Information processing in the different
Basal Ganglia sub regions

Thesis submitted for the degree of
"Doctor of Philosophy"

by
Avital Adler

Submitted to the Senate of the Hebrew University of
Jerusalem

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This work was carried out under the supervision of

Prof. Hagai Bergman
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Abstract

The basal ganglia (BG) are a group of sub-cortical nuclei, which together with the cortex, work to execute habitual and goal directed behaviors requiring motor, cognitive and limbic structures. Experimental and theoretical studies have suggested that the BG nuclei implement a reinforcement learning algorithm in order to maximize gained reward. However, it is not clear how information is processed along this network, thus enabling it to perform its functional role. I therefore studied the information processing along the BG nuclei in response to appetitive and aversive behavioral events and in different brain states.

To this end, I recorded the activity of single cells in different areas of the BG of behaving monkeys in two states: while they were engaged in a classical conditioning paradigm involving rewarding (food), aversive (air-puff) and neutral (neither) outcomes and while their eyes were open vs. when they were closed. I compared the neuronal activity within and across BG regions; namely, the input stage of the BG, the striatum and its pallidal downstream targets, the central nucleus of the BG, the external segment of the globus pallidus (GPe) and the output stage of the BG, the internal segment of the globus pallidus (GPi).

In primates the striatum is divided into three territories: the putamen, caudate and ventral striatum (VS) which all converge onto the pallidum and onto the same motor pathway. This parallel organization suggests that there are multiple and competing control systems over behavior in the BG network. To explore which mechanism(s) enables the different striatal domains to encode behavioral events and control behavior, I compared the neural activity across striatal territories during the performance of the classical conditioning task.

I found that although neurons in all striatal territories displayed similar temporal modulations of their discharge rates to the behavioral events, their correlation structure was profoundly different. Specifically, putamen neuron pairs displayed increased correlations compared to the closer to zero correlations in the caudate and VS. The synchronization only observed between putamen cells transiently increased following the behavioral events and displayed different correlation dynamics to rewarding vs. neutral/aversive cues. These results indicate that the increased neural correlations observed solely in the putamen enabled efficient information encoding and were suggestive of a more efficient information transfer
from the putamen to its downstream targets, GPe and GPi, during the performance of a well-practiced task.

Furthermore, I studied how the activity in the striatum contributed to the activity generated in its downstream target, in the GPe. I examined the response profile and dynamical behavior of striatal and GPe neurons during the performance of the classical conditioning task. Both cell populations displayed sustained average activity to cue presentation. However, the population average response of striatal cells was composed of three distinct response groups which were temporally differentiated and fired in serial episodes along the trial. The population average response of GPe cells was composed of two response groups which overlapped in their time of activation and displayed persistent activity also at the single cell level. These results therefore support a functional, and not just anatomical convergence of striatal response groups on to GPe cells.

Finally, current anatomical models of the BG network predict reciprocal discharge patterns between the GPe and GPi. However, physiological studies revealing similarity in the transient responses of GPe and GPi neurons cast doubts on these predictions. I found that both pallidal populations exhibited decreased discharge rates in the "eye closed" state accompanied by elevated values of the coefficient variation (CV) of their inter-spike interval (ISI) distributions. In addition, the pallidal discharge modulations were gradual, starting prior to closing of the eyes. Thus, changes in GPe and GPi discharge properties were positively correlated, suggesting that the sub-thalamic nucleus and/or the striatum are the main common driving force for both pallidal segments.

Together these results suggest that in a well-practiced behavior, the BG network is organized such that interconnected sub networks in the putamen encode behavioral events and enable efficient information transfer to downstream BG structures.
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Introduction

The basal ganglia (BG) have traditionally been associated with the brain's motor systems (DeLong and Georgopoulos, 1981; Albin et al., 1989), partly due to BG related disorders such as Parkinson's and Huntington disease. However, studies of the BG during both normal functioning and neurodegenerative disorders have revealed that they are also involved in cognitive and limbic as well as emotional functions (Bevan et al., 1996; Haber and Knutson, 2010). Today, the BG are customarily viewed as a reinforcement learning system which play a crucial role in associative conditioning and habitual behavior. However, the neural correlates of RL with BG activity remain fundamental, partly open questions. This research was aimed at studying the normal information processing mechanisms along the BG to better understand how information related to valence is encoded in the BG and is translated to the observed response. In what follows I review the major anatomical components of the BG, the suggested functional role of these anatomical components and a suggested computational model for BG activity. Finally, I outline the goals and aims of this research.

Basal Ganglia anatomy

The BG neural network is commonly viewed as two functionally related subsystems, the main axis and the neuromodulators (Parent and Hazrati, 1995; Bar-Gad and Bergman, 2001). The main axis is, to a large extent, a feed-forward network in which projections from all cortical areas, the amygdale and the hippocampus are integrated within the input structures of the BG, the striatum and the subthalamic nucleus (STN). These structures project to the output nuclei of the BG; i.e., the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). The output nuclei affect behavior via their projections to the thalamus and then to the frontal cortex, or via projections to brainstem pre-motor nuclei. Transmission of information within the BG occurs both directly from the striatum to the GPi/SNr and indirectly through the external segment of the globus pallidus (GPe) and STN (Albin et al., 1989; Haber et al., 2011). The BG main axis is characterized by an anatomical convergence where the number of neurons is largely reduced from one stage to the
The majority of neurons in the BG, and in the striatum specifically, are GABAergic neurons. The principle projection neurons of the striatum are the medium spiny neurons (MSNs) which constitute up to 95% of the striatal cells (depending on the species, Tepper et al., 2008). They receive their vast excitatory input from the cortex and the thalamus and they project to both pallidal segments and to the SNr. In addition, the MSNs emit a dense local axon collateral system that innervates other spiny cells, creating a lateral inhibitory network (Tepper et al., 2004;Tepper et al., 2007). The striatum also contains several populations of a-spiny interneurons, all but one of which are GABAergic (Tepper and Bolam, 2004). Two major groups of interneurons, which have well-characterized electrophysiological properties, are the fast spiking parvalbumin (PV) expressing GABAergic interneurons (FSIs) and the tonically active cholinergic interneurons (TANs, Kawaguchi et al., 1995).

The other related BG subsystem is made up of the neuromodulators which modulate information processing along the main axis, primarily by regulating the plasticity of the cortico-striatal synapse (Calabresi et al., 2000;Reynolds et al., 2001). The two major components belonging to the neuromodulator group are the dopaminergic neurons located in the midbrain (substantia nigra pars compacta and ventral tegmental area neurons, Arbuthnott and Wickens, 2007) and the cholinergic interneurons located in the striatum (TANs, Calabresi et al., 2000).

The basal ganglia as an actor-critic reinforcement learning model

The BG have been suggested to implement a reinforcement learning (RL) algorithm. RL systems adjust their behavior in order to maximize the frequency, the magnitude, or both, of the reinforcing events they encounter over time (Houk et al., 1995;Sutton and Barto, 1998). The computational problem of RL is to find a policy (i.e. mapping from states of the environment to actions) that will maximize the total amount of (discounted) reward expected in the future. One of the most compelling models of RL is the actor-critic architecture because it has many similarities with the biological (both anatomical and functional) structure of the BG network. According to this model, there are two separate components. One component is the actor which stores and updates the policy, or the stimulus-response or stimulus-response-reward associations, such that actions associated with greater reward are more frequently chosen. The
other, the critic, generates a temporal difference (TD) prediction error signal when there is a discrepancy between the predictions and the actual reinforcements. This prediction error is used by the actor as a teaching signal to update the predictions of future rewards and to adjust the behavioral policy. In fact, the actor-critic architecture resembles the anatomical structure of the BG, where the neuromodulators, or particularly dopaminergic neurons of the midbrain correspond to the critic, and the actor corresponds to the BG main axis; i.e. the cortex-striatum-pallidum-frontal cortex axis (Houk et al., 1995; Suri and Schultz, 1998). Specifically, dopaminergic neurons have been shown to increase their discharge rates when conditions were better than expected (Schultz et al., 1997). Furthermore, these cells initially responded to the delivery of food reward; however, with conditioning they later shifted their response from the actual food delivery to the external cues predicting the delivery of food (Hollerman and Schultz, 1998). Based on these and other studies (Fiorillo et al., 2003; Nakahara et al., 2004; Morris et al., 2004; Bayer and Glimcher, 2005) the phasic activity of dopamine neurons was suggested to encode the temporal difference prediction error in the positive domain. In addition, it has been shown at the cellular level that dopamine plays a crucial role in cortico-striatal plasticity, thus modulating synaptic efficacy to reinforce behavior (Reynolds et al., 2001; Surmeier et al., 2007; Calabresi et al., 2007). The striatum, according to the actor-critic model, presumably corresponds to the main actor component where associations between states and actions are established and modified. Animal studies have shown that the activity of striatal MSNs is modulated by reward related events; namely, by the expectation, the amount and the probability of reward (Apicella et al., 1991; Kawagoe et al., 1998; Shidara et al., 1998; Cromwell and Schultz, 2003; Balleine et al., 2007). The BG were therefore modeled as a RL system where dopaminergic neurons transmit their teaching message to the striatum in order to reshape the behavioral policy.

The striatum – anatomy and function

The striatum is the primary input stage of the BG main axis and the principal source of innervations to BG downstream structures (Haber et al., 2011). It has been segregated into the dorsal striatum (DS) which includes the caudate and putamen nuclei, and into the ventral striatum (VS) which includes the nucleus accumbens and
the medial and ventral portions of the caudate and putamen. The afferent projections of the striatum are derived from three major sources: cerebral cortex, thalamus and brainstem, primarily from dopaminergic and serotonergic cells (Haber et al., 2011). These afferent projections terminate in a functional topographic manner (Parent, 1986) whereby the dorsal striatum, mainly the putamen and caudate, receive cortical input from sensory-motor and associative areas respectively, and the ventromedial striatum receives input from limbic cortical areas, from the amygdala and the hippocampus (Haber, 2003). Mainly lesion studies have suggested that these subregions play different functional roles. The VS has been found to be implicated in reward and motivation (Everitt et al., 1991; Cardinal et al., 2002) whereas the DS was involved in motor and cognitive control (Packard and Knowlton, 2002). Therefore the striatum cannot be addressed as a single entity anatomically or functionally under the actor-critic model. These findings suggest that the DS corresponds to the actor while the VS to the critic. Alternatively, the VS could be part of the actor subsystem which couples valence with action.

Studies have shown that the DS is directly involved in decision-making processes via integration of sensorimotor, cognitive and motivational information within distinct cortico-striatal circuits (Balleine et al., 2007). DS neurons have been demonstrated to encode action-outcome associations based on expected reward value (Delgado et al., 2000; O’Doherty et al., 2004; Delgado et al., 2005; Samejima et al., 2005; Haruno and Kawato, 2006; Lau and Glimcher, 2007). However, the DS can be further segregated into the dorsolateral (DLS) or sensorimotor striatum, homologous to the putamen in primates, and into the dorsomedial (DMS) or association striatum, homologous to the caudate. In fact, lesions (or inactivation) of the dorsolateral striatum (putamen) but not of the dorsomedial striatum (caudate) shift habitual to goal directed behavior (Miyachi et al., 1997; Yin et al., 2004; Yin et al., 2006). Correlatively, lesions to the caudate but not to the putamen cause goal directed actions to become habitual (Miyachi et al., 1997; Yin et al., 2005a; Yin et al., 2005b). Furthermore, in primates (Williams and Eskandar, 2006) it has been shown that caudate activity in response to outcome presentation is strongly correlated with the rate of learning, whereas putamen activity is correlated with the learning curve itself. Moreover, stimulation of the caudate significantly enhances the rate of learning, whereas stimulation of the putamen has no effect. In rodents it has been shown that the caudate is preferentially involved in the initial stages of visuo-motor learning whereas the putamen is engaged
later on in training (Yin et al., 2009). Another study found that the activity in the putamen and caudate developed concurrently but with different dynamics (Thorn et al., 2010). These studies therefore describe a shift in activation of single cells, in which learning of new motor responses activates the caudate but well-learned motor sequences activate the putamen (Miyachi et al., 2002). The current supposition thus holds that more gradually acquired, stimulus-based automatic (habitual) behavior is mediated by the putamen, whereas more flexibly acquired, outcome-based goal directed behavior is mediated by the caudate (Balleine et al., 2007).

As for the VS, pharmacological, molecular and physiological studies have implicated it in reward and to a lesser extent in aversive related processing (Carlezon, Jr. and Thomas, 2009). Lesion experiments have suggested that the VS mediates the motivational valence of stimuli (Everitt et al., 1991; Parkinson et al., 2000; Cardinal et al., 2002). Electrophysiological studies have shown modulations in the discharge rates of VS projection neurons in response to both reward delivery and reward expectation (Apicella et al., 1991; Shidara et al., 1998; Hassani et al., 2001). Furthermore, VS neurons respond (mainly by excitation) to the cues predicting rewarding and aversive stimuli with relative selectivity (Roitman et al., 2005).

Despite these apparent differences no electrophysiological study has compared the response properties of all striatal subregions in primates performing the same behavioral task.

**Research goals and thesis outline**

My research was aimed at studying information processing along the BG. In particular, I examine the ways in which appetitive and aversive behavioral cues are encoded in the BG, specifically in the different main axis (actor) nuclei, and are translated to the observed response. In the thesis I first target the information processing within the striatum and compare the encoding of behavioral events across striatal subregions. Next, I examine information processing along the main axis, first from the striatum to the GPe and then the relationship between GPe and GPi activity. Finally I examine how the activity of striatal interneurons (critic component) modulates the activity of striatal projection neurons (actor component), i.e., striatal output. Thus the main objectives of my thesis can be summarized as follows:
I. **Information processing within the input stage of the main axis – striatum**

Lesion studies have suggested that the differences in the anatomy and connectivity of striatal sub regions are reflected in their functionality (Alexander et al., 1986; Cardinal et al., 2002; Yin et al., 2004; Yin et al., 2006). The current view of striatal sub region functionality suggests that the putamen is involved in habitual behavior, the caudate in goal directed behavior, and the VS in reward and motivation (Yin and Knowlton, 2006; Graybiel, 2008; Redgrave et al., 2010). As these parallel functional systems can lead to the same behavioral outcome/response, they are likely to converge onto the same lower level motor structures (Redgrave et al., 2010) suggesting multiple controllers over behavior. Assuming that a behavior is regulated by a single control system raises the question as to what mechanism(s) enables one particular striatal domain to control behavioral output (Daw et al., 2005), or alternatively, whether and how information is differentially encoded within these cortico-striatal loops leading to different behavioral outcomes. My study was designed to test the following hypotheses. First, information could be differentially conveyed from striatal sub regions to downstream targets via different single cell discharge rate modulations. Second, there could be a shift in the organization of the functional connectivity within one of the regions and not the others that would alter synchrony but not firing rates. Finally, modulation in both single cell activity and neuronal synchrony may not be mutually exclusive and could operate together to differentially encode the behavioral events across striatal sub regions. To test these hypotheses I trained two monkeys on a classical conditioning task involving rewarding (food), aversive (air-puff) and neutral (neither) outcomes for several months. Once the monkeys were over trained (possibly habituated) on the task I performed extracellular recordings of MSNs and TANs from the three sub regions of the striatum (putamen, caudate and VS). Following this, I analyzed the data using two complementary levels: modulations of firing rates to the behavioral events and the correlation structure of simultaneously recorded pairs of striatal neurons. I found that neurons in the different striatal sub regions displayed similar response profiles in terms of firing rate modulation. However, their correlation structure was profoundly different, hinting at the formation of
interconnected sub networks solely in the putamen during the performance of a well-practiced task.

II. **Information processing along the main axis – striatum to GPe**

The BG are commonly viewed as two subsystems: the main axis which according to RL model corresponds to the executor of the system, i.e., the actor, and the neuromodulators, which correspond to the teacher of the system, i.e., the critic. It has been demonstrated that this anatomical division is also reflected in the activity of these populations which respond diversely to significant behavioral events. The neuromodulators, specifically midbrain dopamine neurons and striatal TANs (presumably cholinergic interneurons), display a homogeneous transient response, thus providing the main axis with a single dimensional teaching message (Schultz, 1998; Bar-Gad et al., 2003; Joshua et al., 2008). Neurons in the main axis, however, specifically in the GPe, GPi and SNr, exhibit diverse activity with an average sustained response that enables a large information capacity and has been attributed to action preparation processes (Mink, 1996; Bar-Gad et al., 2003; Joshua et al., 2009b). Striatal projection neurons (MSNs), as the input stage to the main axis which heavily project to the GPe, GPi and SNr are expected to display sustained activity. However, studies that examined the role of primate MSNs in both movement initiation and decision making have found mostly sharp transient activations to movement onset (Crutcher and DeLong, 1984a; Crutcher and DeLong, 1984b; Hikosaka et al., 1989; Watanabe et al., 2003), cue presentation (Kawagoe et al., 1998; Cromwell and Schultz, 2003; Samejima et al., 2005) and reward delivery (Apicella et al., 1991). I therefore aimed to study the ways in which MSN activity contributed to the sustained activity generated in their downstream target, in the pallidum. I recorded and compared the response profile and dynamical behavior of several BG neural populations (MSNs, TANs and GPe neurons) from monkeys while they were engaged in a classical conditioning task (as in goal I). I found three different encoding schemes implemented by these BG neural populations which helped explain GPe sustained activity and pointed to a functional convergence from the striatum to the GPe.

III. **Information processing along the main axis – GPe and GPI reciprocity**

According to the classic view of the BG, transmission of information occurs
both directly from the striatum to the GPi/SNr and indirectly through the GPe and STN (Haber et al., 2011). Striatal origins of the direct and indirect pathways are oppositely affected by D1 and D2 dopamine receptors (Gerfen et al., 1990;Surmeier et al., 2007). This therefore implies reciprocal discharge patterns between GPe and GPi neurons. GPe-GPi reciprocality has been supported by anatomical findings showing inhibitory connections from the GPe to the GPi (Hazrati et al., 1990;Sato et al., 2000;Kita, 2001;Kita, 2007) and by studies on human patients with Parkinson's disease and its primate model (MPTP treated monkeys, Miller and DeLong, 1987;Filion and Tremblay, 1991;Hutchinson et al., 1997;Levy et al., 2001). However, physiological studies conducted on normal animals have not supported this view (Elias et al., 2008), in that the discharge of neurons in both pallidal segments was similarly, rather than oppositely, modulated during the execution of movements (Georgopoulos et al., 1983;Nambu et al., 1990;Turner and Anderson, 2005) and reward-related events (Gdowski et al., 2001;Arkadir et al., 2004;Joshua et al., 2009b). To shed light on these conflicting results I recorded and compared the spontaneous activity of GPe and GPi neurons in different brain states; specifically, upon closing and opening of the eyes. I found that the changes in GPe and GPi discharge properties upon closing and opening of the eyes were positively correlated. These results suggest that the sub-thalamic nucleus and/or the striatum are the main common driving force for both pallidal segments.

IV. Information processing between main axis (actor) and neuromodulators (critic) neurons

The vast majority of striatal cells are the MSNs, striatal projection neurons (Tepper et al., 2008). However, their activity and hence striatal output is highly affected by the proportionally small population of a-spiny interneurons (Kawaguchi et al., 1995;Kreitzer, 2009). Two major groups of interneurons, which have well-characterized electrophysiological properties and have been extensively studied, are the fast spiking parvalbumin (PV) expressing GABAergic interneurons (FSIs) and the tonically firing large cholinergic interneurons (TANs). The in-vivo activity of TANs has been amply investigated in behaving primates, whereas that of FSIs has been described mainly in rodents. Both interneuron cell types have been shown to be
imperative to normal striatal functioning (Pisani et al., 2007; Gittis et al., 2011). To define the functional relationship between their activity and that of the MSNs that mediate striatal output I recorded and analyzed the simultaneous spiking activity of MSN–TAN or MSN–FSI pairs. I found a different correlation structure between MSN-TAN and MSN-FSI neuron pairs which suggests that the two interneuron populations play a different role in modulating MSN (striatal output) activity.
Methods

A full description of the methods used in this research can be found in the methods sections of the published manuscripts (Results section of the Thesis). In this chapter I briefly summarize the behavioral states/paradigm and the recording technique.

Behavioral states

The results described below were taken from behaving monkeys in one of two states: while they were engaged in a classical conditioning paradigm and while their eyes were open vs. when they were closed.

For the first state, two monkeys (G and Le, Macaque fascicularis, male 4.5 Kg and female 3 Kg) were engaged in a classical conditioning task involving rewarding, aversive and neutral outcomes. Nine or six (monkey G and Le respectively) different fractal cues were introduced to the monkeys, each predicting a different outcome. Three/ two (monkey G and Le respectively) cues (reward cues) predicted a liquid food outcome. Three/ two (monkey G and Le respectively) cues (aversive cues) predicted an air puff outcome and the remaining three/two (monkey G and Le respectively) cues (neutral cues) were never followed by either food or air puff. Trials were followed by a variable inter trial interval (ITI) of 5-6 s. The rewarding and aversive cues were differentiated by the magnitude or intensity of the liquid food or air puff, respectively. The neutral cues were differentiated by a change in the duration of the ITI (-2/0/+2s to ITI duration). Under this condition we recorded the neuronal activity in two areas of the BG network: the external segments of the pallidum (GPe) and the striatum. Recordings were performed following an extensive period of training (G for 4 months; Le for 3 months) on the behavioral task. Results of this state are reported in chapters one, two and four of the results section.

