FSIs, which are thought to control MSN firing on millisecond timescales (Damodaran et al. 2015). FSIs receive input from many of the same external sources as MSNs (Fino and Venance 2011) and are thus able to encode behaviorally relevant information. Indeed, we found that FSI firing rate was correlated to licking and running to an even greater degree than MSNs, and FSIs were more likely to be classified as discriminating units than MSNs. Discriminating FSIs were also more likely to be correlated with discriminating MSNs over non-discriminating MSNs. Many studies have shown that FSIs provide feedforward GABAergic inhibition of MSN spiking activity (Gittis et al. 2010; Koos and Tepper 1999; Mallet et al. 2005; Taverna et al. 2007). We found 0.75% of FSI-MSN pairs exhibited significant cross correlations in vivo, providing evidence for direct interactions between these two subpopulations of striatal neurons. It is interesting to note that another study did not find any significant cross correlations between these cell types in vivo (Gage et al. 2010). This suggests that FSI-MSN interactions may also occur over long timescales relative to single
action potentials because of polysynaptic network effects in the striatum. FSI-FSI connectivity is complex as it involves both chemical and electrical synapses (Berke 2011; Fukuda 2009; Kita et al. 1990; Russo et al. 2013), whose collective influence on network activity in the intact brain, or behavior is not well understood.

Another possible mechanism by which MSN activity can be correlated is through direct MSN-MSN connections. Despite a well-known effect of lateral inhibition among MSNs (Czubayko and Plenz 2002; Taverna et al. 2004; Tunstall et al. 2002), the role of this inhibitory coupling in striatal computation is still unclear (Tepper et al. 2008). Our cross correlation analysis found very few significant low latency interactions among nearly 30,000 MSN pairs (only 0.1%), suggesting that individual MSN-MSN interactions are weak compared to other factors that influence MSN activity (Jaeger et al. 1994). Furthermore, inhibitory post-synaptic potentials (IPSPs) between MSNs have been found to be weak (Tunstall et al. 2002) relative to IPSPs evoked by FSIs on neighboring MSNs (Koos and Tepper 1999). Interestingly, the ratio of these IPSPs is in the same order of magnitude as the ratio of significant FSI-MSN to MSN-MSN cross correlation pairs in our study, suggesting that cross correlations are sensitive to synaptic strength between two neurons. Thus, our work appears to show that MSN-MSN interactions are sparsely detected using cross correlation analysis, and that FSI-MSN coupling is 7.5 times more prevalent at the level of individual cell pairs. However, because of the abundance of MSNs in the striatum compared to FSIs, which only represent ~1% of the total population (Berke 2011), the cumulative effect of lateral inhibition may have a significant impact on striatal microcircuit dynamics, particularly during periods when large groups of MSNs are synchronized (Carrillo-Reid et al. 2009).

Finally, temporal correlations in striatal microcircuits are believed to strongly depend on neuromodulatory signals such dopamine and acetylcholine. Dopamine has a well-known role in modulating MSN activity and plasticity (Gerfen and Surmeier 2011). Abnormally low levels of dopamine found in Parkinson’s disease have been linked to excessive synchrony of striatal
ensembles (Jaidar et al. 2010), as well as altered FSI-MSN connectivity (Gittis et al. 2011), both of which could also significantly impact temporal correlations. Cholinergic interneurons are sparsely distributed in the striatum, but have been shown to significantly impact striatal activity (English et al. 2012). In addition to modulating the release of dopamine (Cachope et al. 2012; Threlfell et al. 2012) and GABA (Nelson et al. 2014) from midbrain dopaminergic terminals, cholinergic signaling has also been shown to regulate the efficacy of corticostriatal input in eliciting action potentials in MSNs (Perez-Ramirez et al. 2015; Shen et al. 2005). Cholinergic interneurons are thought to correspond to putative TAN units (Aosaki et al. 1994). We found only 1 significant MSN-TAN cross correlation out of over 2000 pairs (<0.05%), which appears consistent with TANs influencing MSN activity on long timescales relative to single action potentials. On the other hand, significant FSI-TAN cross correlation events were relatively common (9/381, 2.4%). Coupling between these cells (Koos and Tepper 2002) may indirectly influence MSN correlations as well. However, the role of interactions between TANs and other interneurons in coordinating MSN dynamics remains unclear.