In the second state we used digital video cameras to record the monkeys' facial movements. Video analysis was carried out on custom software to identify periods when the monkeys' eyes were closed. We then divided the recording times into two distinct periods, depending on the state of the monkeys' eyes. Periods when the monkeys' eyes were closed continuously for more than 1s were considered "eye closed" states. Other periods were considered as the "eyes open" state. Two monkeys
(W and La, Vervet, female 4.5 Kg and Macaque fascicularis, female 4 Kg) were used in this condition. Under this condition we recorded the neuronal activity in three areas of the cortex-BG network: the primary motor cortex (M1) and the external and internal segments of the pallidum (GPe and GPi respectively). Results of this state are reported in chapter three of the results section.

**Recording and data acquisition**

After the training period, the monkeys were operated under full anesthesia and in sterile conditions. In the surgery, an MRI-compatible head holder and recording chamber were attached to the monkeys' heads. The head holder enabled immobilization of the head during recording. During recording sessions eight glass-coated tungsten microelectrodes were advanced separately into the different targets of the basal ganglia. Spike activity was sorted and classified online using a template-matching algorithm. In the striatum, I recorded the activity of both projection neurons and interneurons and classified the units according to anatomical location, extracellular waveform, firing rate and firing pattern. Recorded neurons were subjected to offline quality analysis before being included in the final database. Additionally, in order to assess the monkeys' behavior, I monitored their mouth (monkeys La, G and Le) and eye (monkeys W, La, G and Le) movements by an infrared reflection detector and computerized digital video cameras, respectively.
Results

Chapter details:

I. Encoding by synchronization in the primate striatum.
   Avital Adler, Inna Finkes, Shiran Katabi, Yifat Prut and Hagai Bergman.

II. Temporal convergence of dynamic cell assemblies in the striato-pallidal network.
   Avital Adler, Shiran Katabi, Inna Finkes, Yifat Prut and Hagai Bergman.

III. Neurons in both pallidal segments change their firing properties similarly prior to closure of the eyes.
   Avital Adler, Mati Joshua, Michal Rivlin-Etzion, Rea Mitelman, Odeya Marmor, Yifat Prut and Hagai Bergman.

IV. Different driving modes of striatal cholinergic and GABAergic interneurons.
Results chapter number I.

*Encoding by synchronization in the primate striatum.*

Avital Adler, Inna Finkes, Shiran Katabi, Yifat Prut and Hagai Bergman.

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Encoding by synchronization in the primate striatum

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Abbreviated title: Neural correlations in striatal sub regions

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Abstract:

Information is encoded in the nervous system through the discharge of single neurons as well as through their synchronization. The striatum, the input stage of the basal ganglia, is divided into three territories: the putamen, caudate and ventral striatum (VS) which all converge onto the same motor pathway. This parallel organization suggests that there are multiple and competing control systems over behavior in the basal ganglia network. To explore which mechanism(s) enables the different striatal domains to encode behavioral events and control behavior, we compared the neural activity of phasically active neurons (presumably projection neurons, MSNs) and tonically active neurons (TANs, presumed cholinergic interneurons) across striatal territories from monkeys during the performance of a well-practiced task. Although neurons in all striatal territories displayed similar spontaneous discharge properties and similar temporal modulations of their discharge rates to the behavioral events, their correlation structure was profoundly different. The distributions of signal and noise correlation of pairs of putamen MSNs were strongly shifted towards positive correlations and these two measures were correlated. In contrast, MSN pairs in the caudate and VS displayed symmetrical, near-zero signal and noise correlation distributions. Furthermore, only putamen MSN pairs displayed different noise correlation dynamics to rewarding vs. neutral/aversive cues. Similarly, the noise
correlation between TAN pairs was stronger in the putamen than in the caudate. We suggest that the level of synchronization of the neuronal activity and its temporal dynamics differentiate the striatal territories and may thus account for the different roles striatal domains play in behavioral control.

Introduction

The striatum, the input stage of the basal ganglia (BG), is known to be involved in motor, cognitive and emotional functions (Graybiel, 2008; White, 2009; Balleine et al., 2009). In primates, the striatum is partitioned into three sub regions: the caudate nucleus, the putamen nucleus and the ventral striatum (VS). The caudate and putamen together comprise the dorsal striatum (DS) and the VS includes the nucleus accumbens and the medial and ventral portions of the caudate and putamen (Haber et al., 2011). This double or tri-partite division of the striatum is based on both anatomy and functionality (Parent and Hazrati, 1995). However, it is not clear if/ how striatal sub regions differentially encode behavior.

Anatomically, the afferent projections of the striatum are derived from the cerebral cortex, thalamus and brainstem, all of which terminate in a functional, topographic manner in the striatum (Parent, 1986). For example, the putamen and caudate receive cortical input from the sensory-motor and associative areas respectively (Ragsdale, Jr. and Graybiel, 1981; Haber et al., 2011), whereas the ventral striatum receives input from the limbic areas of the frontal cortex, the amygdala and the hippocampus (Haber, 2003).

Functionally, lesion studies have suggested that the ventral striatum is implicated in reward and motivation (Everitt et al., 1991; Cardinal et al., 2002), whereas the dorsal striatum is involved in motor and cognitive control (Packard and Knowlton, 2002). Furthermore, within the dorsal striatum, lesions (or inactivation) of the dorsolateral striatum (putamen) but not of the dorsomedial striatum (caudate) shift habitual to goal directed behavior (Miyachi et al., 1997; Yin et al., 2004; Yin et al., 2006). Correlatively, lesions to the caudate but not to the putamen cause goal directed actions to become habitual (Miyachi et al., 1997; Yin et al., 2005a; Yin et al., 2005b).

These parallel anatomical and functional cortico-striatal loops suggest multiple controllers over behavior. Assuming that a behavior is regulated by a single control system raises the question of what mechanism enables one particular striatal domain
to control behavioral output (Daw et al., 2005; Redgrave et al., 2010). A small number of studies have attempted to answer this question and achieve better understanding of striatal functionality by electrophysiological recordings from different striatal sub regions. Typically these studies have involved instrumental conditioning (Miyachi et al., 2002; Williams and Eskandar, 2006) primarily of rodents (Yin et al., 2009; Kimchi et al., 2009; Thorn et al., 2010) and have compared striatal sub regions at the level of single cell firing properties. However, no electrophysiological study has compared the response properties of all three sub regions in primates performing the same behavioral task. Furthermore, there are no studies comparing striatal sub regions using correlation measures of simultaneously recorded neurons. It therefore remains unclear whether and how information is differentially encoded within these cortico-striatal loops, or the ways in which this could lead to different behavioral outcomes. Our study was therefore designed to test the possibility that neuronal synchronization enables the encoding of behavioral events, both in the spatial domain (striatal sub regions) and the temporal domains (during the performance of a behavioral task).

Materials and methods
Two monkeys (Macaque fascicularis, G male, 4.5 kg; L female, 3 kg) were used in this study. All experimental protocols were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and the Hebrew University guidelines for the use and care of laboratory animals in research. The experimental protocols were approved and supervised by the Institutional Animal Care and Use Committee (IACUC) of the Hebrew University and Hadassah Medical Center. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) internationally accredited institute. Surgery procedures, data-recording methods and single cell analysis were described in detail in previous manuscripts (Adler et al., 2010; Adler et al., 2012). Here we present them briefly and describe the methods not used in the previous manuscripts.

Behavioral task
The monkeys were trained (G, for 4 months; L, for 3 months) on a classical conditioning task (Fig. 1a) and were engaged in the task during recordings of the neuronal activity. Briefly, each trial began with the presentation of a visual cue (full-screen fractal images generated using the Chaos Pro 3.2 program; www.chaospro.de
and displayed on a 17" LCD monitor, 50 cm in front of the monkeys' faces) for a period of 2 seconds. The cues were immediately followed by an outcome which could be one of three categories: liquid food in the reward trials, air puff (directed at both eyes) in the aversive trials or neither in the neutral trials. The beginning of the outcome state was signaled by one of three sounds (duration, 80 ms) that discriminated the three outcome categories. Trials were followed by a variable inter-trial interval (ITI) of 5-6s. In each category there were three/two (monkey G and L respectively) different visual cues. In the rewarding and aversive trials the cues were differentiated by the magnitude or intensity of the liquid food or air puff respectively. In the neutral trials the cues were differentiated by a change in the duration of the ITI (-2/0/+2 s to ITI duration). In total there were nine/six (monkey G and L respectively) different visual cues; three/two (monkey G and L respectively) for each outcome category. In some of the analyses we combined the trials within each outcome category and present the results for the rewarding trials (which include all amounts of liquid food), the aversive trials (which include all air puff intensities) and the neutral trials. Visual fractal cues and auditory sounds were randomized between monkeys.
Figure 1: Behavior and recording during the classical conditioning task

a) Left: Classical conditioning paradigm. Visual cues were presented for 2s and predicted the delivery of food (reward trials, upper row), air puff (aversive trials, third row) or only sound (neutral trials, second row). The trial outcome epoch was followed by a variable inter trial interval (ITI) of 5-6s. Right: Normalized behavioral response (average ± SEM, solid line and shaded envelope, respectively). The monkeys’ licking and blinking behavioral responses were normalized between 0 and 1. In each time bin (20 ms) the licking and/or the blinking response (x) was transformed by (x-min)/(max-min), where min and max are the minimal and maximal values of the response over all time bins. Ordinate: normalized licking response minus normalized blinking response. Abscissa: time. Time zero: cue presentation followed by outcome delivery at time 2 seconds. Blue is for reward trials, green for neutral and red for aversive.

b) Recording sites: a representative coronal section +3 mm from the anterior commissure (adapted from Martin and Bowden, 2000). Eight electrodes were advanced separately into one or two of the three sub regions of the striatum. P for putamen, C for caudate and V for ventral striatum.

c) An example of six simultaneously recorded units from the putamen. Each row is for a single electrode. Left: 4 second analog trace of extracellular recording filtered between 300 and 6000 Hz. Right: examples of spike waveforms. The
spike waveform plot includes 100 superimposed 4 ms waveforms selected randomly from the whole recording time of the cell. Black: MSNs. Gray: TANs.

**Magnetic Resonance Imaging**

We estimated the stereotaxic coordinates of the physiological recordings using MRI scans. Following surgery, an MRI scan (General Electric or Siemens 3 Tesla system, T2 sequence) was performed with five tungsten electrodes at accurate coordinates of the recording chamber \( \text{Y,X = (6.0), (0,-6), (0,0), (0,6), and (-6,0) in mm from the chamber center} \). We then aligned the two-dimensional MRI coronal scans with the sections of the atlas of *Macaca fascicularis* (Martin and Bowden, 2000). We performed an additional MRI scan at the final stage of the recording period to verify our coordinate system and to rule out a significant brain shift. We used additional physiological parameters (as described below) to identify the putamen, caudate and VS. At the completion of the experiments and after a recovery period we send the monkeys for rehabilitation to the Ben-Shemen Primate Sanctuary, operated by the Israeli Primate Sanctuary Foundation (IPSF)- http://www.monkeypark.co.il/; http://ipsf.org.il/?section=51&item=117. We therefore did not make micro lesions at the end of each electrode track and cannot report post-mortem histology.

**Recording and data acquisition**

During recording sessions, the monkeys' heads were immobilized. Licking and blinking behavior was recorded by an infrared reflection detector (Dr. Bouis, Freiburg, Germany) and video computerized analysis (Mitelman et al., 2009). Neuronal activity was recorded by eight glass-coated tungsten microelectrodes (impedance at 1KHz=0.3-0.8Mohms) that were advanced separately (EPS, Alpha-Omega Engineering, Nazareth, Israel) into the striatum (AC-1 to AC+4). We identified the putamen and caudate sub regions by mapping the medial-lateral plane and using the typical axonal activity of the internal capsule as a divider between the two regions. The axonal activity of the internal capsule is characterized by extremely sharp and narrow waveforms deflected upwards (initial positive) as opposed to the wide, downward deflections (initial negative phase) of somatic action potential spikes (Lemon, 1984). We identified the ventral striatum by lowering the vertical position of the electrodes at least 4 mm below the first identification of the striatum. The average vertical distance of the units from the first identification of the striatum was 1.04±0.04
mm (average ± SEM) for putamen cells, 1.08±0.05 mm for caudate cells and 4.56±0.03 mm for VS cells. In each recording session we targeted either the lateral part of the striatum (i.e., 4 electrodes in the dorsolateral striatum, the putamen and 4 electrodes in the ventral striatum) or the medial part (i.e., 4 electrodes in the dorsomedial striatum, the caudate and 4 electrodes in the ventral striatum). Spike activity was sorted online using a template matching algorithm (ASD, Alpha Omega Engineering) by two experimentalists (each responsible for no more than 4 electrodes).

Recorded neurons were subjected to offline quality analysis which included tests for rate stability, refractory period and waveform isolation (Hill et al., 2011). First, the firing rate was graphically displayed as a function of time and the largest continuous segment of stable data was selected for further analysis. Second, cells in which more than 0.02 of the total ISIs were shorter than 2 ms were excluded from the database. Third, only cells with an isolation score (Joshua et al., 2007) above 0.7 were included in the database. Finally, only single cells recorded for more than 18/21 minutes (monkey L and G respectively) during task performance and only pairs of cells simultaneously recorded on two different microelectrodes for more than 21/30 minutes (monkey L and G respectively) during task performance were included in the database.

**Single cell analysis**

We conducted a clustering analysis to differentiate the activity of single MSNs into groups of cells with similar response profiles (Adler et al., 2012). Briefly, we first characterized the neural responses to the behavioral events by the post stimulus time histogram (PSTH). Next we ran a principal component analysis on the normalized PSTHs. We used the projection of the data points on the first two principal components to represent the response vector of each cell and applied the K-means algorithm on the cells' principal component representation. We ran the analysis on all MSNs recorded in all striatal territories together, as well as on the MSNs recorded in each territory separately. We obtained three temporally distinct response profiles; i.e. clusters (Fig. 4) when we ran the analysis on both the entire striatal MSN population as well as separately within each territory. Eighty percent of the cells were classified into the same temporal cluster in the two runs. We present the data below when analyzing all MSNs recorded in all striatal territories together.
**Pair-wise correlation analysis: signal correlation**

The signal correlation measures the similarity of the responses to behavioral events (i.e., PSTHs) of two neurons. Here, we used a time window of 2 s starting at event (i.e., cue and outcome) onset. For each neuron we computed the PSTHs in 100 ms bins (without smoothing) for all behavioral events. We combined all PSTHs of a single cell into one matrix with rows (n=9 and 6 for monkey G and L respectively) for each behavioral event and columns (n=20) for each 100 ms time bin. For each column (time bin), we subtracted that column's mean and then flattened the matrix (column after column) into a single vector. For each pair of simultaneously recorded neurons, we computed the signal correlation by calculating the correlation coefficient of these two vectors. Since a noise correlation can significantly bias the signal correlation, we applied a correction procedure (Rothschild et al., 2010). Briefly, we calculated the covariance of the average responses using the single trial responses; however, we removed the simultaneous trial terms from the sum to achieve a non-biased estimate of the signal correlation. We obtained similar results with and without the correction (data not shown). We also calculated the signal correlation for all pairs, including pairs which were not simultaneously recorded and were therefore likely to be further from one another, and obtained similar results. The data displayed below were not subjected to the noise correlation correction procedure and refer to simultaneously recorded pairs.

**Pair-wise correlation analysis: noise correlation**

The noise correlation is a measure of the similarity of the trial-to-trial variability (around the average PSTH) of two neurons. Here, we computed the spike counts in 2000 ms bins following the onset of all behavioral events for each neuron for all trials. For each event we computed the average spike count over all trials and then subtracted this mean from the trial to trial spike counts. We then combined all spike counts from all behavioral events into a single vector. For each pair of simultaneously recorded neurons (by definition, the noise correlation can be calculated only for simultaneously recorded neurons), we computed the noise correlation by calculating the correlation coefficient of these two vectors.
Pair-wise correlation analysis: JPSTH analysis

The noise correlation measures the similarity of the trial to trial variability over large 2000 ms bins. To characterize the temporal dynamics of the noise correlation during the behavioral epochs we calculated the Joint PSTH (JPSTH). We first calculated the raw JPSTH, in which the \((t_1,t_2)\) time bin was the count of the number of times there was a coincident event in which neuron number one spiked in time bin \(t_1\) and neuron number two spiked in time bin \(t_2\) in the same trial. To correct for rate modulations we calculated the PSTH predictor matrix, which is the product of the single neuron PSTHs. The JPSTH was calculated by subtracting the PSTH predictor from the raw matrix (Aertsen et al., 1989; Joshua et al., 2009) in bins of 50 ms and smoothed with a two-dimensional Gaussian window with a SD of 50 ms (single bin). We also computed the JSPTH using a shift predictor and obtained similar results (data not shown). The data from the two monkeys were grouped since no significant differences were detected between the individual monkeys. Data analysis was performed on custom software using MATLAB V7.

Results

Behavioral task and neuronal database

We recorded striatal neuronal activity from two monkeys while they were engaged in a classical conditioning task. The monkeys were introduced to visual fractal images which predicted either a food outcome in the rewarding trials, an air puff in the aversive trials or neither in the neutral trials (Fig. 1a, left panel). Recording sessions followed an extensive training period of several months. Thus, during recordings the monkeys were over-trained on the task, were familiar with the visual cues and displayed the appropriate anticipatory licking and blinking behavior (Fig. 1a, right panel). We used conventional physiological methods to record the spiking activity of striatal phasically active neurons - presumably striatal medium spiny projection neurons (MSNs) and striatal tonically active neurons - presumably cholinergic interneurons (TANs). Classification of striatal cells was done online based on the spike waveforms, firing rate and pattern (Berke et al., 2004; Sharott et al., 2009) and was validated off line (compare Figure 2 with Figure 8; note the different ranges of the X-axes). Recordings were made from the three territories of the anterior striatum, putamen, caudate and ventral striatum (Fig. 1b) and from two to eight electrodes simultaneously (see Fig. 1c for an example of simultaneous recording of six
electrodes in the putamen). Overall, the recordings of 896 MSNs (334, 265, 287 in the putamen, caudate and VS respectively), 309 TANs (145, 125, 39 in the putamen, caudate and VS respectively), as well as 617 (337, 148, 132 in the putamen, caudate and VS respectively) MSN to MSN and 110 (72, 38 in the putamen and caudate respectively) TAN to TAN pairs satisfied our analysis inclusion criteria (see Materials and methods) and are reported here.

**MSNs in different striatal sub regions display similar spontaneous spiking characteristics**

We compared the spiking parameters of MSN spontaneous discharges (during the ITI period) across regions. The MSN average spontaneous firing rates (1.06±0.06, 0.77±0.04 and 1.31±0.07 spikes/s ± SEM in the putamen, caudate and VS) were similar but still significantly different across all three territories (one- way ANOVA p<0.05, Fig. 2a). The coefficient of variation of the inter-spike interval (1.54±0.49, 1.6±0.5 and 1.53±0.4 in the putamen, caudate and VS), which is a measure of firing pattern, did not differ between the sub regions (Fig. 2b). Finally, the length of the MSN spike waveforms, calculated as the duration from the first negative peak to the next positive peak of the extra-cellular recorded action potential, (1.39±0.04, 1.6±0.09 and 1.39±0.04 ms ± SEM in the putamen, caudate and VS) was longer in the caudate than in the putamen and VS (one- way ANOVA p<0.05, Fig. 2c). Thus although there were differences in the cells' spiking parameters, these were not consistent across the different measures of spontaneous activity and therefore did not indicate that the MSNs recorded in the different striatal sub regions represented different types of cells. More importantly, they cannot explain our results below. Next we describe and compare the encoding of the task events by the different striatal sub regions, first at the level of firing rate modulations and then at the level of static and dynamic modulations in the correlations between pairs of simultaneously recorded neurons.
Figure 2: MSNs in the different striatal sub regions display similar spiking parameters

a) Distribution of average MSN spontaneous (ITI) firing rates by striatal sub regions. Abscissa: firing rate in Hz. Firing rate was calculated for each unit as the total number of spikes divided by the duration of the full time segment used for analysis. The firing rate is displayed in logarithmic scale for clarity due to the low firing rates and wide range (0.01-7.8 Hz) of MSN discharge rates. Ordinate: ratio of cells. First row: MSNs recorded in the putamen (n=344), second row: caudate (n=265) and third row: ventral striatum (VS, n=287).

b) Distribution of the coefficient of variation (CV) of the inter spike intervals of MSN in the striatal sub regions. Abscissa: CV. Ordinate: fraction of cells. Same conventions as in a.

c) Distribution of average MSN waveform length by striatal sub regions. Abscissa: spike waveform length calculated as the duration from the first negative peak to the next positive peak of the extra-cellular recorded action potential. Ordinate: fraction of cells. Same conventions as in a.
MSNs in striatal different sub regions display similar response profiles

MSNs in all three striatal regions were highly modulated by the task (84.8% of putamen MSNs showed significant rate modulations, 78.1% of caudate and 77.7% of VS) and displayed increases and/or decreases in discharge rates in response to the task events. When we compared the average response profile across regions we found several differences (Fig. 3). However, as reported previously, MSNs display very diverse activity to significant behavioral events, which implies that the average population response is not the best estimate of neuronal activity (compare Fig. 3 column b, which displays the population average response and Fig. 3 column c, which displays the average over the absolute value of the responses). In order to better characterize the diverse modulations in MSN firing rate, we clustered the cells into groups with similar response profiles (Adler et al., 2012). Figure 4a displays the average response profiles of striatal MSNs to the three task events separately for each response cluster (columns) and for each sub region (rows). The MSNs in all three regions best fit three response groups (clusters) that were differentiated primarily by their temporal profile (Fig. 4a). Clusters one, two and three could be characterized by "brief excitation", "intermediate excitation" and "delayed prolonged excitation" respectively. The profile of the average response of each cluster was similar across striatal sub regions. We compared (one- way ANOVA, p<0.05) the MSN responses within each cluster across regions using two parameters: the spike count over the two seconds of cue presentation, and the time of peak response (Fig. 4b and c). The only significant difference was between the VS to the putamen and caudate in the third response group (cluster 3). On the other hand, the average spike count and time of peak response were significantly different across the three clusters when pooling over all MSNs (putamen and caudate and VS). Overall, we found significant and similar modulations in firing rates to the task cue events in all striatal territories. The temporal modulations in firing rate in response to the behavioral cues in our classical conditioning task were similar for the putamen, caudate and VS, and the three territories could not be differentiated solely on this basis.

There were small differences in the outcome PSTHs of the different striatal regions (Fig. 4a). However, to avoid possible confounding effects triggered by motor components in this epoch, we targeted our analysis (PSTH and correlation analysis) on cue presentation epoch (time zero to 2 s).
Figure 3: MSN cell population average response

a) Average MSN cell population response (± SEM) to behavioral events. Each row is the average response of MSNs recorded in a single striatal sub region (first; putamen, second; caudate and third; VS). The PSTHs were calculated in 1 ms bins and smoothed with a Gaussian window with a SD of 20 ms. The baseline firing rate, calculated by averaging the firing rate in the last 0.5s of the ITI, was subtracted from the smoothed PSTH. Abscissa: time in seconds. Time zero: cue presentation, time 2s: outcome delivery. The display is extended into the ITI to depict the return of the MSN discharge to baseline levels. Ordinate: firing rate in Hz. Color code: blue, responses to all reward cues; green, responses to all neutral cues; red, responses to all aversive cues. N is for the number of neurons.

b) Average MSN cell population response (± SEM) to behavioral cues alone. Cues were presented at time zero. Same conventions as in a.

c) MSN cell population response to behavioral cues averaged over the absolute value of the PSTHs. The absolute PSTH was calculated for each neuron and then averaged over the entire population. Same conventions as in b.
Figure 4: MSNs in different striatal sub regions display similar response profiles

a) MSN responses (± SEM) to the behavioral events divided into response clusters. Abscissa: time in seconds. Time zero: cue presentation, time 2s: outcome delivery. Ordinate: firing rate in Hz normalized by the ITI discharge rate. Blue RC, reward events; red AC, aversive events; green NC, neutral events. Rows: striatal sub region, first for putamen, second for caudate and third for VS (ventral striatum). Columns: average (± SEM, solid line and envelope) response clusters. In each sub-plot, N is for the number of MSNs averaged and the percentage of these units out of all the units in that sub region.
b) Distributions of average MSN spike counts to cue presentation (time zero to 2s only). Each black dot represents an individual MSN. Red dots stand for population means. p is for putamen, c for caudate and v for VS. Asterisk marks statistically significant difference, one-way ANOVA, \( p < 0.05 \).

c) Distributions of time of peak response for all MSNs. Same conventions as in b.

**MSN pairs in the putamen, but not in the caudate or ventral striatum, exhibit positive signal and noise correlations**

The synchronization of neural activity plays a major role in encoding and decoding information in the nervous system (Schneidman et al., 2003; Averbeck et al., 2006). The signal correlation measures the similarity between the average responses of a pair of neurons to behavioral events. Its values range from plus one through zero to minus one, for highly correlated through non-correlated to anti-correlated response profiles respectively. The noise correlation measures the correlation between the deviations of the trial to trial discharge rate from the average responses spanning the same range (1 to -1) as the signal correlation (Lee et al., 1998; Averbeck and Lee, 2004; Joshua et al., 2009).

Figure 5 shows the distributions of the signal and noise correlation (left and middle columns, calculated over all behavioral events; i.e., cue presentation and outcome delivery) for all simultaneously recorded MSN pairs for each sub region (rows). We found that the distributions of the signal correlation for caudate and VS MSN pairs were symmetrical around zero (Fig. 5b and c). Indeed there was a similar ratio of pairs that had a significant (t test: \( p < 0.05 \)) positive and negative correlation (Fig. 5b and c, inset). Similarly, the distributions of the noise correlations for the caudate and VS MSN pairs were also symmetrical around zero and were very narrow, as evidenced by the smaller ratio of pairs with a significant correlation. On the other hand, the distributions of both the signal and noise correlation of putamen MSN pairs (Fig. 5a) had a strong tail towards positive correlation values and a higher percentage of pairs displayed a significant positive correlation (compared to the negatively correlated putamen MSN pairs and the caudate and VS). The distributions of signal and noise correlations of putamen MSN pairs were significantly different from those of the caudate and VS pairs (one- way ANOVA, \( p < 0.05 \) for both signal and noise). To confirm that the differences between the putamen and caudate/VS could not be attributed to the higher number of putamen MSN pairs, we also compared the
distributions (one-way ANOVA) when randomly selecting 150 putamen pairs. We repeated this process 1000 times and found that the signal and noise distributions of the partial sampling of putamen pairs were significantly different from those of the caudate and VS on almost all runs (97-100% of the runs, depending on the comparison; signal vs. noise and caudate vs. VS).

Finally, Figure 5, right column, depicts the correlation between the two measures of neuronal synchrony (signal and noise correlation). There was a strong linear relationship (F-test, \( p<0.05 \)) between the signal and noise correlation of putamen MSN pairs; i.e., when a pair of putamen MSNs tended to have a high signal correlation, they also tended to have a high noise correlation and vice versa (similar results were obtained using type 2 regression, data not shown). Although there was also a linear relationship (F-test, \( p<0.05 \)) between the two measures for MSN pairs in the caudate and VS, it was not as strong, as shown by the differences in R square values and the slope of the linear fit.

To further confirm that the increased correlation found solely in putamen MSN pairs was not a result of systematically recording closer cells in that sub region, we compared the vertical distance between all cell pairs. Note that only pairs recorded on different electrodes were included in this study. The vertical distance for cell pairs in the VS (0.38±0.03 average±SEM) was significantly shorter than the putamen (0.55±0.03 average±SEM, one-way ANOVA, \( p<0.05 \)). The vertical distance for cell pairs in the caudate (0.42±0.03 average±SEM) did not differ from the other two regions. Although there was a difference in the vertical distance of the cell pairs, it was shorter in the VS which would suggest an opposite bias in space.

We also calculated the noise correlation during the ITI period (using the last 1s of the ITI). Although noise correlations during the ITI period were lower than noise correlations during task performance, a similar trend was observed. Namely, the noise correlation distribution of MSN pairs in the putamen was more positively skewed compared to the caudate or VS (\( p<0.05 \), one-way ANOVA) and a higher percentage of pairs displayed a significant positive correlation. Furthermore, we found a significant correlation between the noise correlation in the putamen MSN pairs during task performance and during the ITI period (\( R^2=0.38, p<0.05 \)).

Last, we also calculated the signal correlations for pairs that were not simultaneously recorded (n=20736, 11248 and 13741 pairs in the putamen, caudate and VS respectively) and found a similar distribution as when taking the simultaneously
recorded pairs alone (data not shown). Furthermore, there was no relationship between the correlation measures for a pair of neurons and their response profile; i.e. their cluster identity (data not shown).

Figure 5: MSNs in the putamen, but not in the caudate or ventral striatum, display positive signal and noise correlations

a) Distributions of the signal (left column) and noise (middle column) correlations for putamen MSN pairs. Abscissa: correlation coefficient values, Ordinate: ratio of pairs. Inset: ratio of pairs that had a significant positive (gray) or negative (black) correlation. Right column: the correlation between the signal (abscissa) and noise (ordinate) correlation. N is for the number of MSN pairs. Lower right: $R^2$ and $a$ (slope) of the linear fit.

b) Same as in a for the caudate MSN pairs.

c) Same as in a for the ventral striatum MSN pairs.
Putamen MSN pairs display different noise correlation dynamics to different behavioral events

The noise correlation presented in Figure 5 was calculated on the entire trial epoch (cue presentation or outcome delivery) as a single bin (2 s each) and did not relate separately to the different behavioral events. Next we examined the time course of the correlation along the trial and compared the modulation in synchronized activity between the different behavioral events. We thus calculated the Joint Peri Stimulus Time Histograms (JPSTHs, Aertsen et al., 1989; Vaadia et al., 1995) corrected for rate modulations triggered by the behavioral events for all simultaneously recorded MSN pairs.

Figure 6 shows the JPSTH averaged over all MSN pairs for each striatal region (rows) and for each behavioral event (columns). The main diagonal of the JPSTH captures the time course of the modulation in correlations (with zero delay) beyond those predicted by the changes in firing rates; i.e., it depicts the dynamics of the noise correlation (calculated in 50 ms bins). The data show a significant increase in correlation along the main diagonal of the JPSTH for putamen MSN pairs for all behavioral events (Fig. 6a, t-test, \(p<0.05\)). This result is consistent with the positive noise correlation between putamen MSN pairs (Fig. 5a). Second, the JPSTH analysis of putamen MSN pairs revealed different dynamics for the different behavioral events. Whereas the increase in correlation in the reward event was transient and occurred about one second after cue presentation, the increase in correlation for the aversive and neutral events was more sustained and lasted throughout the remaining time of cue presentation. The modulations along the main diagonal were significantly different between the rewarding and aversive/neutral events (paired t-test, \(p<0.05\)). To ascertain whether these changes in correlation did not simply reflect the discharge rate modulations, we examined the predictor matrix and found it did not account for the differences found in the pattern of the JPSTH between the rewarding and aversive/neutral events (Fig. 7). Specifically, we found that although there was an immediate and sharp increase in discharge rate for all behavioral events, the increase in correlation for the reward event was steep and transient whereas the increase in correlation for the aversive and neutral events was moderate and sustained. Finally, we did not find an oscillatory component in the synchronization between putamen MSN pairs.
Figure 6: Putamen MSN pairs display different dynamics of noise correlation in the different behavioral events

a) Population JPSTH of putamen MSN pairs (n=337). Left column: reward trials, middle column: neutral trials, right column: aversive trials. Time zero: cue presentation followed by outcome delivery at time 2 seconds. The different JPSTHs have the same color scaling (color bar on the right) to enable comparison of the different behavioral events.

b) Population JPSTH of caudate MSN pairs (n=148).

c) Population JPSTH of ventral striatum MSN pairs (n=132). a, b and c have the same color bar to enable comparison of striatal sub regions.
Figure 7: Dynamics of noise correlation of putamen MSN pairs do not reflect rate modulations

a) Common rate modulation: diagonal of the PSTH predictor (± SEM, shaded envelope) for putamen MSN pairs (n=337). Time zero: cue presentation followed by outcome delivery at time 2 seconds. Color code: blue, reward cues; green, neutral cues; red, aversive cues.

b) Zero lag noise correlation: JPSTH diagonal (± SEM, shaded envelope) for putamen MSN pairs (n=337). Same conventions as in a.

Putamen TAN pairs display a stronger noise correlation

TANs in the different striatal sub regions displayed very similar spontaneous spiking parameters (except for slower average discharge rates in the VS than in the putamen and caudate, one-way ANOVA p<0.05, Fig. 8) as well as similar response profiles (Fig. 9).
Figure 8: TANs in the different striatal sub regions display similar spiking parameters


b) Distribution of coefficient of variation (CV) of the inter spike intervals of TANs in the three striatal sub regions. Abscissa: CV. Ordinate: fraction of cells. Same conventions as in a.

c) Distribution of average TAN waveform length by striatal sub regions. Abscissa: spike waveform length calculated as the duration from the first negative peak to the next positive peak of the extra-cellular recorded action potential. Ordinate: fraction of cells. Same conventions as in a.
Figure 9: TANs in different striatal sub regions display similar response profiles

a) Average TAN population responses (± SEM) to behavioral events. Time zero: cue presentation, time 2s: outcome delivery. Same conventions as in Figure 3a.

b) Distributions of time of peak and minimum response and of average spike counts to cue presentation for all TANs. Each black/gray/blue dot represents an individual TAN. Red dots stand for population means. p is for putamen, c for caudate and v for VS. The distributions were not different across striatal
sub regions (one-way ANOVA). Color code: black is for time of minimum response, gray is for time of peak response and blue is for average spike counts.

We therefore examined the correlations between simultaneously recorded TAN pairs as was done for the MSN pairs. Although we identified and recorded TANs in the VS, they were harder to detect in this sub region (especially in comparison to the dorsal striatum). Hence our database included fewer single TANs from the VS (N=39) and no pairs (Bernacer et al., 2007, for the differential distribution of cholinergic interneurons in the striatum). Our correlation analysis of TAN to TAN pairs thus only included the striatal putamen and caudate sub regions.

As we showed elsewhere for putamen TAN pairs (Joshua et al., 2009) and extend here to caudate TAN pairs, the distributions of their signal and noise correlations were extremely skewed to the right (Fig. 10a), which is consistent with their stereotyped responses and synchronized activity (Graybiel et al., 1994; Joshua et al., 2008 and Fig. 9). However, as was the case for the MSN pairs, the TAN pairs also displayed differences in synchrony between the putamen and the caudate. Specifically, more putamen TAN pairs had a significant positive noise correlation (Fig. 10a inset), stronger correlation values for single pairs (Fig. 10a, histogram) and a stronger linear relationship between the signal and noise correlation measures (Fig. 10a right column). Due to the difference in the number of pairs and in order to enable a better comparison between the putamen and the caudate, we also examined the signal and noise distributions when we relaxed our inclusion criterion (time of simultaneous recording reduced from 21 and 30 minutes in monkeys L and G respectively to 15 and 21 minutes), thus increasing the number of TAN pairs in the caudate to 61. Similar results (differences between regions) were obtained. The distributions (signal and noise) were significantly different between regions (t-test, p<0.05).

The JPSTHs of TAN pairs did not reveal significant modulations along the main diagonal (except for the reward event, t-test, p<0.05 Fig. 10b). However, the data supported the difference in the noise correlation distributions of TAN pairs described above, where the diagonal of the JPSTH of putamen TAN pairs differed from that of caudate TAN pairs for all behavioral events (t-test, p<0.05).
Figure 10: Noise correlation between TAN pairs is stronger in the putamen than in the caudate

a) Distributions of signal (left column) and noise (middle column) correlations and the correlation between the two measures (right column) for TAN pairs recorded in the putamen (first row, n=72) and caudate (second row, n=38). Same conventions as in figure 5.

b) Population JPSTH of TAN pairs. First row for putamen, second for caudate. Same pair numbers as in a, same conventions as in Figure 6.
Discussion

In this study we compared the encoding of well-learned rewarding, aversive and neutral cues across striatal sub regions. We conducted two complementary analyses; namely, modulations of firing rates as related to the behavioral events and the correlation structure of simultaneously recorded pairs of neurons. We found that most MSNs and TANs in all striatal territories displayed significant modulations in firing rates to the task. Furthermore, the response profiles of TANs and MSN clusters were similar across striatal territories. The most pronounced difference across regions was revealed in the correlation structure. MSN pairs in the putamen displayed a positive average signal and noise correlation, which contrasted with the (closer to zero) correlations in the caudate and VS. In the putamen the signal and noise correlations were correlated. Furthermore, putamen MSN pairs displayed different dynamics in their discharge co-variation (modulations of noise correlation) to the different behavioral events (reward vs. aversive and neutral). The correlation structure of TAN pairs supported the results found for the MSN pairs, in that TAN pairs in the putamen displayed stronger noise correlations than those found in the caudate. Hence our results indicate that the increased neural correlations observed solely in the putamen enabled efficient information encoding and were suggestive of a more effective information transfer from the putamen to their downstream targets during the performance of a well-practiced task.

Neural correlations reflect the different roles of striatal sub regions

Previous studies have suggested that the differences in the anatomy and connectivity of striatal regions is reflected in their functionality (Alexander et al., 1986; Cardinal et al., 2002; Yin et al., 2004; Yin et al., 2006). This supposition assumes that the putamen is involved in habitual behavior, the caudate in goal directed behavior, and the VS in reward and motivation (Yin and Knowlton, 2006; Graybiel, 2008; Redgrave et al., 2010). As these parallel functional systems can lead to a single behavioral outcome/response, they are likely to converge onto the same lower level motor structures (Redgrave et al., 2010). This therefore raises the question of what processing mechanism enables one striatal system to mediate behavior over the others.

Previous studies have compared the activity in striatal regions solely at the level of rate coding by examining single cell discharge (e.g. Yamada et al., 2004; Williams and
Eskandar, 2006; Ding and Gold, 2012). These and other studies described a shift in activation of single cells, in which learning of new motor responses and the initial stages of learning activated the caudate, but well-learned motor sequences and advanced stages in training activated the putamen (Miyachi et al., 2002; Yin et al., 2009).

We hypothesized that rate coding and neural synchronization, as the means for encoding the behavioral events, can occur separately or together (Eggermont, 1990; Abeles, 1991; Ginzburg and Sompolinsky, 1994; Ben-Yishai et al., 1995; Panzeri et al., 2002; Averbeck and Lee, 2004; Averbeck et al., 2006). We therefore compared the neuronal activity in striatal regions in the same over-trained behavioral task on both levels. We found that cells in all regions were highly modulated by the task and displayed similar response profiles. However, we found an increase in the neuronal correlations solely in the putamen region. Since overtraining promotes habit formation (Dickinson, 1985; Colwill and Rescorla, 1988; but see, Holland et al., 2008), our task involved a more habitual rather than goal directed behavior. Thus the increase in neural correlations in the putamen may have reflected the differences in the functional roles of striatal territories. The increased synchronization found between putamen TAN-TAN and MSN-MSN pairs could have supported the efficient information flow from the putamen neuromodulators to the putamen projection neurons (Threlfell et al., 2012) and from the putamen to its downstream BG structures during an over-trained, possibly habitual task. We did not find evidence for increased correlation in the putamen during performance of a different, learning task (data not shown). However, these data were restricted and therefore future studies will need to ascertain whether the increase in correlation is indeed specific to the task or an innate property of the putamen. Finally, recent anatomical studies have revealed that the cortico-striatal projections often display a dorsal-ventral gradient and do not simply obey the caudate/putamen/VS boundaries (Calzavara et al., 2007). Our results sustain a functional dorsolateral vs. medioventral segregation of the striatum (Voorn et al., 2004); however, they are still in line with the description of diffuse and integrative cortico-striatal projections (Haber et al., 2011).
Differential synchronization level of MSNs in response to behavioral cues may enable efficient information transfer from the putamen

We found that putamen MSN pairs displayed positive signal and noise correlations. Moreover, there was a linear relationship between the two measures. A positive signal correlation is usually interpreted as originating from common input, whereas a positive noise correlation is usually interpreted as reflecting local synaptic connections (Gawne and Richmond, 1993; Lee et al., 1998). Positive noise correlations have been associated with decreased information coding (Zohary et al., 1994). However, once the network is not completely homogeneous in terms of the signal correlation (as in our case), the noise correlation should not necessarily limit information (Averbeck and Lee, 2004; Shamir and Sompolinsky, 2006).

Our finding of a strong linear relationship in the putamen between the two correlation measures suggests a model of functional interconnected sub networks which share common inputs, a model that has been put forward for cortical networks (Yoshimura et al., 2005; Rothschild et al., 2010). According to this model, during over-trained behavior, the putamen network is organized such that interconnected neuronal assemblies convey synchronized input to their downstream target. The transfer of information can be more reliably propagated by synchronous neuronal activity rather than by single cell rate modulations (Bruno and Sakmann, 2006; Bruno, 2011), thus enabling the putamen to control behavior over the caudate and VS.

Additionally, we examined the dynamics of the noise correlation with the different behavioral events using JPSTH analysis. JPSTH can be used to identify temporal modifications in the functional connectivity of a circuit as a result of behavioral stimuli and context (Espinosa and Gerstein, 1988; Aertsen et al., 1989; Ahissar et al., 1992; Vaadia et al., 1995; Cohen and Newsome, 2008). We found that putamen MSN pairs displayed increased correlations following cue presentation. Furthermore, the temporal profile of the modulation in the rewarding condition differed from that observed in the aversive and neutral conditions. This differential encoding suggests that the correlation structure of the network may be informative as regards the encoding of the task and is coherent with our previous observation of dopaminergic neuron pairs (Joshua et al., 2009). These JPSTH results, together with our other correlation measures, suggest dynamic changes in the strength of the input to putamen MSNs during over-trained behavior, both from a common source (dopaminergic, cortical, thalamic, etc.) and/or recurrent connections and based on the valence. It
further suggests a more efficient information transfer from the putamen compared to the caudate and VS during a well-practiced task.

Possible confounding factors in the correlation analysis
Several factors may limit the interpretation of correlation analysis, and specifically JPSTH analysis. One such factor is the common activation of the neurons by an unmeasured event; e.g., the motor related responses of BG neurons (Ben Shaul et al., 2001; Arkadir et al., 2002). We found that MSN pairs in the putamen alone, but not in the caudate or VS, displayed dynamics along the main diagonal of the JPSTH. However, the licking and blinking behavioral responses were similar when recordings were obtained from either region. Furthermore, although putamen MSNs may have a strong motor component in their response, MSNs in the caudate have also been shown to encode motor components, especially oro-facial and oculomotor movements (Hikosaka, 2007). Finally, we obtained similar results for TAN pairs, which displayed higher correlations in the putamen than in the caudate, but unlike the MSNs, the TANs do not have a motor component in their response (Kimura et al., 1984; Morris et al., 2004).