In conclusion, large-scale neural recordings enabled an examination of temporal correlations among hundreds of electrophysiologically defined striatal neurons in animals undergoing reward conditioning. We identified a population of cue discriminating striatal neurons that were more highly correlated to each other than to non-discriminating units. These results suggest that discriminating units represent a functionally specialized ensemble with a higher occurrence of shared connections from both external as well local sources. Thus, temporal correlations among specialized neurons may help to pattern a strong output signal that is sent to downstream basal ganglia nuclei in order to facilitate behavior.
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AUTHOR CONTRIBUTIONS

K.I.B. and S.C.M. conceived the experiments, designed the silicon microprobes, built the data acquisition system, analyzed data, and wrote the manuscript. K.I.B. carried out the surgical procedures, behavioral training and electrophysiological recordings. V.M. electroplated silicon microprobes, performed histology, fluorescence imaging, and image analysis. P.G. assisted with the head fixation system development and shared expertise on in vivo physiological experimentation.

COMPETING INTERESTS

The authors declare no competing interests.


Roitman MF, Wheeler RA, and Carelli RM. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron 45: 587-597, 2005.


**FIGURE LEGENDS**

**Figure 1.** Head restrained mice demonstrate single-session discrimination learning. *A:* Experimental setup and trial schematic. Head-fixed mice were placed on a spherical treadmill and were presented with olfactory cues and liquid rewards. Breaks in the infrared beam positioned in front of the lick tube were used to detect licking activity and treadmill velocity was monitored using an optical mouse (not shown). Trials consisted of either 1 s of odor (CS+) followed by a 1.5 s pause and a reward, or a different 1 s odor (CS-) followed by no outcome. *B:* Licking activity rasters during CS+ and CS- trials for one representative animal. Shaded rectangles represent the olfactory cue presentation period. Black and red tick marks indicate individual licks during trials with correct and incorrect responses, respectively. Red triangle indicates the time of reward delivery. *C:* Learning curves for all animals (*n*=9) showing the mean probability of licking after CS+ (black) and CS- (red) trials in blocks of 25 trials. A two-way ANOVA, repeated measures revealed a significant effect of trial block (*p*=0.003) and a significant interaction between trial types (*p*=0.0016, *p*<0.05, Sidak’s test for multiple comparisons). *D:* Evolution of the mean discriminatory behavior rate in blocks of 25 trials (*p*=0.0021, one-way, repeated measures ANOVA). Error bars represent SEM.

**Figure 2.** Large-scale striatal recordings with silicon microprobes. *A:* Illustration of the two 256 electrode silicon microprobe designs used to record in the striatum. Each silicon prong contains a high-density electrode array, with the geometry shown in magnified images of the tips. Short scale bars represent 10 μm. *B:* Fluorescence image of silicon microprobe tracks (white) embedded in a TH-labeled section of the striatum (orange). White outline represents the perimeter of the striatum. Scale bar represents 0.5 mm. *C:* Samples of measured signals from 10 representative recording sites, filtered offline from 600 to 6500 Hz. Columns show simultaneously recorded data from two sets of adjacent recording sites. *D:* Distribution of signal-
to-noise ratios for all recorded units used in the study. E: Scatter plot of the log of baseline firing rate versus spike waveform trough-to-peak time with color representing putative cell identity. Gray circles denote unclassified units. F: (left) Representative waveforms of three putative cell types, medium spiny neurons (MSNs), fast spiking interneurons (FSIs) and tonically active neurons (TANs) identified in this study. (right) Corresponding mean firing rate during CS+ trials for each representative unit depicted at left aligned to the cue onset. Shaded rectangle represents CS+ odor cue delivery time. Red triangles indicate reward delivery. Rates are averaged over all correctly performed trials. G: Mean baseline firing rate across all recorded MSNs, FSIs, and TANs in the study (p<0.05, Bonferroni-corrected t-test). H: Percentage of each cell class that comprised the combined dataset.

Figure 3. Identification of a cue-discriminating subpopulation of striatal MSNs. A: Mean baseline subtracted and normalized firing rates for 841 MSNs obtained from 9 animals during correct CS+ trials (top) and correctly withheld CS- trials (center). Units in both plots are sorted by latency to peak firing during CS+ trials (top plot). Cues are presented between 0 and 1 s, indicated by colored rectangles. Reward delivery during CS+ trials is indicated with the red triangle. Bottom panel shows the mean baseline subtracted firing rate for all neurons depicted in the heat plots. The orange rectangle represents the odor delivery time. B: Mean baseline subtracted firing rate for two MSNs during correct CS+ trials (blue) and correctly withheld CS- trials (magenta). Top: a representative discriminating MSN defined by differential firing between the CS+ and CS- trial conditions. Bottom: a representative non-discriminating MSN. Discrimination was determined on the interval from 0 to 2.5 s following cue onset. C: Pie chart showing the mean fraction of cue-discriminating MSNs. D: Mean value per animal of baseline subtracted firing rate between 0 and 2.5 s during correct CS+ trials for discriminating and non-discriminating MSNs (p=0.0004, paired t-test). E: Mean Pearson correlation coefficient between spiking activity of discriminating and non-discriminating MSNs, and lick rate (p=0.0002, paired t-
test) or treadmill velocity (p=0.9, paired t-test). Each point represents one animal. Lines between points represent paired data from individual animals. All error bars represent SEM.