Another confounding factor is related to trial to trial variability along the recording session that results from latency co-variation, state excitability changes, changes in the monkey's arousal, motivation and other factors (Brody, 1999; Cohen and Newsome, 2008; Joshua et al., 2009). We cannot control for all the possible factors to which the cells might respond. However, we compared the results of the correlation analysis across the three territories and the behavioral context did not differ. Moreover, recording from the VS was done simultaneously with the recording in the putamen. Therefore, external causes for the observed changes should have affected both the putamen and VS.

Concluding remarks
In this study we examined the functional connectivity of the striatal network using correlation methods. The correlation structure revealed here suggests how the striatal multiple controller system determines which mode of control is used. We showed that in a well-practiced behavior, the striatal network is organized such that interconnected sub-networks in the putamen encode behavioral events. Thus the synchronized
activity solely in the putamen enables it to efficiently convey information to downstream BG structures during over-trained, possibly habitual, behavior.

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Results chapter number II.

*Temporal convergence of dynamic cell assemblies in the striato-pallidal network.*

Avital Adler, Shiran Katabi, Inna Finkes, Yifat Prut and Hagai Bergman.

Behavioral/Systems/Cognitive

Temporal Convergence of Dynamic Cell Assemblies in the Striato-Pallidal Network

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The basal ganglia (BG) have been hypothesized to implement a reinforcement learning algorithm. However, it is not clear how information is processed along this network, thus enabling it to perform its functional role. Here we present three different encoding schemes of visual cues associated with rewarding, neutral, and aversive outcomes by BG neuronal populations.

We studied the response profile and dynamical behavior of two populations of projection neurons [striatal medium spiny neurons (MSNs), and neurons in the external segment of the globus pallidus (GPe)], and one neuromodulator group [striatal tonically active neurons (TANs)] from behaving monkeys. MSNs and GPe neurons displayed sustained average activity to cue presentation. The population average response of MSNs was composed of three distinct response groups that were temporally differentiated and fired in serial episodes along the trial. In the GPe, the average sustained response was composed of two response groups that were primarily differentiated by their immediate change in firing rate direction. However, unlike MSNs, neurons in both GPe response groups displayed prolonged and temporally overlapping persistent activity. The putamen TANs stereotyped response was characterized by a single transient response group. Finally, the MSN and GPe response groups reorganized at the outcome epoch, as different task events were reflected in different response groups.

Our results strengthen the functional separation between BG neuromodulators and main axis neurons. Furthermore, they reveal dynamically changing cell assemblies in the striatal network of behaving primates. Finally, they support the functional convergence of the MSN response groups onto GPe cells.

Introduction

Most models of the basal ganglia (BG) nuclei suggest they implement a reinforcement learning (RL) algorithm (Houk et al., 1995; Sutton and Barto, 1998). One of the most compelling RL models is the actor-critic architecture because it bears similarities to the structure of the BG network. The actor component stores and updates stimulus–response associations, such that actions associated with greater cumulative reward are more frequently chosen. The critic component generates a temporal difference prediction error signal when there is a discrepancy between predictions and actual reinforcements. This is used as a teaching message by the actor to adjust behavior. Hence, the analogy with the BG, where the neuromodulators correspond to the critic, and the actor corresponds to the BG main axis; i.e., the cortex-striatum-pallidum-frontal cortex axis (Houk et al., 1995; Suri and Schultz, 1998).

It has been demonstrated that the BG neuromodulators and main axis neurons respond diversely to behavioral events in line with their different functional roles. The neuromodulators, specifically midbrain dopaminergic neurons and striatal cholinergic interneurons, display a homogeneous transient response, thus providing the main axis with a single-dimensional teaching message (Schultz, 1998; Bar-Gad et al., 2003; Joshua et al., 2008). Neurons in the main axis, however, specifically in the globus pallidus external segment (GPe) and the BG output structures, exhibit diverse activity with an average sustained response that enables a large information capacity (Mink, 1996; Bar-Gad et al., 2003; Joshua et al., 2009b). Possibly these long duration responses are similar to the set-related activity described in the cortex (Kojima and Goldman Rakic, 1982; Miyashita, 1988; Wise and Kurata, 1989; Funahashi et al., 1993; Fuster, 1999; Romo et al., 1999), which has been attributed to action preparation processes.

The striatum is the primary input stage of the BG main axis and the principal source of innervation to BG downstream structures (Haber et al., 2011). The main projection neurons of the striatum are the medium spiny neurons (MSNs), which make up the vast majority of the striatal cells (Tepper et al., 2007). Studies that examined the role of primate MSNs in both movement initiation and decision making have found mostly sharp transient activations to movement onset (Crutcher and DeLong, 1984a, 1984b; Hikosaka et al., 1989; Watanabe et al., 2003), cue present-
sounds were randomized between monkeys. Nevertheless, striatal MSNs are predicted to display sustained activity. This would correspond to their functional role in the BG network and previous observations of the GPe and BG output nuclei (Arkadir et al., 2004; Wichmann and Kliem, 2004; Joshua et al., 2009b).

To deepen our understanding of information processing along the BG main axis, we need to characterize the response profile and dynamic behavior of the MSNs, and specifically the ways in which MSNs contribute to the sustained activity generated in the pallidum. We thus recorded and compared the activity of several BG neural populations (MSNs, striatal cholinergic interneurons, and GPe neurons) from monkeys while they were engaged in a classical conditioning paradigm with long cues that predicted the trial outcome.

Materials and Methods

Two monkeys (*Macaca fascicularis*, G, male, 4.5 kg; and L, female, 3 kg) were used in this study. All experimental protocols were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and the Hebrew University guidelines for the use and care of laboratory animals in research. The experimental protocols were approved and supervised by the Institutional Animal Care and Use Committee of the Hebrew University and Hadassah Medical Center. The Hebrew University is an accredited institute of the Association for Assessment and Accreditation of Laboratory Animal Care International.

Behavioral task. The monkeys were trained (G for 4 months; L for 3 months) on a classical conditioning task (Fig. 1a). Each trial began with the presentation of a visual cue (full-screen fractal images generated using Chaos Pro 3.2 program (www.chaospro.de) and displayed on a 17 inch LCD monitor, 50 cm in front of the monkeys’ faces) for a period of 2 s. The cues were immediately followed by an outcome which could be one of three categories: liquid food (reward trials, upper row), air puff (aversive trials, third row), or only sound (neutral trials, second row). The trial outcome was followed by a variable ITI of 5–6 s. In total there were nine and six (G and L, respectively) different visual cues; three and two (G and L, respectively) for each outcome category. We thus recorded and compared the activity of several BG neural populations (MSNs, striatal cholinergic interneurons, and GPe neurons) from monkeys while they were engaged in a classical conditioning paradigm with long cues that predicted the trial outcome.

![Figure 1. Behavioral task.](image)

**Figure 1. Behavioral task.** a. Classical conditioning paradigm. Visual cues were presented for 2 s and predicted the delivery of food (reward trials, upper row), air puff (aversive trials, third row), or only sound (neutral trials, second row). The trial outcome epoch was followed by a variable ITI of 5–6 s. b. Normalized licking behavior (average ± SEM, solid line and shaded envelope, respectively) as recorded by an infrared reflection detector directed at the monkeys’ mouths. Time 0, cue presentation followed by outcome delivery at time 2 s; blue, reward trials; green, neutral; red, aversive trials; N, number of trials. c. Blinking behavior (average ± SEM) as processed by the video signal, detecting within each recording frame the state of the monkeys’ eyes (open, 0; closed, 1). In each time bin (20 ms) we calculated the fraction of trials with eyes closed. Same conventions as in b.

**Table 1. The neuronal database**

<table>
<thead>
<tr>
<th>Inclusion criterion</th>
<th>N</th>
<th>Fraction of ISI &lt;2 ms</th>
<th>Isolation score</th>
<th>Stable recording time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNs</td>
<td>344</td>
<td>0.0006 ± 0.0001</td>
<td>0.86 ± 0.004</td>
<td>49 ± 1.58</td>
</tr>
<tr>
<td>TANs</td>
<td>145</td>
<td>0.0001 ± 0.00002</td>
<td>0.92 ± 0.006</td>
<td>50 ± 2.36</td>
</tr>
<tr>
<td>GPe</td>
<td>179</td>
<td>0.0003 ± 0.0003</td>
<td>0.94 ± 0.004</td>
<td>47 ± 2.33</td>
</tr>
</tbody>
</table>

Recording quality parameters: (average ± SEM) of putamen and GPe neurons included in the study.

Surgery and magnetic resonance imaging. After the training period, the monkeys were operated on under full anesthesia and in sterile conditions. In the surgery, an MRI-compatible Cilux head holder (Crist Instrument) and a square Cilux recording chamber (AlphaOmega) with a 27 mm (inner) side, located above a burr-hole in the skull, were attached to the monkeys’ heads. The recording chamber was attached to the skull tilted 45° laterally in the coronal plane with its center targeted at the stereotaxic coordinates of the left anterior putamen (G: A16, L5, H1; L: A17, L6, H1; Martin and Bowden, 2000). Analgesia and antibiotics were administered during surgery and continued for 2 d postoperatively. Recording began after a postoperative recovery period of 5 d. We estimated the stereotaxic coordinates of the physiological recordings using MRI scans. The MRI scan (General Electric 3 tesla system, T2 sequence) was performed with five tungsten electrodes at accurate coordinates of the recording chamber [Y,X = (6,0), (0,−6), (0,0), (0,6), and (−6,0) in mm from the chamber center]. We then aligned the two-dimensional MRI
coronal scans with the sections of the atlas of *Macaca fascicularis* (Martin and Bowden, 2000). We performed an additional MRI scan at the final stage of the recording period to verify our coordinate system. At the end of the experiment, the chamber and head holder of monkey G were removed, the skin was sutured, and after a recovery period the monkey was sent to a primate sanctuary (http://monkeypark.co.il). Experiments on monkey L are still in progress.

All surgical procedures were performed under aseptic conditions and general isoflurane and N₂O deep anesthesia. The MRI procedure was performed under Dormitor and Ketamine light anesthesia.

**Figure 2.** Methodology of clustering analysis: K means. 

*a*, MSN responses to task cue events. In each subplot, each row is the color coded Z-PSTH of a single MSN to the presentation of the rewarding cues (first column, time 0–2 s) followed by Z-PSTH to the presentation of the aversive cues (second column) and to the neutral cues (third column). *N* = 344 neurons. Each subplot presents grouping of the Z-PSTHs into different numbers of clusters (*K* = 1–5) delineated by a white horizontal line. Visual inspection of the cells’ responses falling into different numbers of clusters served as one of our criteria for choosing *K*. 

*b*, Error as a function of *K* (number of clusters) when clustering was performed based on all cue events. Error was defined as the within-cluster sums of distances of data points to centroid summed over all *Ks*. 

*c*, Silhouette (degree of similarity of each point to points in its own cluster compared with points in other clusters) as a function of *K* (number of clusters).

Recording and data acquisition. During recording sessions, the monkeys’ heads were immobilized and eight glass-coated tungsten microelectrodes (0.3–0.7 MΩ impedance at 1000 Hz) were advanced separately (EPS, Alpha Omega Engineering) into the striatum and the GPe. In the striatum we targeted the putamen (*AC* = 1 to *AC* + 4) which has been shown to be involved in active movements or responses to sensory stimulation in the arm, leg, and face areas (Crutcher and DeLong, 1984a; Alexander and DeLong, 1985; Kandel et al., 2000). In the GPe, we recorded from the entire spatial nucleus span. The electrical activity was amplified with a gain of 5 K and bandpass filtered with a 1–6000 Hz
four-pole Butterworth filter and continuously sampled at 25 kHz by 12 bits, ±5 V range, analog-to-digital converter (AlphaMap, Alpha Omega Engineering). Spike activity was sorted online using a template matching algorithm (ASD; Alpha Omega Engineering) by two experimenters. Classification of putamen cells into MSNs, tonically active neurons (TANs), presumably cholinergic interneurons, fast spiking interneurons (FSIs), and other unidentified types of striatal neurons was done online based on the spike waveforms, firing rate, and pattern (Berke et al., 2004; Sharott et al., 2009) and was validated offline. GPe cells can be categorized into two subgroups (DeLong, 1971), one of which has a high-frequency discharge rate (in this study >20 Hz, HFD) typically interrupted by long pauses (Elias et al., 2007). The other is characterized by a low-frequency discharge rate which usually includes short bursts. Here, we targeted the HFD population of GPe cells.

Recorded neurons were subjected to offline quality analysis which included tests for rate stability, refractory period, waveform isolation, and recording time (Hill et al., 2011). First, the firing rate was graphically displayed as a function of time and the largest continuous segment of stable data was selected for further analysis. Second, cells in which >0.02 of the total ISIs were shorter than 2 ms were excluded from the database. Third, only cells with an isolation score (Joshua et al., 2007) >0.7 were included in the database. We ran the analysis on a subset of the cells with an isolation score >0.85 (N = 213 MSNs, average ± SEM of isolation score: 0.92 ± 0.002, N = 162 GPe neurons, isolation score: 0.95 ± 0.002, N = 123 TANs, isolation score: 0.94 ± 0.003) and obtained similar results (data not shown). Finally, only cells that fulfilled the above criteria for >18 min of task performance were included in the database. Inclusion criteria did not include significant responses to task events. Average (±SEM) values of the quality parameters in the final database are given in Table 1.

To assess the monkeys’ behavior, we monitored their mouth and eye movements, licking and blinking, respectively. Licking movements were monitored using an infrared reflection detector (Dr. Bouis Devices). The infrared signal was filtered between 1 and 100 Hz by a bandpass four-pole Butterworth filter, and sampled at 1.56 kHz. This was then normalized by subtracting the average infrared signal in the last 0.5 s of the ITI and dividing by the maximum value of the infrared signal. Blinking movements were monitored using infrared digital video cameras (AVer-s 2.54, AVer Information) which recorded the monkeys’ facial movements at 50 Hz. Video analysis was performed on custom software to identify periods when the monkeys’ eyes were closed (Mitelman et al., 2009).

Clustering analysis. For each cell, activity was calculated for two different epochs of the trial (cue presentation and outcome delivery) and for the three different task categories (reward, neutral, and aversive).

Neural responses to behavioral events were first characterized by their poststimulus time histogram (PSTH). The PSTHs were calculated in 1 ms bins and smoothed with a Gaussian window with a SD of 20 ms (we repeated the analysis by smoothing the PSTHs with a Gaussian window with a SD of 2 ms and received similar results). The baseline firing rate was calculated by averaging the firing rate in the last 0.5 s of the ITI and was subtracted from the smoothed PSTH. Next we Z-score transformed each PSTH (Z-PSTH) by subtracting the mean baseline corrected PSTH and dividing by the SD of the PSTH. Note that since the Z-PSTHs were computed by subtracting the mean discharge rate of the PSTH from the original PSTH and then dividing by the SD of the PSTH, a negative Z score did not necessarily imply that the discharge rate was below the original PSTH and then dividing by the SD of the PSTH. Note that since the

average sustained response versus putamen MSN and GPe average sustained response. a, GPe cell population average response (±SEM) to behavioral cues presented at time 0. The PSTHs were calculated in 1 ms bins and smoothed with a Gaussian window with SD of 20 ms. The baseline firing rate, calculated by averaging the firing rate in the last 0.5 s of the ITI, was subtracted from the smoothed PSTH. For the GPe population, we averaged over the absolute value of the PSTHs. Abscissa, time in seconds; ordinate, firing rate in Hz. Blue, responses to all reward cues; green, responses to all neutral cues; red, responses to all aversive cues. N = 179 neurons. b, TAN population average response (±SEM) to behavioral cues presented at time 0. Same conventions as in a. N = 145 neurons. c, MSN population average response (±SEM) to behavioral cues presented at time 0. Same conventions as in a. N = 344 neurons.

Figure 3. Putamen TANs average transient response versus putamen MSN and GPe average sustained response. a, GPe cell population average response (±SEM) to behavioral cues presented at time 0. The PSTHs were calculated in 1 ms bins and smoothed with a Gaussian window with SD of 20 ms. The baseline firing rate, calculated by averaging the firing rate in the last 0.5 s of the ITI, was subtracted from the smoothed PSTH. For the GPe population, we averaged over the absolute value of the PSTHs. Abscissa, time in seconds; ordinate, firing rate in Hz. Blue, responses to all reward cues; green, responses to all neutral cues; red, responses to all aversive cues. N = 179 neurons. b, TAN population average response (±SEM) to behavioral cues presented at time 0. Same conventions as in a. N = 145 neurons. c, MSN population average response (±SEM) to behavioral cues presented at time 0. Same conventions as in a. N = 344 neurons.

Clustering analysis. For each cell, activity was calculated for two different epochs of the trial (cue presentation and outcome delivery) and for the three different task categories (reward, neutral, and aversive).

Neural responses to behavioral events were first characterized by their poststimulus time histogram (PSTH). The PSTHs were calculated in 1 ms bins and smoothed with a Gaussian window with a SD of 20 ms (we repeated the analysis by smoothing the PSTHs with a Gaussian window with a SD of 2 ms and received similar results). The baseline firing rate was calculated by averaging the firing rate in the last 0.5 s of the ITI and was subtracted from the smoothed PSTH. Next we Z-score transformed each PSTH (Z-PSTH) by subtracting the mean baseline corrected PSTH and dividing by the SD of the PSTH. Note that since the Z-PSTHs were computed by subtracting the mean discharge rate of the PSTH from the original PSTH and then dividing by the SD of the PSTH, a negative Z score did not necessarily imply that the discharge rate was below the baseline rate.

We performed principal component analysis separately for each task event [six in total: three categories (reward, neutral, and aversive), times two epochs (cue presentation and outcome delivery)]. The principal components (eigenvectors) of the covariance matrix were sorted according to their explained variance (eigenvalues). The first two principal components had the highest explanatory power, whereas the following components did not make a substantial contribution. We therefore used the projection of the data points on the first two principal components (times six task events) to represent each cell.

To cluster the cell responses we applied the K-means algorithm on the cells' principal components representation. The K-means algorithm uses an iterative refinement procedure to minimize the sum, over all clusters,
K) silhouette score (Fig. 2c). Last, we visually inspected the Z-PSTHs grouped into K clusters (Fig. 2a), and confirmed our visual impression with the other two measures (error rate and Silhouette analysis).

We ran the K-means algorithm twice, once representing each cell with the principal components of the cue events and again with those of the outcome events.

For each cluster, we determined the fraction of cells that had a significant response. We calculated the mean (baseline) and SD of the PSTH of the last 3 s of the ITI (ITI-SD) using the same number of trials as in the studied PSTH and identified time segments in which there was a deviation of the PSTH from the baseline firing rate that exceeded three times the ITI-SD. A response was considered significant if the duration of the deviant segment was more than 60 ms (three times the SD of the smoothing filter). Finally, for each cell that had a significant response, we identified the delay in ms from presentation of the cue to the start of a significant increase/decrease in firing rate.

The data from the two monkeys were grouped since no significant difference was detected between the individual monkeys. Data analysis was performed on custom software using MATLAB V7.

**Results**

**Behavioral task and neuronal database**

Two monkeys were trained on a classical conditioning task (Fig. 1a) that involved three categories of visual cues predicting different outcomes: rewarding (food), aversive (air puff), and neutral (no outcome). The monkeys were trained on the behavioral task before recording and data acquisition. During recordings the monkeys, which were familiar with the different cues, displayed the appropriate anticipatory behavior (Fig. 1b,c). While the monkeys were engaged in the task we recorded the spiking activity of putamen phasically active neurons (presumably striatal projection neurons or MSNs), putamen TANs (presumably cholinergic interneurons), and GPe high-frequency discharge projection neurons. Recordings were made from up to eight electrodes simultaneously. Only neurons that passed the study inclusion criteria (see Materials and Methods, Recording and data acquisition and Table 1; N = 344 MSNs, 145 TANs, and 179 GPe neurons) were included in the analysis database.

**Different populations in the BG display transient versus sustained average responses**

We first examined the cells’ responses to the cue events at the population level. Figure 3 displays the average response of all three populations to the presentation of the visual cues. GPe high-frequency discharge neurons increased and decreased their firing rates to cue presentation; we therefore averaged over the absolute response (Joshua et al., 2009b) and obtained an average sustained response that lasted throughout the entire 2 s of cue presentation (Fig. 3a). By contrast, TANs displayed a homogeneous bipolar transient response (Fig. 3b). This persistent GPe versus transient TANs response is expected from neurons along the BG main axis compared with neuromodulators and in line
with previous observations (Joshua et al., 2008, 2009b). Next, we examined the average response of the low-frequency discharge MSNs and found it was also sustained (Fig. 3c). Thus, BG neural populations display transient (TAN) versus sustained (MSN and GPe) average responses. To understand the functional relationship between MSNs and GPe neuronal activity, we further investigated the response profile and dynamic behavior of the MSNs at the single cell level (see below).

Average persistent response of MSNs consists of cell assemblies firing sequentially along the trial

Examining the responses of all putamen MSNs separately revealed a wide range of modulations in both the direction (increases vs decreases in firing rates) and temporal profile (Fig. 2a, upper left). We therefore used clustering to separate the activity of single MSNs into groups of cells with similar response patterns. We conducted a principal component analysis on the normalized PSTHs ($Z$-PSTHs) and used the $K$-means algorithm to classify the cells into different response groups (Fig. 2). The responses of the MSNs best fit three clusters. Figure 4a presents the color-coded $Z$-PSTHs (one row for each cell) ordered by the clustering analysis. The clusters were differentiated primarily by the neurons’ temporal response profile. Neurons belonging to the first cluster displayed a sharp and immediate increase in firing rate in response to cue presentation (average $\pm$ SEM delay to significant increase in firing rate, 252.4 $\pm$ 34.95 ms). Neurons belonging to the third cluster displayed a more moderate increase in firing rate that was delayed after cue onset (average $\pm$ SEM delay to significant increase in firing rate 835.2 $\pm$ 25.01 ms) and lasted for the remainder of the cue presentation. For some cells, this elevation in rate was preceded by a decrease in firing rate (average $\pm$ SEM delay to significant decrease in firing rate, 223.55 $\pm$ 26.74 ms). Neurons belonging to the second cluster displayed an intermediate response to cue presentation both in the magnitude and in the timing of the response (average $\pm$ SEM delay to significant increase in firing rate, 403.14 $\pm$ 23.85 ms). Figure 4b depicts the average PSTHs separately for each MSN cluster. Finally, Figure 4c displays the fraction of cells which had a significant response (both increases and decreases in firing rate) for each cluster separately. This analysis confirms that MSNs in the different clusters responded at different times during cue presentation. Thus, the average persistent response of the MSNs to cue presentation was composed of three major cell assemblies that fired in sequential episodes along the trial.

The results of the PSTH clustering were supported by a correlation analysis of cue related activity (Fig. 4d). Cells belonging to the same cluster tended to display a high positive correlation, whereas those in different clusters displayed a negative or no correlation.

We also clustered the MSN responses for each cue event (reward, aversive, and neutral) separately and compared the results with the original clustering, which was performed using all cues together (data not shown). Three similar sequentially switching response groups were found. Thus, MSNs displayed a general pattern of response to significant behavioral events (i.e., cluster 1, 2, or 3) regardless of which valence triggered the response. The difference between values was coded by the amplitude of change in firing rate (Figs. 3c, 4b). In this article we address the general result of three temporally distinct response groups and not the coding of valence by the MSNs.

Figure 5. TANs display homogeneous responses to cue presentation. a, TAN responses to task cue events. Same conventions as in Figure 4a. N = 145 neurons. Cells are randomly ordered. b, Population average responses ($\pm$ SEM) to cue presentation (time 0). Same conventions as in Figure 4b. c, Fraction of TANs with a significant response (both increases and decreases in firing rate) to task cue events. Same conventions as in Figure 4c. d, Correlation coefficient of the $Z$-PSTH matrix in a. Same conventions as in Figure 4d.
TANs display a single homogeneous response pattern

We performed the same clustering analysis on the responses of the TANs to cue presentation. TANs displayed a homogeneous response, as previously described (Graybiel et al., 1994; Joshua et al., 2008, 2009a) and as expected from their functional role as neuromodulators. The clustering analysis suggests accordingly that their responses conformed to a single response pattern (Fig. 5). Clustering analysis of the baseline activity of the TANs (starting 1 s after cue presentation) also did not reveal robust clustering of the TAN activity (data not shown).

Average persistent response of GPe cells is composed of persistent response at the single cell level

The responses of GPe cells, on the other hand, best fit two clusters (Fig. 6). The two GPe clusters primarily reflected the neurons’ immediate change in firing rate. Neurons belonging to the first cluster displayed a sharp increase in firing rate to cue presentation (average ± SEM delay to significant increase in firing rate, 275.47 ± 21.5 ms). Neurons belonging to the second cluster displayed an immediate decrease (average ± SEM delay to significant decrease in firing rate, 276.82 ± 23.39 ms). However, most neurons, whether they belonged to the first or second cluster, had a prolonged component in their response after the sharp immediate increase/decrease in firing rate that lasted for the remainder of the cue presentation (Fig. 6b,c). This contrasted with the response groups found for putamen MSNs, where each response group dominated a different epoch in cue presentation time in a sequential manner (compare Fig. 4c with 6c). Thus, GPe neuronal activity was divided into two response groups that overlapped in time.

MSNs response profile does not reflect the neurons’ intrinsic properties or spatial layout

There is heterogeneity in the intrinsic properties of the MSNs, the most prominent probably being D1 versus D2 dopamine receptor expression (Gerfen et al., 1990; Gradinaru et al., 2009; Bateup et al., 2010). The D1/D2 heterogeneity is coupled with the expression of substance P or enkephaline as a cotransmitter, intrinsic excitability and morphological properties (Day et al., 2008; Gertler et al., 2008) and with different targets in the BG network. It could have been that MSNs belonging to different response clusters represent different types of cells. In our study design, which involved extracellular recordings from behaving monkeys, we were not able to tag or characterize single cells (Belujon et al., 2010; Flores-Barrera et al., 2010). However, we could examine the spiking parameters of the cells, which are often used to classify cells recorded in the same location into different types (for example, in our study, MSNs vs TANs). There was no significant difference (one-way ANOVA) in the average firing rates or in the average length of the spikes’ waveform between the MSN clusters (Fig. 7a,c). Although there was a difference (one-way ANOVA, p < 0.05) in the coefficient of variation of the interspike interval of cells belonging to the first cluster compared with the second and third cluster, it was very weak (Fig. 7b). As for the MSNs, cells in the two GPe clusters were not differentiated by their spiking
parameters (Fig. 7d–f). The TANs unimodal distribution of spiking parameters is shown for comparison in Figure 7g–i.

In addition, we did not observe a spatial layout of the MSNs response clusters; i.e., there was a representation of all three clusters in all recording coordinates (Fig. 8a). We further examined whether the probability that a pair of MSNs will have the same response profile decreases with the spatial distance between the cells. We calculated the fraction of pairs of cells belonging to the same cluster when the cells were recorded simultaneously and from the same electrode, when they were recorded simultaneously but from different electrodes, and when they were recorded in different recording sessions. The estimated distance between the neurons was \( <100 \mu m \) for the pairs recorded from the same electrode, \( <2 \text{ mm} \) for the pairs recorded in the same session, and \( >2 \text{ mm} \) for the pairs recorded in different sessions. There was a small decrease in the fraction of pairs belonging to the same cluster when the estimated distance between neurons increased (Fig. 8b). Nevertheless, the fraction of pairs belonging to the same cluster did not change significantly when cells were recorded in the same or in different recording sessions (\( \chi^2 \) test). As for the MSNs, we did not observe a spatial layout of the GPe response clusters (Fig. 8c). The fraction of GPe neuron pairs belonging to the same cluster was not significantly different when cells were recorded in the same or in different sessions (\( \chi^2 \) test, Fig. 8d).

Thus, the formation of the MSNs and GPe response clusters could not be accounted for by the cells’ intrinsic firing properties or spatial layout within the nuclei.

The formation of clusters is dynamic

Up to now we have described modulations of MSN activity following cue presentation. Next, we studied whether the formation of MSNs assemblies remained constant throughout different parts of the trial (i.e., as expected from fixed anatomical clustering). Figure 9a displays the \( Z \)-PSTHs of the MSNs ordered by the clustering analysis as in Figure 4a, but this time including the responses at the outcome epoch. There was no apparent clustering in the MSNs’ responses to outcome delivery. We therefore performed the same clustering analysis using the outcome events and found that cells were reorganized into different groups of clusters (Fig. 9b). For example, if a cell belonged to the first cluster (transient early response) in the cue epoch, its response to the outcome epoch could be associated with any of the three response groups found in the outcome epoch and vice versa (Fig. 9c,d).

Hence, the formation of MSN assemblies in the putamen was dynamic and probably did not reflect the anatomical or the neurochemical properties of the MSNs. The GPe neurons were less responsive in the outcome epoch of the trial; however, similar dynamic clustering was also found (data not shown).
Discussion

In this study we presented three different encoding schemes of visual cues associated with rewarding, neutral, and aversive outcomes by three BG neuronal populations. TANs displayed an average transient sharp response, whereas GPe neuron and MSN responses were sustained. At the single cell level, TAN activity was stereotyped and associated with one cluster of response profiles. GPe neurons and MSNs, on the other hand, were classified into two and three response groups, respectively. The two GPe response groups reflected their bipolar change in firing rate. In both groups, however, there was sustained activity at the single cell level. The average persistent activity observed in the MSNs was composed of three cell assemblies that fired in serial episodes along the trial. Finally, the formation of MSN cell assemblies was dynamic, as the cells were reorganized into different response groups at different trial epochs.

Is the actual number of clusters arbitrary?

Here we used the $K$-means algorithm to cluster the cell responses. The number of clusters ($K$) is an input parameter to the algorithm, which thus raises the question: What is the appropriate choice of $K$? Our aim was to find the smallest number of clusters to represent the cell responses. We ran the analysis several times, each with different assignment for $K$, and used three parameters as the criteria for choosing the optimal $K$ (for details see Materials and Methods, Clustering analysis). Finally, we chose $K = 3$ clusters as the best fit for the MSNs data and $K = 2$ clusters as the best fit for the GPe data. We believe the number of response clusters is not arbitrary but represents separate subpopulations of cells with clear distinctive functions. Nonetheless, the main result of this study—the MSN persistent population activity is composed of dynamically changing cell assemblies whereas the GPe persistent population response is composed of persistent single cell responses—is not affected by the exact number of clusters chosen.

Possible different sources of innervation of different MSN response groups

MSNs throughout the entire putamen are innervated by both the cortex and the CM-Pf complex of the thalamus (Smith et al., 2004; Haber et al., 2011). The thalamostriatal synapses have not been studied as extensively as the corticostriatal synapses although they constitute $40–50\%$ of the glutamatergic synapses formed on MSN dendrites (Smith et al., 2004; Doig et al., 2010). Physiologically, these two pathways are presumed to have different functional roles. It is hypothesized that the corticostriatal projections are involved in cognitive and motivational properties of associative learning (Graybiel, 2000), whereas thalamostriatal projections supply information about behaviorally significant sensory events involved in arousal and attention (Matsumoto et al., 2001).

Anatomically, these synapses have been shown to share similar morphology and to converge onto single MSNs (Raju et al., 2006). However, recently it was demonstrated that repetitive stimulation of the cortical or the thalamic pathways lead to distinct patterns of spiking activity in the MSNs. Cortical stimulation generated a postsynaptic depolarization that grew and then maintained with little subsequent decline. Thalamic stimulation, on the other hand, generated a postsynaptic depolarization that steadily declined in amplitude (Ding et al., 2008). In this study we showed that MSNs belonging to different response clusters were differentiated primarily by their temporal profile. Neurons belonging to the different response clusters were uniformly distributed within the putamen and were not characterized by different spiking parameters, suggesting they are not separated by their neurochemical properties. This is in line with recent results showing that D1 and D2 receptors expressing MSNs undergo the same form of dopamine-dependent synaptic plasticity (Bagetta et al., 2011) and cholinergic-dependent D2/A2A modulation (Tozzi et al., 2011). Thus, different pathways (cortical vs thalamic) may possibly drive the different MSN response groups and could convey distinct information and lead to different functions. We cannot, however, rule out other factors possibly differentiating the cells; this topic requires further inquiry.
Functional convergence of MSNs onto GPe

It has been suggested that the GPe acts as more than simply a relay nucleus but rather as an integration site for different behaviorally relevant neuronal circuits (Bolam et al., 2000; Kita, 2007). Anatomically, the large pallidal neurons are characterized by long and sparsely branched dendritic arborizations. The arborizations are discoidal in shape and are perpendicular to the main bundle of the afferents of striatal axons (Yelnik et al., 1984; Kita and Kitai, 1994). Thus, GPe cells receive input from wide regions of the striatum (Percheron et al., 1984). Furthermore, the massive reduction in the number of cells from the striatum to the GPe implies convergence of many MSNs onto single GPe neurons (Percheron and Filion, 1991; Oorschot, 1996). Physiological findings support information convergence at the GPe level and have shown coding of both movement direction and reward prediction by single pallidal neurons (Gdowski et al., 2001; Arkadir et al., 2004). At the striatal level, the findings are more contradictory with reports of convergent representation of limbic and motor information on the one hand (Parthasarathy et al., 1992; Levy et al., 1997; Kawagoe et al., 1998) and a lower degree of convergence up to separate representations on the other (Flaherty and Graybiel, 1991; Cromwell and Schultz, 2003; Schmitzer-Torbert and Redish, 2004). Together, these findings could imply a greater degree of convergence at the GPe compared with the striatal level. We found that the average persistent response of the MSNs was composed of three major cell assemblies whose response patterns were temporally differentiated and were sequentially active along the trial. The average persistent response observed in the GPe, however, was composed of sustained activity at the single cell level resulting in an overlap in the time of activation between the two response clusters. Our results thus support the funneling model with anatomical convergence of MSNs onto GPe and point to functional convergence as well. In addition, they are in line with stronger convergence at the GPe compared with the striatal level.

The GPe response clusters were differentiated primarily by the change in the cells’ response direction, which exhibited both decreases and increases in firing rates. Firing rate decreases are expected by striatal convergent GABAergic innervations. The source of GPe increases in firing rates could be the excitatory convergent information received from the subthalamic nucleus (Hazzari and Parent, 1992; Shink and Smith, 1995; Hanson et al., 2004). However, other alternatives could explain the GPe response profiles, such as opposing effects of D1 versus D2 receptors expressing MSNs and/or striatal inhibitory connections along with disinhibition by axon collaterals within the GPe (Kita and Kitai, 1994; Parent et al., 2000; Sato et al., 2000).

Formation of dynamically changing functional correlations within the striatal MSNs network

The striatum plays a key role in reinforcement and associative learning; however, how information is processed within the striatal network is still open to debate. The dense local axon collateral system found between spiny cells led early studies to hypothesize a functional competition among the neurons which enabled a winner-take-all dynamics (e.g., one action is selected over all other alternatives; Wickens, 1993; Fukai and Tanaka, 1997). However, physiological studies have found these lateral connec-

Figure 9. The formation of MSN clusters is dynamic. a, Single MSN responses to task events. Same as Figure 4a, however for each cell (row) all six task events are presented. RC, reward cue; AC, aversive cue; NC, neutral cue; RO, reward outcome; AO, aversive outcome; NO, neutral outcome. b, Same as in a, however clustering analysis was run on the outcome events. c, d, Cells are clustered differently in cue versus outcome events. Each group of three bars (green, cyan, and brown) represents the MSNs in every cluster in the outcome (c) or the cue (d) events. The separate bars represent distribution of the cells among the clusters in the cue (c) or the outcome (d) events. There is no pattern in the distribution within each cue or outcome cluster; i.e., the formation of clusters changes along the trial.
tions to be sparse, weak, and asymmetrical (Jaeger et al., 1994; Tunstall et al., 2002; Koos et al., 2004; Tepper et al., 2004; Planert et al., 2010) thus casting doubt on competitive inhibition as a key computational process in the information processing of the striatum. Ponzi and Wickens (2010) recently showed that in a model based on a realistic striatal inhibitory network, cells form assemblies that fire in sequential coherent episodes. Cell members of the same assembly showed correlated firing rate fluctuations at behaviorally relevant timescales, whereas cell members in different assemblies were negatively correlated. This simulation was supported by an in vivo experimental study (Carrillo-Reid et al., 2008) showing that a set of neurons in a corticostriatal slice exhibit episodes of recurrent and synchronized bursting. They further demonstrated that blockage of glutamatergic transmission abolished the correlated activity, whereas blockage of GABAergic transmission locked the network into a single dominant state. Our results demonstrate the formation of dynamically changing functional correlations within the striatal MSN network and are thus the first representation of the above dynamics in a behaving primate. Based on previous results and our findings, we hypothesize that the cortical and/or thalamic excitatory input likely generates the coordinated MSN assemblies. Once formed during learning, these assemblies need only a trigger for activation thereafter. However, without lateral inhibitory connections, the network would constantly remain in a certain state. Thus, the response profile and network dynamics of MSNs, as revealed here in behaving primates, reflect the complex interplay between striatal excitatory input and lateral inhibitory connections.

In conclusion, we presented three different encoding schemes by MSNs, GPe neurons, and TANs. Our results strengthen the functional separation between BG neuromodulators and the main axis by showing that MSNs display an average sustained response as expected by their functional role in associative learning. Furthermore, these results point to the strong functional convergence of MSNs onto GPe cells, leading to the average sustained response of single GPe neurons. Finally, the MSNs display a response profile and dynamic behavior that incorporates the elements required for information processing in a dynamic network of cell assemblies.

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Results chapter number III.

*Neurons in both pallidal segments change their firing properties similarly prior to closure of the eyes.*

Avital Adler, Mati Joshua, Michal Rivlin-Etzion, Rea Mitelman, Odeya Marmor, Yifat Prut and Hagai Bergman.

INTRODUCTION

The basal ganglia (BG) are usually defined in terms of the cortico–basal ganglia (BG) network predict reciprocal discharge patterns between the external and internal segments of the globus pallidus (GPe and GPi, respectively), as well as cortical driving of BG activity. However, physiological studies revealing similarity in the transient responses of GPe and GPi neurons cast doubts on these predictions. Here, we studied the discharge properties of GPe, GPi, and primary motor cortex neurons of two monkeys in two distinct states: when eyes are open versus when they are closed. Both pallidal populations exhibited decreased discharge rates in the “eye closed” state accompanied by elevated values of the coefficient of variation (CV) of their interspike interval (ISI) distributions. The pallidial modulations in discharge patterns were partially attributable to larger fractions of longer ISIs in the “eye closed” state. In addition, the pallidial discharge modulations were gradual, starting prior to closing of the eyes. Cortical neurons, as opposed to pallidal neurons, increased their discharge rates steeply on closure of the eyes. Surprisingly, the cortical rate modulations occurred after pallidal changes. Moreover, the early, unexpected changes in the pallidum are better explained by a subcortical rather than a cortical loop through the BG.

Neurons in Both Pallidal Segments Change Their Firing Properties Similarly Prior to Closure of the Eyes

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Adler A, Joshua M, Rivlin-Etzion M, Mitelman R, Marmor O, Prut Y, Bergman H. Neurons in both pallidal segments change their firing properties similarly prior to closure of the eyes. J Neurophysiol 103: 346–359, 2010. First published October 28, 2009; doi:10.1152/jn.00765.2009. Current anatomical models of the cortico–basal ganglia (BG) network predict reciprocal discharge patterns between the external and internal segments of the globus pallidus (GPe and GPi, respectively), as well as cortical driving of BG activity. However, physiological studies revealing similarity in the transient responses of GPe and GPi neurons cast doubts on these predictions. Here, we studied the discharge properties of GPe, GPi, and primary motor cortex neurons of two monkeys in two distinct states: when eyes are open versus when they are closed. Both pallidal populations exhibited decreased discharge rates in the “eye closed” state accompanied by elevated values of the coefficient of variation (CV) of their interspike interval (ISI) distributions. The pallidial modulations in discharge patterns were partially attributable to larger fractions of longer ISIs in the “eye closed” state. In addition, the pallidial discharge modulations were gradual, starting prior to closing of the eyes. Cortical neurons, as opposed to pallidal neurons, increased their discharge rates steeply on closure of the eyes. Surprisingly, the cortical rate modulations occurred after pallidal modulations. However, as in the pallidum, the CV values of cortical ISI distributions increased in the “eye closed” state, indicating a more bursty discharge pattern in that state. Thus changes in GPe and GPi discharge properties were positively correlated, suggesting that the subthalamic nucleus and/or the striatum constitute the main common driving force for both pallidal segments. Furthermore, the early, unexpected changes in the pallidum are better explained by a subcortical rather than a cortical loop through the BG.

The cortex exhibits changes in its patterns of activation in different brain states, such as during slow-wave sleep and under anesthesia (Contreras and Steriade 1997; Evarts 1964; Hobson and Pace-Schott 2002; Steriade et al. 1993; Webb 1976). Recent studies have even reported changes in cortical activation that were associated with “resting” states (Poulet and Petersen 2008) and with the eye state (McAvoy et al. 2008). The cortex has a strong influence on the spontaneous discharge properties of neurons in the BG (Aldridge et al. 1990; Nambu et al. 1999; Turner and Anderson 1997) and reward-related events (Ardakiri et al. 2004; Gdowski et al. 2001; Joshua et al. 2009). Thus the similarity in behaviorally triggered responses (Mitchell et al. 1987; Turner and Anderson 1997, 2005) between the GPe and GPi neurons challenges the current models of BG connectivity. We sought to shed light on these conflicting results by further investigating the spontaneous activity of GPe and GPi neurons in different brain states rather than by studying their responses to phasic behavioral events.

The cortex exhibits changes in its patterns of activation in different brain states, such as during slow-wave sleep and under anesthesia (Contreras and Steriade 1997; Evarts 1964; Hobson and Pace-Schott 2002; Steriade et al. 1993; Webb 1976). Recent studies have even reported changes in cortical activation that were associated with “resting” states (Poulet and Petersen 2008) and with the eye state (McAvoy et al. 2008). The cortex has a strong influence on the spontaneous discharge properties of neurons in the BG (Aldridge et al. 1990; Nambu et al. 2000, 2002a; Yoshida et al. 1993) and in fact the cortico–BG loop architecture suggests that discharge modulation of basal ganglia neurons should follow the cortical changes. However, subcortical loops from the midbrain via the thalamus have the capacity to influence activity in the BG (McHaffie et al. 2005). To provide further insights into the relative role of subcortical versus cortical drive of the BG, we
compared the spontaneous discharge properties of neurons in the cortex (primary motor cortex [M1]) and both pallidal segments in the transition between different brain states—upon closing and opening of the eyes.