**Figure 4.** Mapping discriminatory activity across the striatal cross-section. A: Outline of the cross-section of the striatum spatially divided into a 4x3-compartment grid. Values represent the total number of recorded MSNs allocated into each of the grid’s compartments based on the estimated recording position of each unit. B: Mean baseline subtracted firing rates for all MSNs positioned in each of the 12 boxes illustrated in A. Color conventions are identical to Fig. 3A. C: Combined map of the location of discriminating (blue) and non-discriminating (gray) MSNs recorded in all mice. Centers of all recordings were all aligned along the dotted line. D, left panel: Difference between the mean correct CS+ and CS- firing rates for all neurons binned by their mediolateral recording position. Right panel: Difference between the mean correct CS+ and CS- firing rates for all neurons binned by their dorsoventral recording position. Correlations were performed between position and difference in rate for MSNs pooled from all recordings (n=841).

**Figure 5.** Evolution of activity in discriminatory MSNs during training. A: Mean baseline subtracted firing rate for all discriminating MSNs during the period starting 1s before cue onset until reward delivery for all CS+ trials (blue) and all CS- trials (magenta). Each panel depicts firing activity for each trial type in blocks of 25 trials. Color conventions are identical to Fig. 3A. B: Same as A for non-discriminating MSNs. C: Mean firing rate difference between CS+ trials and CS- trials for discriminating and non-discriminating MSNs in blocks of 25 trials. A two-way, repeated measures ANOVA revealed a significant effect of trial block (p<0.0001) and population (p=0.0003) and a significant interaction between the two factors (p<0.0001). Averages were computed across individual animals, n=9. Error bars represent SEM.
Figure 6. Correlated resting state activity in the striatum. A: Sample data depicting resting state identification. The black circles and magenta traces represent individual licks and running speed on the treadmill, respectively. Blue shaded regions label a 5 second window following cue onset. Gray shaded regions depict resting periods that would be concatenated with other resting periods for resting state analysis. B: Mean resting correlation coefficient for all MSN pairs plotted as a function of pairwise distance (p<0.0001, one-way ANOVA). Data are binned in 0.1 mm increments. C: Mean resting correlation coefficient for all MSN pairs plotted as a function of the pair’s signal correlation during correct CS+ trials. Binned data show a strong relationship between these parameters, and unbinned data are also correlated (n=23758 pairs, permutation test for correlations). Removing the outlier point in the left-most bin did not change the significance of the correlation (p<0.0001, r=0.104, permutation test for correlations). D: Mean signal correlation coefficient for significantly correlated and non-correlated MSN pairs during spontaneous activity in the resting state. Points represent mean values of individual animals (p=0.0008, paired t-test, n=9). E: Probability of finding significant resting correlations among discriminating MSN pairs, and between discriminating to non-discriminating MSN pairs (p=0.0096, paired t-test). Points represent the fraction of pairs spaced within 0.025 to 1 mm recorded from individual animals. F: Mean resting state firing rate of discriminating MSNs and non-discriminating MSNs (p=0.011, paired t-test). Points represent the mean rate in individual animals. G: Resting state correlation probabilities among discriminating MSN pairs and between discriminating to non-discriminating MSN pairs calculated during resting times that occurred in different blocks of the recording. Each trial block represents resting periods detected within blocks of 25 CS+ trials. H: Resting firing rates for discriminating and non-discriminating MSNs calculated during blocked resting periods. I: Mean probability of finding significant pairwise resting correlations, as a function of the firing rate of each cell in the pair. Color scale represents significant resting correlation probability. J: Resting state correlation probabilities among pairs of discriminating and between pairs of discriminating to non-discriminating MSNs that had firing
rates less than or equal to 1 Hz (p=0.042, paired t-test). K: Mean resting state firing rate of discriminating MSNs and non-discriminating MSNs having firing rates < 1 Hz. (p=0.3, paired t-test). Lines between points represent paired data from individual animals. All error bars are SEM.