METHODS

Animals

Two monkeys (W: Vervet, Cercopithecus aethiops, female 4.5 kg; and L: Macaque fascicularis, female 4 kg) were used in this study. Monkey L was trained on a probabilistic classical conditioning task (Joshua et al. 2008, 2009). At the end of the experiment after a recovery period, monkey L was sent to a primate sanctuary (http://www.ipsf.org.il). Monkey W was not engaged in a behavioral task and was trained only to sit quietly in the primate chair. Monkey W was systematically treated with MPTP after recording in the normal state (Rivlin-Etzion et al. 2008) and its neural activity was also recorded after development of the clinical signs of PD. It was perfused through the heart with saline followed by a 4% paraformaldehyde solution within 30 min of its death, which occurred 13 days from the first MPTP injection. The MPTP data are not included in this report.

All experimental protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Hebrew University guidelines for the use and care of laboratory animals in research. The experimental protocols were approved and supervised by the Institutional Animal Care and Use Committee of the Hebrew University and Hadassah Medical Center. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care International accredited institute. Materials and methods were given in detail in previous studies (Joshua et al. 2008; Rivlin-Etzion et al. 2008). Here we give a brief summary of these methods and provide information on the methods not used in the previous reports.

Recording and data acquisition

During recording sessions, the monkeys’ heads were immobilized and eight glass-coated tungsten microelectrodes were advanced separately (EPS, Alpha-Omega Engineering, Nazareth, Israel) into the arm-related area of the motor cortex (monkey W) and into the GPe or the GPi (monkeys W and L). Pallidal recordings were not restricted to specific areas within the GPe and GPi, but spanned the entire nuclei. Two experimenters controlled the position of the eight electrodes and real-time spike sorting (AlphaMap, ASD, Alpha-Omega).

A titanium screw was implanted above the frontal area of the skull for electroencephalographic (EEG) recordings in monkey L. The recorded EEG was amplified with a gain of 500 and band-pass filtered with a 1-100 Hz four-pole Butterworth filter and sampled at 1.56 kHz. Near-infrared digital video cameras were used to record the monkeys’ facial movements (monkey W: AVC 307R B/W, Avtech systems, Taipei, Taiwan; monkey L: AVer-s 2.54, AverMedia Systems, Taipei, Taiwan). The recordings were made at a sampling rate of 9–13 and 50 frames/s (monkey W and monkey L, respectively). Video analysis was carried out on custom software to identify periods when the monkeys’ eyes were closed (Mitelman et al. 2009). We divided the recording times into two distinct periods, depending on the state of the monkey’s eyes. Periods when the monkey’s eye was closed continuously for >1 s (which is longer than the typical duration of monkey blinks, 50-ms down phase; Baker et al. 2002) were considered “eye closed” states. Other periods (including short, <1 s periods with closed eyes, i.e., suspected blinks) were considered as “eye open” states.

EEG data analysis

EEG data from all recording days were visually inspected and we discarded any day in which the signal was contaminated with artifacts (mainly due to cross talk between recording channels). The final analysis was run on 18 of 26 recording days. The recorded EEG signal was digitally low-pass filtered at 50 Hz using an 11th-order Butterworth zero-shift filter. Subsequently, the EEG signal was divided into segments according to the distinction between “eye open” and “eye closed” states. To compare the amplitude of the EEG waves between states we calculated the root mean square (RMS) of the signal in each state for each day. We compared the RMS in the two states using a paired Wilcoxon signed-rank test. The power spectrum of the EEG signal was calculated separately in each state using a fast Fourier transform with a window size of 2 s, 50% overlap, and a frequency resolution of 0.2 Hz. The power spectral density in each state was normalized (divided by the mean power of all 1- to 50-Hz frequencies) and averaged across days.

Neuronal data analysis

Recorded spike trains were subjected to an off-line quality analysis, which included tests for discharge rate stability, refractory period, spike waveform isolation, and recording time. As a first step, the firing rate was graphically displayed and visually inspected. The largest continuous segment of data with a stable discharge rate was selected for further analysis. To rule out the possibility that cells with very large modulations in discharge rate due to transitions between the “eye open” and “eye closed” states had been excluded, we repeated the analysis using the cells’ entire recording durations and obtained similar results (data not shown). Second, cells in which >0.02 of the total interspike intervals (ISIs) were <2 ms were excluded from the database (Fee et al. 1996). Third, only pallidal and M1 cells with an isolation score (Joshua et al. 2007) >0.8 and 0.7, respectively, were used. We repeated our analysis on the subpopulation of M1 neurons that had isolation scores >0.8 (n = 68 cells) and obtained similar results to those reported in the following text (data not shown). Fourth, for the pallidal population of cells, only high-frequency discharge neurons (i.e., discharge rate in the “eye open” state >20 Hz) were included in the database. This was done to exclude GPe low-frequency discharge bursts (DeLong 1971), which may represent a different population of GPe neurons (François et al. 1984; Kita and Kitai 1994). Finally, the recording time (after off-line quality analysis) of each cell was segmented into “eye open” versus “eye closed” states; thus each cell had multiple distinct recording segments in each state. Only cells that fulfilled the aforementioned inclusion criteria (discharge stability, isolation quality, and refractory period) for >240 s and had ≥5 s (not necessarily continuous) of recording in each state (“eye open” and “eye closed”) were included in the database. Monkey L was engaged in a behavioral task during recording sessions. However, even during behavioral sessions, the monkey paused in its task from time to time and could close its eyes. These periods were used for the analysis in this report. Thus in monkey L the average recording duration of neurons was 3,162 and 256 s in the “eye open” and “eye closed” states, respectively. In monkey W, which was not engaged in a behavioral task, the average recording duration of the neurons was similar in the two states and was 267 and 342 s in the “eye open” and “eye closed” states, respectively (see details in Table 1).

Discharge rate analysis

We calculated the mean discharge rate for each neuron in the “eye open” and “eye closed” states and tested the difference in discharge rate between the two states at both the single-cell and the population level. At the single-cell level, for each neuron we first computed the discharge rates in 1-s bins separately for the “eye open” and “eye closed” states and then found the absolute difference between the means of these
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<th>ISIs Open</th>
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<tr>
<td>64</td>
<td>0.64</td>
<td>0.05 (0–0.009)</td>
<td>0.024 (0–0.009)</td>
</tr>
<tr>
<td>102</td>
<td>0.60</td>
<td>0.04 (0–0.007)</td>
<td>0.024 (0–0.009)</td>
</tr>
</tbody>
</table>

Recording statistics were calculated separately for each monkey (W and L) and for each of the four cortical regions. Values in parentheses are the 99th percentile. For each cell, the average discharge rate in the “eye open” state was calculated as the average deviation of the single cells’ PSTHs from their baseline discharge rates.

**Discharge pattern analysis**

We used the coefficient of variation (CV; SD to the mean) of the ISIs to assess the changes in discharge patterns. As described earlier, each cell had multiple distinct recording segments in each state with different durations. At the single-cell level we used a resampling (bootstrap) method to test the significance of the changes in discharge patterns. At the population level, we tested the differences in discharge rates using a paired Student’s t-test comparing the mean discharge rates in the “eye open” state with those in the “eye closed” state.

In all three populations (M1, GPe, and GPi), we tested whether the average discharge rates were significantly different in the “eye open” and “eye closed” states. We also performed this analysis on neurons not recorded during task performance and obtained similar results (data not shown).
In addition, to better characterize the differences in discharge pattern between the two states, we looked at the skewness and kurtosis values of the ISI distributions. We used the logarithm of the ISI to calculate the skewness and kurtosis values, since the logarithmic transformation makes the ISI distributions independent of timescale and more symmetric (Bhumbra and Dyball 2004) and therefore compensates for the different durations of recording segments in each state. We used the same inclusion criteria as for the CV calculation. In addition, in all three populations (M1, GPe and GPi) we corrected for the bias in skewness and kurtosis estimation by random shuffling (bootstrap) with 2,000 random replications (Efron and Tibshirani 1993). We tested the difference in skewness and kurtosis values between the “eye open” and “eye closed” states at the population level using a paired Student’s t-test.

We evaluated the time course of the changes in pallidal discharge pattern between the “eye open” and “eye closed” states by calculating the CV for each neuron over a fixed number of intervals around closing and opening of the eyes. For each neuron, each spike train was divided into a series of 50 ISIs (~1 s for the pallidal cells). CV values were computed on the moving window of 50 ISIs, with an overlap of 40 ISIs (80% window size), and assigned to the time of the first spike creating the 50- ISI window. The CV values were then interpolated to create a continuous time series with a resolution of 100 ms and averaged across trains. Next, to obtain a population time course of the changes in CV values we averaged the CV time course across cells. Only cells that had at least one full CV time course were included in the population average (as in the PSTH analysis). We repeated this analysis for all neural populations (M1, GPe and GPi) using a moving window of 5 ISIs, with an overlap of 4 ISIs to compute the CV.

We did not detect any significant difference between the individual monkeys; therefore we grouped their results according to structure and analysis type. Significance level was set to 5% in all statistical tests. Data analysis was carried out on custom software using MATLAB V7 (The MathWorks, Natick, MA).

**RESULTS**

**Segmentation of recording times into “eye open” and “eye closed” states is reflected in EEG**

We recorded neuronal activity in three areas of the cortex–BG network: the primary motor cortex (M1) and the external and internal segments of the pallidum (GPe and GPi, respectively). In addition, video cameras were used to record the monkeys’ facial movements. We divided the recording times into two distinct periods depending on the state of the monkey’s eyes: “eye open” versus “eye closed” states (for details see METHODS). Figure 1A shows an example of the raw eye state and the segmentation into “eye open” and “eye closed” states (first and second rows).

Early electroencephalographic (EEG) recordings from human subjects showed slow large-amplitude EEG waves when the subjects were closing their eyes and relaxing. These large EEG waves were absent when the subjects opened their eyes (Buzsáki 2006; Nunez and Srinivasan 2006). Indeed we found a difference in the EEG signal between the two states. Figure 1A shows an example of the EEG recorded in the two states (third row), as well as its corresponding root mean square (RMS) values (fourth row) and spectral density (fifth row). In the “eye closed” state the EEG signal was characterized by high-amplitude waves reflected in larger RMS values. We averaged the RMS values in the open/closed states separately for each recording day (Fig. 1B). The difference between the averaged RMS values was highly significant (P < 0.001, Wilcoxon signed-rank test). In addition, the EEG power distribution averaged across days (Fig. 1C) was concentrated in different frequency bands in the two states. These differences in the EEG signal suggest that the distinction between “eye closed” and “eye open” states is indicative of different brain states. We tend to assume that these different brain states reflect different arousal levels. However, since we did not record neck EMG, multiple-contacts EEG, or other physiological parameters required for a definitive assessment of arousal level (Schulz 2008; Silber et al. 2007), we restricted ourselves to the more conservative definition of brain states.

**Neuronal database**

Out of 732 cells recorded, 174 neurons from the GPe, 75 neurons from the GPi, and 102 neurons from M1 met the quality and inclusion criteria and were studied in both states (Table 1; for details see METHODS).

Figure 2 depicts three examples of neurons recorded in the “eye open” (first row) and “eye closed” (second row) states, one for each area (column). The GPe neuron in Fig. 2A showed a decrease in discharge rate in the “eye closed” state (second row) compared with the “eye open” state (first row). This change was accompanied by an increase in the frequency of pauses and by a heavier tail of the ISI distribution (third row). Figure 2B shows an example of a GPi neuron, which like the GPe neuron decreased its discharge rate in the “eye closed” state (second row) and had a higher frequency of long ISIs (third row). Finally, the M1 neuron in Fig. 2C, unlike the two pallidal neurons, increased its discharge rate in the “eye closed” state and exhibited a burstier discharge pattern (compare second row with first row).

To summarize, these illustrative cells exhibited differences in discharge rates and in discharge patterns between the eye open/closed states; in the following text we give a detailed description of the changes found in discharge variables both at the single-cell and at the population level of all neurons recorded. We do not present the cross-correlation functions of the recorded neurons since the typically short duration of each segment in the “eye closed” state (Table 1) did not enable us to reliably assess the changes in the cross-correlation functions of simultaneously recorded pairs of neurons (especially for M1 low-discharge-rate neurons).

**Opposite rate modulations of pallidal and cortical neurons in the transition to the “eye closed” state**

Figure 3 shows the mean discharge rates in the “eye open” and “eye closed” states of all recorded neurons in the three neuronal
populations. High-frequency discharge (HFD) neurons in the GPe and GPi displayed similar rate modulations in the transition from the “eye open” to “eye closed” state. Most of the neurons in the GPe and GPi showed significantly lower mean discharge rates in the “eye closed” state compared with those in the “eye open” state and only a few cells showed the reverse (Fig. 3, A, B, and D). Our pallidal recordings were not restricted to specific areas within the GPe and GPi, but spanned the entire nuclei, and this effect size was uniformly distributed. At the population level the mean discharge rate was also significantly lower in the “eye closed” state in both areas (Table 2 and Fig. 3, D and E).

M1 neurons displayed a less profound change compared with pallidial neurons in their mean discharge rates on closure of the eyes. Moreover, this change was in the opposite direction from the pallidal decrease in discharge rate in the “eye open” state. Most of the neurons in the GPe and GPi showed significantly lower mean discharge rates in the “eye closed” state compared with those in the “eye open” state and only a few cells showed the reverse (Fig. 3, A, B, and D). Our pallidal recordings were not restricted to specific areas within the GPe and GPi, but spanned the entire nuclei, and this effect size was uniformly distributed. At the population level the mean discharge rate was also significantly lower in the “eye closed” state in both areas (Table 2 and Fig. 3, D and E).

M1 neurons displayed a less profound change compared with pallidial neurons in their mean discharge rates on closure of the eyes. Moreover, this change was in the opposite direction from the pallidal decrease in discharge rate at the single-cell level, many M1 neurons showed a significant increase in their mean discharge rate in the transition from the “eye open” to the “eye closed” state, whereas only a few showed a decrease (Fig. 3, C and D). This difference was also significant at the population level (Table 2 and Fig. 3, D and E). M1 neurons showed more diverse discharge rate changes compared with the pallidum (Fig. 4) and thus the difference between the two states, at the population level, was weaker (Fig. 3E). However, at the single-cell level, the relatively small absolute changes in M1 discharge rates constituted a substantial relative change (due to M1 low basic discharge rates). Thus on average M1 neurons increased their firing rates on closure of the eyes by 0.46 spike/s and 61.83%, whereas GPe and GPi neurons decreased their average discharge rates by 8.02 and 8.34 spikes/s and 10.28 and 9.64%, respectively.

To appreciate the time course of the changes in discharge rates we examined the averaged PSTHs aligned at closing and opening of the eye. In both the GPe and GPi the discharge rate changes were gradual, starting (10–25 s) prior to eye closing and continuing after eye opening (Fig. 5, A and B and Supplemental Fig. S1 for continuously recorded neurons). Unlike the pallidal rate modulation, the dynamics of discharge rate changes in M1 neurons was not gradual (Fig. 5C and Supplemental Fig. S1), corresponding to the time course of changes in EEG (Fig. 5D). The analysis performed earlier used a population average; therefore it is possible that individual pallidial cells had abrupt discharge rate transitions (e.g., from high to low firing rates) whose timing differed between cells, thus leading to a population average with a smooth transition. We therefore examined and analyzed (change point analysis; 1 The online version of this article contains supplemental data.)
FIG. 2. Pallidal and M1 cortical neurons have different discharge properties in the “eye open” state compared with the “eye closed” state. A: example of neuronal activity from a single GPe cell from monkey L. Top row: raster in the “eye open” state. Middle row: raster in the “eye closed” state. Each raster contains 15 traces of 5 s each. Traces are not necessarily consecutive. Below each raster is an example of a 1-s analog trace of extracellular recording filtered between 300 and 6,000 Hz. An asterisk marks the trace in the raster from which the analog example is taken. Below the 1-s analog trace are examples of spike waveforms. The spike waveform plot includes 100 superimposed 1.5-ms waveforms selected randomly from the whole recording time of the cell. Last row: histogram of the logarithm of the interspike intervals (ISIs) in the 2 states (bin width 0.1 ms). B: example of neuronal activity from a single GPi cell from monkey L. Same conventions as in A. C: example of neuronal activity from a single cell in the arm related area of the primary motor cortex of monkey W. Same conventions as in A. spike waveform duration = 2 ms. GPe and GPi, external and internal segments of the globus pallidus.
Gallistel et al. (2004) the time course of discharge rate changes at the level of single trials/cells and found that the population mean reflected the single elements in the network (data not shown). However, the strong variability in the duration of single trials did not enable us to reliably assess the time course at the single-cell level and this possible confounding effect should be further examined in future studies.

**Pallidal and cortical neurons have increased discharge variability in the “eye closed” state**

We further looked at the discharge patterns of the neurons in the “eye open” and “eye closed” states using the coefficient of variation (CV) of the ISI histogram. The CV is a measure of the regularity of firing (CV = 1 for a Poisson process, <1 for a more regular process, and >1 for a bursty firing pattern).

In M1, sufficient data (see METHODS) to calculate the CV were available for 88 cells. These M1 cells displayed diverse CV values, ranging from 0.68 to 4.13. Many neurons showed a significant increase in their CV value in the transition from the “eye open” to the “eye closed” state, whereas only a few showed a significant decrease (Fig. 6, C and D). This difference was highly significant at the population level of the CV (Table 3 and Fig. 6, D and E) and implied a more bursty discharge pattern in the “eye closed” state. In addition, the ISI distributions showed a more predominant occurrence of shorter ISIs (<10 ms) in the “eye closed” state (see example of ISI distributions in Fig. 2C, last row) as well as less negative skewness values in accordance with higher discharge rates and more bursty firing patterns (Supplemental Fig. S2).

As in the cortex, in both pallidal areas CV values were significantly larger in the “eye closed” state (Fig. 6, A and B) compared with the “eye open” state both at the single-cell level (Fig. 6D) and at the population level (Table 3 and Fig. 6, D and E). We examined the difference in skewness and kurtosis values of the ISI distributions between the “eye open” and “eye closed” states in each neuronal area. The surprise of a P value of 0.05 is 2.996; note the value for all neuronal areas is far greater.

**TABLE 2. Discharge rate statistics**

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of Cells</th>
<th>“Eye Open”</th>
<th>“Eye Closed”</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPe</td>
<td>174</td>
<td>75.63 ± 24.08 (22.8–143.86)</td>
<td>67.61 ± 22.43*** (18–130.55)</td>
</tr>
<tr>
<td>GPi</td>
<td>75</td>
<td>82.02 ± 23.66 (38.28–156.45)</td>
<td>73.68 ± 21.15*** (34.41–134.45)</td>
</tr>
<tr>
<td>CTX</td>
<td>102</td>
<td>3.18 ± 3.79 (0.1–15.68)</td>
<td>3.65 ± 4.21*** (0.11–18.32)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with ranges in parentheses. Discharge rate statistics were calculated separately for each neural population. ***(P < 0.001, paired t-test. GPe and GPi, external and internal segments of the globus pallidus, respectively; CTX, cortex."

**FIG. 3.** Pallidal and cortical neurons have opposite rate modulations in the “eye closed” state. A: discharge rates (in Hz) of GPe neurons (n = 174). Each point represents the average discharge rate of a single neuron in both states. Abscissa: average discharge rate in the “eye closed” state; ordinate: average discharge rate in the “eye open” state. Black diagonal line is the equality line, i.e., points above this line represent cells for which the discharge rate in the “eye open” state > “eye closed” state. Color code: red is for cells where the discharge rate was significantly higher in the “eye open” state (P < 0.05, bootstrap). Blue is for cells where the discharge rate was significantly lower in the “eye open” state. Gray is for cells where the difference was not significant. Small black arrow points to the GPe cell in Fig. 2A. B: discharge rates of GPi neurons (n = 75). Same conventions as in A. Black arrow points to the GPi cell in Fig. 2B. C: discharge rates of motor cortex neurons (n = 102) in logarithmic scale. Same conventions as in A. Black arrow points to the M1 cell in Fig. 2C. D: summary of percentage of cells with significant differences in discharge rate between “eye open” and “eye closed” states for all 3 neuronal areas. Color code is the same as in A. E: surprise value (minus the logarithm of the statistical P value, Student’s paired t-test) of the differences in discharge rates between “eye open” and “eye closed” states in each neuronal area. The surprise of a P value of 0.05 is 2.996; note the value for all neuronal areas is far greater.

**TABLE 3.** Summary of percentage of cells and implied a more bursty discharger pattern in the “eye closed” state. In addition, the ISI distributions showed a more predominant occurrence of shorter ISIs (<10 ms) in the “eye closed” state (see example of ISI distributions in Fig. 2C, last row) as well as less negative skewness values in accordance with higher discharge rates and more bursty firing patterns (Supplemental Fig. S2). As in the cortex, in both pallidal areas CV values were significantly larger in the “eye closed” state (Fig. 6, A and B) compared with the “eye open” state both at the single-cell level (Fig. 6D) and at the population level (Table 3 and Fig. 6, D and E). We examined the difference in skewness and kurtosis values of the ISI distributions between the “eye open” and “eye closed” states (Supplemental Fig. S2). In the GPe and in the GPi, both parameters were significantly larger (Student’s
compared with that in the GPi, despite their similar discharge
differences between GPe and GPi neurons, where the duration
sion, on the one hand, our "pause" analysis further revealed the
0.0568) according to the "surprise" method (Fig. 7
P
absolute threshold definition, and marginally significant (
5.71 (Table 3). Furthermore, in the GPi skewness values were
not normal (Supplemental Fig. S2). These differences are concor-
dant with the basic "pause" characteristic and heavy-tailed ISI
distributions of the GPe neurons as opposed to the GPi (DeLong
1971; Elias et al. 2007).

We therefore looked at specific parameters of the pauses, in
particular the percentage of the pauses of the total ISIs and
their mean duration in the "eye open" and "eye closed" states.
To identify the pauses we used three different definitions of
pauses: two absolute thresholds that are frequently used as
definitions of GPe pauses (ISIs >300, 500 ms; DeLong 1971) and
an algorithm that maximizes the surprise function to detect
pauses ("surprise" method; Elias et al. 2007). We also intro-
duced a short absolute threshold of 150 ms (to include the long
ISIs of the GPi). In the GPe, according to all four definitions,
the percentage of pauses of the total ISIs was significantly
larger in the "eye closed" state (Fig. 7A). The mean duration of
the pauses was also significantly longer (in three of the four
definitions; Fig. 7B), although the difference was small. In the
GPi the percentage of pauses of the total ISIs was significantly
larger in the "eye closed" state, only according to the 150-ms
absolute threshold definition, and marginally significant (P =
0.0568) according to the "surprise" method (Fig. 7C). The
mean duration of the GPi long ISIs in the "eye open" state did
not differ significantly from the "eye closed" state. In conclu-
sion, on the one hand, our "pause" analysis further revealed the
differences between GPe and GPi neurons, where the duration
of the long silent intervals was much longer in the GPe
compared with that in the GPi, despite their similar discharge
rates (68 and 76 vs. 74 and 82 spikes/s in GPe and GPi,
respectively, in the eye closed/open states, Table 2). On the
other hand, the "pause" analysis suggests that the change in
discharge pattern observed in the transition between the "eye
open" and "eye closed" states in both pallidal segments can be
attributed primarily to the rise in the frequency of pauses (or
long silent intervals) and not to the duration of these pauses.

Finally, we tested the time course of the changes in the pallidal
discharge pattern by examining the dynamics of the changes in
CV values (Fig. 8 and Supplemental Fig. S3). Estimates of CV
values and their dynamics were affected by the size of the analysis
moving window (thus the low discharge rate of M1 neurons did
not enable a reliable estimation of fast changes in their discharge
pattern). The discharge of pallidal cells was characterized by rare
(<1%, Fig. 7) long ISIs. Therefore CV values calculated using
short windows mainly represent common short ISIs, whereas
larger analysis windows enable detection of effects created by the
long ISIs. Changes in the pallidal CV values calculated using a
large analysis window (50 ISIs, ~1 s; Fig. 8) were gradual,
exhibiting kinetics similar to that observed in the discharge rate
modulations (Fig. 5). The changes in the pallidal CV values
calculated using the shorter window size (5 ISIs, Supplemental
Fig. S3), however, were less profound and displayed a steeper
dynamics. These results suggest that changes in the pallidal
discharge pattern might reflect two processes, differentially affecting
the pallidal short and long ISIs. We examined and analyzed
(change point analysis; Gallistel et al. 2004) the time course of
pallidal discharge pattern changes at the single-cell level and
found that the population mean, most probably, reflected the
single elements in the network (data not shown). However, as in
the discharge rate analysis, the strong variability in the single trial
durations did not enable a robust analysis of the time course at the
single-cell level and thus we defer this analysis for the future.

DISCUSSION

Herein we explored the discharge properties of neurons
in the GPe, GPi, and the motor cortex in two brain states:
"eye open" versus "eye closed." We found that the changes
in GPe and GPi discharge properties were positively
correlated. Pallidal neurons showed a gradual decrease in
discharge rate in the "eye closed" state starting prior to
closing of the eyes. In addition, the CV of ISI distribu-
tions of both GPe and GPi neurons increased in the "eye
closed" state. Increased pallidal CV values were associated

FIG. 4. Pallidal and cortical discharge rate modulations in the "eye closed" state as percentage of their "eye open" baseline discharge rates. A: percentage of discharge rate changes of GPe neurons (n = 174). Histogram depicting the average changes in discharge rates between the "eye open" and "eye closed" states as percentage of discharge rate changes in the "eye open" state. Abscissa: percentage of discharge rate change; ordinate: fraction of cells. Gray horizontal line is in zero percentage change. Arrow points to the average percentage change. Note the mass distribution is to the left of the gray line, i.e., most cells have higher discharge rates in the "eye open" state. B: same as A for GPi neurons (n = 75). C: same as A for cortical neurons (n = 102). Most cells are to the right of the gray (zero) line, i.e., most cells have higher discharge rates in the "eye closed" state. The distribution is wider compared with the distributions in A and B (note the different scales of the x-axis).
with a larger fraction of longer ISIs (>150 ms) in the GPi and with a larger fraction of pauses (e.g., ISIs >300 ms) in the GPe. In the motor cortex the changes in discharge properties were less profound and lagged behind the changes observed in the pallidum. Unlike pallidal neurons, cortical neurons increased their discharge rates steeply in the transition to "eye closed" state. These rapid changes in M1 probably reflect distributed cortical activity, since similar kinetics was found in the frontal EEG recording. Finally, as in the pallidum, the CV of ISI distributions of cortical neurons increased in the "eye closed" state. Although assuming that the eye open/closed states reflected transitions in the monkeys' arousal states (as supported by the EEG analysis), we limited our observation to different brain states, since the study was not designed specifically to test for arousal levels.

Cortex and basal ganglia exhibit different dynamics in their discharge rate modulations

Models of the BG commonly emphasize the cortico–BG functional connectivity and characterize the BG as part of a closed loop circuit connecting all cortical areas through the striatum, pallidum, and thalamus with the frontal cortex (Leibois et al. 2006; Rivlin-Etzion et al. 2006). In our study M1 neurons showed increased firing rates and increased irregularity in their spike trains in the "eye closed" state. These changes could be expected to influence the BG network, specifically on the changes observed in the pallidal discharge variables. However, the dynamics of modulations in discharge rates, as observed at the population level, suggest otherwise. The changes not only in cortical discharge rates but also in the amplitude (RMS values) of the EEG waves were steep and followed the pallidal rate modulations. Since our cortical recordings were
restricted to the arm-related area of M1 and our EEG recording was of a single contact we cannot exclude other cortical areas as the source for the observed pallidal changes. However, we can conclude that the (motor) cortex probably did not drive the changes observed in the pallidum. Subcortical closed loops through the BG, which have a thalamic relay on the input rather than on the return link of the circuit (McHaffie et al. 2005), could better explain our results. The striatum and the STN are widely innervated by the intralaminar nuclei of the thalamus (Smith et al. 2004). These afferents presumably carry information on sensory events of behavioral significance and are likely to transmit information regarding arousal states to the BG network (Coizet et al. 2007; Kimura et al. 2004; Smith et al. 2009). Thus the pallidal changes in activity could be the drivers of the cortical discharge changes in the transitions between “eye open” and “eye closed” states (likely reflecting transitions in arousal levels).

**TABLE 3. Discharge pattern statistics**

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of Cells</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>“Eye Open”</td>
</tr>
<tr>
<td>GPe</td>
<td>174</td>
<td>1.73 ± 1.03 (0.22–5.52)</td>
</tr>
<tr>
<td>GPi</td>
<td>75</td>
<td>0.74 ± 0.15 (0.46–1.11)</td>
</tr>
<tr>
<td>CTX</td>
<td>88</td>
<td>1.52 ± 0.50 (0.68–3.77)</td>
</tr>
</tbody>
</table>

Closure of the eyes probably does not significantly affect dopaminergic input to the striatum

According to the classic model of the basal ganglia (Albin et al. 1989) information is transmitted through direct and indirect pathways from the striatum and into the output nuclei of the BG. Dopamine increases the excitability of striatal D1 direct pathway projecting neurons and decreases the excitability of D2 indirect projecting neurons (Gerfen et al. 1990; Surmeier et al. 2007). However, previous studies (Brown et al. 2009; Monti and Monti 2007; Steinfels et al. 1981, 1983; Trulson 1985) and our unpublished observations suggest that there are no major changes in the discharge rate of dopaminergic neurons or striatal cholinergic tonically active interneurons at different arousal levels (although our study was not designed to specifically test for arousal levels). This is in line with our current results, which show that the vast majority of GPe and GPi neurons exhibit similar changes in discharge rate and pattern in the transition to the “eye closed” state, thus pointing to other features of the basal ganglia functional network, rather than a major modification of the dopamine tonus in the striatum.

**Potential source for GPe and GPi changes in activity in the “eye closed” state**

A possible explanation for the similar changes in both pallidal segments could be a common pathway of innervation to both areas. Striatal medium spiny neuron (MSN) inhibitory projections (Tremblay and Filion 1989) can serve as plausible
candidates. In vivo studies have shown that under anesthesia there are fluctuations in the MSN membrane potential between depolarized “up” states and hyperpolarized “down” states (Mahon et al. 2001; Stern et al. 1998; Wilson 1993; Wilson and Kawaguchi 1996). The latter group also reported fluctuations in membrane potential during slow-wave sleep that were correlated with cortical field potentials (Mahon et al. 2006). These results suggest that there are indeed distinct patterns of MSN activity in different brain states. Increased striatal activity in the “eye closed” state (e.g., due to increased thalamic excitation) could explain the modulation observed in both the GPe and GPi. However, recent results (Berke et al. 2004; Mahon

FIG. 7. GPe and GPi neurons exhibit a larger fraction of long silent intervals (“pauses”) in the “eye closed” state. A: average percentage of pauses out of the total ISIs across all GPe cells in the 2 states. Color code: black, “eye open” state; gray, “eye closed” state. Each pair of bars is calculated according to a different definition of pauses: >150, 300, and 500 ms and according to the “surprise” method (denoted as “S”). Significant difference between states (paired t-test): ***P < 0.01, ****P < 0.001. Error bars stand for 95% confidence interval. B: average duration of pauses across all GPe cells in the eye open/closed states. Same conventions as in A. C: same as in A for the GPi population. Note the different scale of the y-axis. Except for ISIs >150 ms, the values are small or absent due to paucity of GPi cells with ISIs fulfilling these definitions. D: same as in B for the GPi population.

FIG. 8. Time course of the changes in pallidal discharge pattern in the transition between “eye open” and “eye closed” states. A: GPe population CV time course aligned at eye closing (time 0, left column) and at eye opening (time 0, right column). CV values were computed on a moving window of 50 ISIs, with an overlap of 40 ISIs, and were averaged across cells (N is the number of cells averaged). Abscissa: time in seconds; ordinate: CV values. Error bars are SE values. B: same as in A for the GPi population.
et al. 2006) and our unpublished observations do not show clear changes in striatal projection neurons’ discharge rates associated with different arousal levels.

Another candidate for a common pathway that innervates both segments of the pallidum could be STN excitatory projections (Hazrati and Parent 1992; Shink and Smith 1995). Evidence from both animal and human patient studies suggests that STN activity is affected by anesthesia and by the sleep–wake cycle (Magill et al. 2000; Stefani et al. 2006; Urbain et al. 2000; Urestarazu et al. 2009). In anesthetized animals, the discharge properties of STN neurons were found to be strictly related to cortical activity and thus to the sleep–wake cycle (Magill et al. 2000). In addition, evidence from human patients undergoing deep brain stimulation surgery points toward reduced spontaneous activity of STN neurons when the patients’ arousal levels are reduced (Stefani et al. 2006). In fact, blockage of STN neuronal activity has been shown to produce decreased discharge rates and increased frequency of long ISIs in both pallidal segments (Hamada and DeLong 1992; Nambu et al. 2000). However, the mechanism behind this reduction in STN discharge on reduced arousal levels is not clear. One candidate is the cortical drive to the STN. The STN discharge is strongly affected by cortical excitatory inputs (Nambu et al. 2002b); however, the direction and dynamics of the cortical discharge rate modulation in our M1 cortical (and EEG) recordings do not support this reasoning. On the other hand, the STN is innervated by subcortical regions such as the mesopontine tegmentum (Bevan and Bolam 1995) and the intralaminar nuclei of the thalamus (Orieux et al. 2000; Smith et al. 2004), which may have led to decreased firing rates in the STN and in the pallidum.

Finally, another factor that could explain the similar changes in both pallidal segments relates to the intrinsic circuitry of the BG. Recent studies have shown a more complex organization of the internal network of connections than originally thought, with closed feedback loops between the GPe, striatum, and STN, as well as other levels of the basal ganglia–cortical circuits (Bolam et al. 2000; Leblois et al. 2006).

Concluding remarks

The classic view of the cortico–BG network is based on the concept of D1/direct and D2/indirect pathways and predicts a reciprocal relationship between GPe and GPi. However, anatomical studies have revealed a more complex network of connection whereby the BG network is characterized by a closed-loop architecture at many different levels. In addition, previous physiological studies conducted on behaving animals have shown that GPe and GPi neurons display similar transient responses to behavioral and motor events. Our study extends these findings and describes the spontaneous activity of pallidal neurons in two distinct brain states. We observed similar changes in GPe and GPi firing rates, strengthening the evidence against GPe–GPi anatomical reciprocity and revealing that the functional efficacy of the GPe to GPi inhibitory connections is weak. The minimal changes in discharge rates of basal ganglia neuromodulators under different arousal states enable striatal and/or STN common inputs to similarly modify GPe and GPi firing rates. In addition, we observed early changes in pallidal compared with cortical activity on the transition from the “eye open” to the “eye closed” state. These unexpected early changes in the pallidum are better explained by a subcortical rather than a cortical loop through the BG. Nevertheless, the efficacy of the projections between two neural structures is not affected solely by the discharge rates of the neurons under study. Depending on the intrinsic properties of spatial and temporal summation, as well as on the convergence/divergence properties of the network, firing patterns, and neural synchronization may modify the efficacy of neural transmission. Future studies of the cortex–basal ganglia network may shed light on these intriguing questions.

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Arousal modulation of pallidal discharge


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Results chapter number IV.

Different driving modes of striatal cholinergic and GABAergic interneurons.

Results presented in this chapter have not been published yet in the scientific literature.
Introduction

The striatum is the primary input stage of the Basal Ganglia (BG) nuclei. Its medium spiny projection neurons (MSNs) constitute up to 95% of striatal cells (depending on the species, Tepper et al., 2008). However, their activity and hence striatal output is highly affected by the proportionally small population of a-spiny interneurons (Kawaguchi et al., 1995; Kreitzer, 2009). Two major groups of interneurons, which have well-characterized electrophysiological properties and have been extensively studied, are the fast spiking parvalbumin (PV) expressing GABAergic interneurons (FSIs) and the tonically firing large cholinergic interneurons (TANs, Kawaguchi et al., 1995).

The in-vivo activity of TANs has been amply investigated in behaving primates. In associative learning paradigms these cells were shown to pause their tonic firing for a few hundred milliseconds in response to external stimuli that become associated with rewarding (and aversive) outcomes (Apicella, 2002; Joshua et al., 2008). The TAN characteristic pause response was related to the motivational significance of external salient stimuli (Apicella, 2002; Goldberg and Reynolds, 2011) and was thought to originate from thalamic input (Matsumoto et al., 2001; Nanda et al., 2009; Ding et al., 2010; Schulz et al., 2011). The in-vivo extracellular activity of FSIs, on the other hand, has been described mainly in rodents. FSIs were shown to display robust task-related responses in operant conditioning paradigms (Berke, 2011). However, although FSIs are coupled by gap junctions (Kita et al., 1990; Koos and Tepper, 1999), their activity was highly individualized (Berke, 2008; Schmitzer-Torbert and Redish, 2008). FSIs were found to display high sensitivity to cortical input and probably integrate information from diverse cortical areas (Parthasarathy and Graybiel, 1997).

GABAergic FSIs were suggested to provide strong feed-forward inhibition that shape the firing patterns of MSNs (Planert et al., 2010; Gittis et al., 2010). They form powerful perisomatic synapses onto MSNs, as their firing can delay or even block spike generation in spiny cells (Tepper et al., 2008). Nonetheless, recent findings show that this inhibition did not functionally control the precise spike timing of MSNs (Gage et al., 2010). Cholinergic TANs, on the other hand, cannot be simply characterized as having an excitatory or inhibitory effect on MSN activity, but rather have a global modulatory effect (Oldenburg and Ding, 2011).
Both interneuron cell types have been shown to be imperative to normal striatal functioning (Pisani et al., 2007; Gittis et al., 2011). It is therefore important to define the functional relationship between their activity and that of the MSNs that mediate striatal output. We recorded and analyzed the simultaneous spiking activity of MSN–TAN or MSN–FSI pairs and used cross-correlation methods to identify direct synaptic interactions and/or common input drives.

Methods
Two monkeys (Macaque fascicularis, G male, 4.5 kg; L female, 3 kg) were used in this study. Experimental protocols were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and the Hebrew University guidelines for the use and care of laboratory animals in research. The experimental protocols were approved and supervised by the Institutional Animal Care and Use Committee (IACUC) of the Hebrew University and Hadassah Medical Center. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) internationally accredited institute. Behavioral paradigm, surgery procedures, data-recording and analysis methods were described in previous manuscripts (Adler et al., 2012a; Adler et al., 2012b). Here we describe the methods not used previously.

Classification of extracellularly recorded striatal neurons
We used two criteria to distinguish between striatal cell types: cells' average firing rate and extracellular spike waveform duration from first negative peak to the following positive peak (Fig 1a, b). Cells with waveform durations of 0.9-2.5ms and average firing rates <4Hz were classified as presumed MSNs. Cells with waveform durations >2.5ms and average firing rates of 3-15Hz were classified as TANs. Finally cells with waveform durations <0.9ms and average firing rates >4Hz were classified as FSIs. Remaining cells that did not strictly belong to the above groups were discarded and are not reported here. Additional classifications using valley width at half maximum of the spike waveform and coefficient of variation (CV) of the inter spike interval (ISI) received similar identification (data not shown).
Data analysis of single cell responses

Neural responses to behavioral events were characterized by post stimulus time histogram (PSTH) starting at cue presentation and ending 2s after outcome delivery. PSTHs were calculated in 1ms bins and smoothed with a Gaussian window (SD of 20ms). The baseline firing rate was calculated by averaging the firing rate in the last 3s of the ITI and was subtracted from the smoothed PSTH. To determine a significant response in a single PSTH, we calculated the SD of the PSTH of the last 3s of the ITI using the same number of trials as in the studied PSTH and identified time segments in which the deviation from the baseline firing rate exceeded three times the ITI-SD. A response was considered significant only if the duration of the deviant segment was >60ms (three times the SD of the smoothing filter).

Data analysis of cell pairs

Signal correlation (Fig. 2) was calculated as described previously (Joshua et al., 2009; Adler et al., 2012a) between all cell pairs within each population. Spike to spike correlation (Fig. 3) between simultaneously recorded MSN-TAN or MSN-FSI pairs was determined using cross-correlation histograms (CCHs, Perkel et al., 1967). CCHs were computed with 1ms bins for ±2s around the trigger spike and were smoothed using a Gaussian (SD of 10ms). Only cell pairs with minimal isolation quality (>0.7, Joshua et al., 2007) and rate stability that were recorded simultaneously for more than 21 and 30 minutes (monkey L and G respectively) were included in the database. CCHs were computed separately for each task event (and averaged to provide the raw CCH). Raw CCHs were normalized (corrected for common modulation of discharge rate) using both PSTH and shift predictors, which yielded similar results. Only PSTH correction method is presented here. To determine a significant peak/trough in a single CCH we calculated the SD of the last 0.5s in both negative and positive lags of the CCH and identified segments in which the CCH (±1.5s around zero) exceeded three times the SD. A CCH was considered to have a significant peak/trough only if the duration of the deviant segment was >30ms (three times the SD of the smoothing filter). We used additional methods (Abeles, 1982) to determine the significance of the CCH and obtained similar results (data not shown). To determine the skewness of a significant CCH we calculated a symmetry index. This index is found by subtracting the number of significant bins in the negative lag of the CCH from those in the positive lag divided by their sum.
Results

Striatal cell classification and identification

Striatal neuronal activity was recorded from two monkeys engaged in a classical conditioning task. The task involved presentation of visual images (cues) predicting either food outcome in rewarding trials, air puff in aversive trials or neither in neutral trials (Adler et al., 2012a; Adler et al., 2012b). Recordings were made from two to eight electrodes simultaneously in all striatal domains (anterior caudate, putamen and ventral striatum). To test whether and to what extent the activity of striatal interneurons was related to that of striatal projection neurons, we first classified striatal cells into three distinct groups. We separated the signals of single cells recorded extracellularly based on their waveform profiles and their average firing rates (Fig. 1). Of the 1330 neurons that passed our inclusion criterion, 777 were classified as striatal projection neurons (MSNs), 283 were classified as TANs (presumably striatal cholinergic neurons) and 36 as FSIs. As reported previously (Berke et al., 2004; Berke, 2008), the FSIs had the narrowest spike waveform lengths and the fastest average firing rates. TANs had the widest spike waveform lengths and intermediate firing rates. Finally, MSNs displayed an intermediate waveform length and the slowest firing rates. TANs had the lowest values of CV of their ISIs with a very narrow distribution; the CV of the MSNs ISIs was larger and broadly distributed, and the distribution of FSIs CV was intermediate in values and variance (Fig. 1c).