Figure 7. Discriminating FSIs and MSNs form correlated ensembles. A: Mean baseline subtracted and normalized firing rates for all 178 FSIs recorded from 9 animals during correct CS+ trials (top) and correctly withheld CS- trials (center). Units in both plots are sorted by latency to peak firing in the top plot. Cues are presented between 0 and 1 s, indicated by colored rectangles. Reward delivery during CS+ trials is indicated with the red triangle. Bottom panel shows the mean baseline subtracted firing rate for all FSIs depicted in the heat plots. Orange rectangle represents odor delivery time. B: mean fraction of cue-discriminating MSNs and FSIs (p=0.011, paired t-test). C: Mean Pearson correlation coefficient between spiking activity of all MSNs or FSIs, and lick rate, (p=0.016, paired t-test) or treadmill velocity (p=0.012, paired t-test) behavior. D: Probability of finding significant resting correlations among pairs of MSNs and FSIs (p<0.0001, paired t-test). E: Probability of finding significant resting correlations among pairs of discriminating FSIs and MSNs and between pairs of discriminating FSIs and non-discriminating MSNs (p=0.0395, paired t-test). F: Mean resting correlation coefficient for all discriminating FSI and MSN pairs and all discriminating MSN pairs plotted as a function of pairwise distance. A two-way ANOVA revealed a significant effect of population (p<0.0001) and pairwise distance (p<0.0001). Data pooled from all animals are binned in 0.1 mm increments. Lines between points represent paired data from individual animals. Error bars are all SEM.
**Figure 8.** *A, top panel:* Spike time cross correlogram between one pair of MSNs exhibiting significant cross correlation. Blue lines represent upper and lower 99% confidence intervals of the time-jittered cross correlation. *Bottom panel:* Mean jitter subtracted and normalized cross correlogram for all MSN pairs exhibiting significant cross correlation. The fraction (0.1%) indicates the proportion of MSN pairs recorded within 0.025 to 1 mm that exhibited significant cross correlation according to the jitter test. Dotted red lines are aligned to a time lag of 0 s. *B:* Same as A but for FSI pairs. *C:* Same as A but for MSN-FSI pairs. *D:* Same as A but for TAN- FSI pairs. Error bars represent SEM.
Figure 1

A

B

C

D

A figure showing experimental setup and data analysis.

- Panel A: Diagram of experiment setup.
- Panel B: Graph showing trial blocks and distribution.
- Panel C: Graph showing fraction of trials with anticipatory licking.
- Panel D: Graph showing (Hit - False alarm) rate.
Figure 2

A. Type I and Type II signals.

B. Image with signal example.

C. Graph showing signal to noise ratio.

D. Heatmap of firing rates.

E. Scatter plot of logbaseline firing rate vs. trough-peak width.

F. Graph showing firing rate changes over time for MSN, FSI, and TAN.

G. Bar chart showing percentage of units by type.

H. Pie chart showing distribution of units by type.
Figure 3

- A: Heatmaps showing firing rate changes over time for discriminating and non-discriminating MSNs.
- B: Graphs illustrating firing rate changes with CS+ and CS- for discriminating and non-discriminating MSNs.
- C: Pie chart indicating the proportion of discriminating and non-discriminating MSNs.
- D: Scatter plots demonstrating the change in firing rate with licking and running for discriminating and non-discriminating MSNs.
- E: Graph showing the correlation between licking, running, and change in firing rate with discriminating and non-discriminating MSNs.
Figure 4

A

B Medial ←→ Lateral

C

D

LateralMedial

Discriminating

Non-discriminating

CS+

CS-

1.5

Position (mm)

1.5

Position (mm)

5 Hz

Time (s)

CS+ vs CS-

rate difference (Hz)

DorsalVentral

A

B

C

D

LateralMedial

Medial ←→ Lateral

Discriminating

Non-discriminating

r = 0.2

p = 0.0001

r = -0.05

p = 0.18

1

2

Position (mm)

Medial ←→ Lateral

Position (mm)

Ventral ←→ Dorsal
Figure 5
Figure 6

A. Licking speed (Rest and CS+), Evoked speed (CS-), and Licking speed (Rest and CS+)

B. Pairwise distance (mm) and Resting Pearson r

C. Signal Pearson r vs. Resting Pearson r

D. Signal Pearson r vs. Correlation probability

E. Correlation probability vs. Discrim to Non-discrim pairs

F. Firing rate (Hz) vs. Discrim pairs

G. Correlation probability vs. Firing rate (Hz) (Discrim pairs vs. Discrim to Non-discrim pairs)

H. Firing rate (Hz) vs. Trial block (Discrim MSNs vs. Non-discrim MSNs)

I. Neuron 1 firing rate (Hz) vs. Neuron 2 firing rate (Hz)

J. Correlation probability vs. Firing rate (Hz) (Discrim pairs vs. Discrim to Non-discrim pairs)

K. Firing rate (Hz) vs. Trial block (Discrim MSNs vs. Non-discrim MSNs)
Figure 7

A

B

C

D

E

F

Change in firing rate (Hz)

Fraction discriminating units

Correlation probability

Pairwise distance (mm)
Figure 8

A. MSN-MSN

B. FSI-FSI

C. MSN-FSI

D. TAN-FSI

Mean normalized firing probability

38/28452 (0.1%) pairs

63/1221 (5.2%) pairs

77/10323 (0.75%) pairs

93/81 (2.4%) pairs