Figure 1: Striatal cells are clustered into three groups
a) Separation of striatal neuron subtypes. Each dot represents a single neuron colored according to its classification. Red, MSN; blue, TAN; green, FSI.
Distinct population activity patterns of striatal cell sub-classes

Cells in all three sub-populations were highly modulated by the task, particularly to cue presentation (Fig. 2, left column). More than 93% (for all three populations) responded to at least one of the task events (Fig. 2, second column). However, across striatal sub-populations, the cells displayed distinct response profiles. MSNs (Fig. 2a) typically responded with an increase in discharge rate to the visual cues, which started on average 547.22±9.82ms after cue presentation. As reported previously, MSNs displayed highly diverse responses characterized by a symmetrical signal correlation distribution (average signal correlation ± SEM; 0.004±0.0003) and an average sustained response. Unlike MSNs, TANs (Fig. 2b) responded with a very stereotyped and synchronized (average signal correlation ± SEM; 0.12±0.0008) pause and rebound excitation response to cue presentation, which was very sharp and immediate (average ± SEM onset to pause response: 153.17±3.86ms, to excitation: 334.92±4.9ms). FSIs (Fig. 2c) like MSNs, responded mostly with an increase in discharge rates to cue presentation. However this response was more immediate compared to the MSN response (average ± SEM FSI onset time: 251.72±27.45ms, one-way ANOVA, p<0.05; MSN response onset time was different from that of TAN and FSI). In terms of similarity of the neural responses, FSIs were not as diverse as the MSNs (average signal correlation ± SEM; 0.06±0.009). However, they also did not display the highly synchronized activity pattern of TANs that is characteristic of BG neuromodulator groups (Joshua et al., 2009). A one-way ANOVA revealed that the distribution of FSI-FSI signal correlation was different (p<0.05) from that of MSN-MSN and TAN-TAN pairs. To sum up, all three neural populations were highly modulated by the task; however, they differed considerably in their response profile and response synchronization levels.
Figure 2: Striatal MSNs, TANs and FSIs display different response profiles

a) MSN response profile. Left subplot: average response ± SEM (solid line and envelope) to cue presentation (zero seconds) and outcome delivery (two seconds). Ordinate: firing rate in Hz normalized by the ITI discharge rate. Blue, reward events; red, aversive events; green, neutral events. N is for number of cells. Second subplot: distribution of MSNs that had a significant response. Blue, red and green bars: fraction of cells that had a significant response for reward, aversive and neutral events respectively. Black bar: fraction of cells that had a significant response to at least one of the task events. Third subplot: distribution of response onset. Abscissa: time in seconds for significant increase in firing rate. Red line marks the average response onset time. Right subplot: distribution of the signal correlation between all (simultaneously and non-simultaneously recorded) MSN pairs. N is for number of pairs.

b) TAN response profile. Same conventions as in a. In subplot 3: distribution of response onset, left and right columns: latency of significant decrease and increase in firing rate respectively.

c) FSI response profile. Same conventions as in a.

MSNs are differentially correlated with striatal interneurons

Figure 3a and b displays the raw and corrected average cross correlation histograms (CCHs) between striatal MSN-TAN and MSN-FSI pairs (left and right columns
respectively). The CCHs between simultaneously recorded pairs of TANs and MSNs were typically flat. Indeed we found that all (N=379 pairs) but three MSN-TAN pairs were not significantly correlated. We further calculated the average number of added spikes of the reference cell (TAN) to the trigger cell (MSN) around the corrected CCH time window of ±1.5s. A negative value would indicate that whenever the trigger cell spiked, the reference cell was more likely to suppress its discharge, a positive value would indicate the opposite, and zero would imply there was no correlation between the two. As predicted by the average flat CCH, we found the distribution of added spikes for the MSN-TAN pairs to be symmetrical around zero and not significantly different from zero (Z-test, p=0.7, Figure 3c).

Unlike the flat CCHs of MSN-TAN pairs, the MSN-FSI pairs were highly correlated. The average raw CCH of all MSN-FSI pairs (N=66 pairs, Figure 3a right) displayed a very broad positive and asymmetrical peak. Even after normalizing the raw CCHs (Figure 3b) by a PSTH predictor (to compensate for the effects of similar responses, Fig. 2) a broad positive peak remained. Most of the MSN-FSI pairs that displayed a significant CCH (N=29 pairs) had a positive peak (N=24 pairs) and only five had a negative trough. This is evident both in the average CCH (Figure 3a, b) and in the positively skewed distribution of the CCH number of added spikes (Figure 3c, significantly different from zero, Z-test, p<0.05). Finally, most MSN-FSI pairs with a significant CCH depicted an asymmetrical histogram where the peak of the histogram was shifted towards negative values. This implies that the spikes of the trigger cell (MSN) followed those of the reference cell (FSI). We quantified the asymmetry (in the CCHs with significant positive peaks) using a symmetry index (see Methods). Most (20/24) MSN-FSI pairs had a negative symmetry index with an average of -0.31±0.1 (mean±SEM, calculated over both positive and negative indices) indicating a CCH peak that is shifted to the left.
Figure 3: MSNs are differentially correlated with striatal interneurons

a) Raw (with no normalization) cross correlation histograms (CCH) between pairs of striatal interneurons and MSNs averaged over all pairs. Abscissa: time in seconds. The FSI and TAN (trigger cell) discharge is at time zero. Ordinate: conditional firing rate of the MSN (reference cell), given a spike of the interneuron at time zero. Left subplot: TAN-MSN. Right subplot: FSI-MSN. N stands for the number of pairs.

b) Normalized CCH (using the PSTH predictor) averaged over all interneurons to MSN pairs. Same conventions as in a.

c) Distribution of the average number of added spikes of the reference cell in the corrected CCHs around the time window of ±1.5s. Abscissa: number of added spikes. Ordinate: ratio of pairs (note the different y-scales).
Discussion
We simultaneously recorded the spiking activity of striatal projection neurons (MSNs) and interneurons (TANs or FSIs) from monkeys engaged in a classical conditioning task involving rewarding, aversive and neutral cues. All cell types were highly responsive to the behavioral events. However, while the synchronized group of TANs was not correlated with MSNs, the FSIs displayed a broad and asymmetrical peak in their CCH with the MSNs.

MSNs and FSIs are activated by a common input
FSIs project heavily onto MSNs. They are highly sensitive to cortical input and display shorter response latencies than MSNs and were therefore suggested to mediate striatal feed-forward inhibition (Tepper et al., 2008). Our response onset measurements (Fig 2, third column) extend this observation to behaving primates as well. In-vivo studies have demonstrated that single FSI spikes can delay or abolish MSN spikes (Koos and Tepper, 1999;Planert et al., 2010). Furthermore, the FSIs are coupled by gap junctions (Kita et al., 1990;Koos and Tepper, 1999). Together, these properties were interpreted as suggesting that FSIs synchronously inhibit MSNs. However, in line with recent publications (Berke, 2008;Gage et al., 2010), we found that in the primate, the FSI population did not respond similarly to behavioral events. Furthermore, we could not detect narrow troughs in the CCHs. Thus, FSIs did not display robust mono-synaptic inhibition of the MSNs. This is also consistent with theoretical studies indicating that FSI gap junctions have moderate synchronization effects on the FSI network (Hjorth et al., 2009) and with in-vitro studies which reveal FSI-MSN connections show substantial depression during continuous discharge (Klaus et al., 2011). Alternatively, the MSN-FSI CCHs displayed an asymmetrical broad peak which likely originated from a common input to both cell types. It remains to be determined whether the cortical projection to the FSIs is distinct to a certain extent from other cortico-striatal projections (Berke, 2011). Our data suggest that proximal MSNs and FSIs receive similar cortical input. This result is congruent with the concept that FSI feed-forward inhibition expands the dynamical range of afferent input to which the MSNs can respond (Pouille et al., 2009;Gittis et al., 2010). For FSI feed-forward inhibition to regulate the MSN activity dynamic range, FSIs must spike prior to the MSNs in response to afferent stimulation. The asymmetrical CCHs found in our study meet this requirement and thus support arguments for a faster cortical
activation of the FSIs. Therefore, consistent with growing evidence (Berke, 2011; Gittis et al., 2011), we suggest that FSIs have a complex and detailed role in modulating MSN activity rather than broad and nonspecific inhibition.

**Striatal MSNs and TANs are not correlated**

Striatal TANs constitute a very small percentage of striatal cells (Aosaki et al., 1995). Nonetheless, they should have a significant influence over striatal activity via their widespread axonal field that forms muscarinic synapses onto MSNs (Bolam et al., 1984; Bonsi et al., 2011). Thus, anti-cholinergic agents were the first effective pharmacological treatment for Parkinson's disease, and their significant role in the pathophysiology of BG related disorders was highlighted by the dopamine-acetylcholine balance hypothesis (Calabresi et al., 2006; Aosaki et al., 2010). Acetylcholine secreted by TANs can affect MSNs directly by changing the cells' excitability (Kreitzer, 2009; Goldberg et al., 2012) or indirectly by altering the dopaminergic input to the striatum (Threlfell et al., 2012). However, striatal TANs, (like midbrain dopaminergic neurons) probably have widespread influence via volume conductance, rather than by targeted synaptic effects. Thus, they can modulate (in conjunction with the dopaminergic and other modulators of the striatum) the efficacy of the cortico and thalamo-striatal synapses, rather than directly affecting their target neurons' ongoing discharge (Kreitzer, 2009; Higley et al., 2011).

Consistent with this reasoning, we did not find any correlation between the TANs' spiking activity and that of the MSNs, in line with previous primate studies of TAN-MSN (Kimura et al., 2003) and TAN-globus pallidus correlations (Raz et al., 2001). Similarly, the lack of TAN-MSN correlation is coherent with a recent optogenetics study which found that cholinergic interneurons did not directly regulate the firing of MSNs (English et al., 2012).

**Concluding remarks**

Here we presented a differential functional relationship between MSNs and two types of striatal interneurons: TANs, the presumably cholinergic interneurons, and FSIs, the presumably PV expressing GABAergic interneurons. We did not find evidence for direct monosynaptic interactions between the MSNs and either cell type. However, the flat CCHs of MSN-TAN pairs contrasted with the asymmetric broadly peaked CCHs of MSN-FSI pairs. This suggests that the two interneuron populations play a
different role in modulating MSN activity and striatal information processing. Whereas the highly synchronized TANs are likely to have a widespread influence via volume conductance, the less synchronized FSIs appear to be more involved in spatially constrained feed-forward information processing in the striatal network.

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Discussion

In this thesis I studied information processing in the different basal ganglia (BG) sub regions. Specifically I examined the ways in which appetitive and aversive behavioral events are encoded in the BG and translated into the observed response. The results of my thesis are summarized in a series of peer-reviewed journal manuscripts and in an additional chapter of yet unpublished results. The main findings of these results are discussed below.

In the first chapter of the results (Adler et al., 2013) I compared the encoding of well-learned rewarding, aversive and neutral cues across striatal sub regions. I found that neurons in the different striatal sub regions displayed similar response profiles in terms of firing rate modulation. However, their correlation structure was profoundly different. My major finding is the increased correlation between pairs of MSNs recorded in the putamen while the monkeys were engaged in a well-practiced, possibly habitual task. This increased correlation unequivocally contrasted with the closer- to- zero correlation in the caudate and VS. Furthermore, MSN pairs in the putamen displayed different correlation dynamics following the rewarding vs. neutral/aversive predicting cues. The correlation structure of TAN (cholinergic interneuron) pairs supported the results found for the MSN pairs, in that TAN pairs in the putamen displayed stronger correlations than those found in the caudate. These results suggest that the correlation structures of the network represented the different functional roles of striatal sub regions.

In the second chapter of the results (Adler et al., 2012) I compared the encoding of well-learned rewarding, aversive and neutral cues across different BG neural populations. I present three different encoding schemes by putamen MSNs, GPe neurons and putamen TANs. The first two groups are part of the BG actor, whereas the TANs belong to the critic component of the BG network. I showed that in response to cue presentation, both MSNs and GPe neurons displayed an average sustained response. However, the MSNs' average sustained response was composed of three temporally distinct response groups. The GPe neurons' average sustained response, on the other hand, was composed of two response groups which overlapped in time and displayed sustained activity at the single cell level. Finally, the MSNs response groups were dynamic, as different task events were reflected in different
response groups. In comparison to the two projection (actor) neuronal populations, TANs displayed transient activity defined by a single response group. These results support the functional separation between BG main axis (actor) neurons and BG neuromodulators (critic). Furthermore, they support the functional convergence of the MSN response groups onto GPe cells.

In the third chapter of the results (Adler et al., 2010) I describe the changes in discharge properties of cortical (M1) and basal ganglia (GPe and GPi) neurons in two distinct brain states, when the eyes are open vs. when they are closed. The transitions between these two states most likely (as supported by the EEG analysis) reflected a transition between high and low arousal states. I found modulations of discharge rates and discharge patterns in the transitions between the "eye open" and "eye closed" states in all three neural populations. The changes in GPe and GPi discharge properties were positively correlated. In addition, changes in cortical discharge rate followed the pallidal rate modulations. These results challenge the current models of basal ganglia connectivity (predicting reciprocal changes of GPe and GPi discharge and earlier modulation of cortical vs. pallidal activity). They suggest that both GPe and GPi neurons are most strongly driven by their afferents from the input stage of the basal ganglia (striatum and STN), whereas the GPe inhibitory projections to the GPi play a minor role in these physiological states.

Finally, in the last chapter of the results I examined the relationship between BG main axis neurons and neuromodulators. I used cross-correlation methods to identify direct synaptic interactions and/or common input drives between pairs of striatal MSNs and TANs or MSNs and FSIs. I did not find evidence for direct monosynaptic interactions between the MSNs and either cell type. Nevertheless, although the MSN-TAN pairs were not correlated, MSN-FSI pairs were highly correlated. Moreover, the cross-correlation between pairs of MSNs and FSIs were broad and asymmetrical, suggesting that the spikes of MSNs follow those of FSIs. Thus both FSI and MSN are driven by a common, most likely cortical, input; however, the FSI can provide feed-forward inhibition to the MSN network.
Neural correlations support a differential encoding of behavioral events across striatal sub regions

Previous studies have compared the activity in striatal sub regions solely at the level of rate coding by examining single cell discharge rate modulations (e.g. Yamada et al., 2004; Williams and Eskandar, 2006; Ding and Gold, 2012). These and other studies described a shift in the activation of single cells, in which learning of new motor responses and the initial stages of learning activated the caudate, but well-learned motor sequences and advanced stages in training activated the putamen (Miyachi et al., 2002; Yin et al., 2009). Different activity patterns were also found when comparing the dorsal and the ventral striatum (Takahashi et al., 2007). Based on these and other studies (Yin et al., 2004; Yin et al., 2006) striatal sub regions were presumed to have different functional roles. The putamen was suggested to be involved in motor and habitual behavior, the caudate, was associated with cognitive goal directed behavior, and the ventral striatum (VS) related to motivation. As these parallel functional systems can lead to a single behavioral response, they are likely to converge onto the same lower level motor structures (Redgrave et al., 2010). This therefore raises the question as to what processing mechanism enables one striatal system to mediate behavior over the others. My underlying assumption was that neuronal activity should be better correlated with behavior in whichever system produced it. My hypothesis was that synchronization and rate coding as the means for neuronal activity could occur separately or together. I found that most cells in all striatal regions were highly modulated by the task and displayed similar response profiles. However, I found an increase in the neural correlations (in both MSN and TAN pairs) solely in the putamen region. Since overtraining promotes habit formation (Dickinson, 1985; Colwill and Rescorla, 1988), my task involved a more habitual rather than goal directed behavior. Thus the increase in neural correlations in the putamen may have reflected the differences in the functional roles of striatal territories. Furthermore, transfer of information can be more reliably propagated by synchronous neuronal activity than by single cell discharge rate modulations (Bruno and Sakmann, 2006; Bruno, 2011). Therefore, the increased synchronization found between putamen TAN to TAN and MSN to MSN pairs supported the efficient information flow from the putamen neuromodulators to the putamen projection neurons (Threlfell et al., 2012) and from the putamen to its downstream BG structures.
during an over-trained, possibly habituated, task. It thus enabled the putamen functional system to control behavior over the caudate and VS. Two complementary questions remain unanswered: the first is whether the increase in correlation is indeed specific to the task or an innate property of the putamen. The second is whether the increase in correlation represents a general encoding scheme of the striatal network; as such we would expect to find increased correlations in the caudate (and not putamen) on a more goal directed task. I did not find evidence for increased correlation in the putamen during performance of a different learning task (unpublished results). However, these data were restricted and therefore future studies will need to ascertain whether the increase in correlation is indeed specific to the task or an innate property of the putamen and whether a similar synchronization pattern emerges in the caudate or VS under different conditions.

**Convergence (vs. divergence) of information from the striatum onto the GPe**

There is an ongoing debate regarding the interaction of information from multiple sources flowing through the basal ganglia (Bergman et al., 1998). One view argues for convergence (funneling) of information (Percheron et al., 1994) whereas a different view favors segregated parallel circuits with minimal interactions between the parallel pathways (Alexander et al., 1986). According to the first view, neurons in the GPe and output nuclei receive many common inputs. There is considerable anatomical evidence in support of this view. Pallidal neurons are characterized by long and sparsely branched dendritic arborizations. The arborizations are discoidal in shape and are perpendicular to the main bundle of the afferents of striatal axons (Yelnik et al., 1984; Kita and Kitai, 1994). Thus, GPe cells receive input from wide regions of the striatum (Percheron et al., 1984). Furthermore, the strong reduction in the number of neurons from the striatum to the GPe implies extensive convergence or funneling at the pallidal level (Percheron and Filion, 1991; Oorschot, 1996). Physiological findings also support convergence of inputs from remote striatal neurons to a focal area in the pallidum (Kimura et al., 1996). Moreover, single pallidal neurons have been shown to encode both movement direction and reward prediction (Gdowski et al., 2001; Arkadir et al., 2004).
On the other hand, there is anatomical and physiological evidence suggesting that the main circuits passing through the BG remain separate. Studies using retrograde tracing from the pallidum to the cortex show organization of discrete circuits (Hoover and Strick, 1993). In addition there is anatomical evidence of topographic organization in the inputs to pallidal neurons (Chang et al., 1981; Bevan et al., 1997). Physiologically, it has been demonstrated that pallidal neurons receiving inputs from different cortical areas are segregated (Yoshida et al., 1993). Finally, the uncorrelated pallidal activity (Nini et al., 1995) suggests that pallidal neurons do not share many common inputs from the striatum.

I found that pallidal neurons and putamen MSNs both displayed on average a sustained response to the task behavioral events (Adler et al., 2012). However, the average persistent response of the MSNs was composed of three major cell assemblies whose response patterns were temporally differentiated and were sequentially active along the trial. The average persistent response of GPe neurons, on the other hand, was composed of two response groups which overlapped in their time of activation and displayed sustained activity also at the single cell level. These results thus support the funneling model with anatomical convergence of MSNs onto the GPe and point to functional convergence as well.

**Not all BG critics are the same**

The BG are customarily viewed anatomically and functionally as two related subsystems – the main axis and the neuromodulators which modulate the information processing along the main axis. Most models of the BG suggest they implement a RL algorithm (Houk et al., 1995; Suri and Schultz, 1998). By analogy to the actor-critic architecture of RL models, BG neuromodulators (specifically dopaminergic neurons and TANs) correspond to the critic, and BG main axis neurons (i.e., the cortex-striatum-pallidum-frontal cortex axis neurons) correspond to the actor. We and others have previously demonstrated that BG neuromodulators and main axis neurons responded diversely to significant behavioral events (Joshua et al., 2009a). Midbrain dopaminergic neurons and putamen TANs displayed a very sharp, transient and synchronized response, thus providing the main axis with a single-dimensional teaching message (Schultz, 1998; Bar-Gad et al., 2003; Joshua et al., 2008). On the other hand, neurons in the main axis, specifically in the GPe and BG output structures (GPi and SNr), exhibited diverse activity with an average sustained response that
enabled a large information capacity (Mink, 1996; Bar-Gad et al., 2003; Joshua et al., 2009b). My results strengthen the functional separation between BG neuromodulators and main axis neurons by extending these diverse activity patterns to a similar yet different task and to additional BG populations. In particular, caudate TANs have also exhibited a homogeneous transient response (Adler et al., 2013, chapter IV) and striatal MSNs (actor component) displayed an average sustained response and diverse activity patterns (Adler et al., 2012; Adler et al., 2013, chapter IV). However, midbrain dopaminergic neurons and striatal cholinergic interneurons (TANs) are not the only modulators of striatal activity (e.g. striatal FSIs). Furthermore, to better understand striatal processing it is crucial to define the functional relationship between the activity of the MSNs and that of its modulators. Acetylcholine secreted by TANs can affect MSNs directly by changing the cells' excitability (Kreitzer, 2009; Goldberg et al., 2012) or indirectly by altering the dopaminergic input to the striatum (Threlfell et al., 2012). Therefore, cholinergic TANs cannot be simply characterized as having an excitatory or inhibitory effect on MSN activity, but rather have a global modulatory effect (Oldenburg and Ding, 2011). Consistent with this reasoning, I did not find any correlations between the TANs' spiking activity and that of the MSNs (chapter IV). Striatal TANs, (like midbrain dopaminergic neurons) probably have widespread influence via volume conductance, rather than by targeted synaptic effects. The lack of a MSN-TAN correlation together with the TANs' synchronized response (Adler et al., 2013, chapter IV), suggest that different TANs (like dopaminergic neurons) provide the MSNs with similar encoding of the behavioral events and modulate the efficacy of the cortico and thalamo-striatal synapses, rather than directly affecting the MSN ongoing discharge (Kreitzer, 2009; Higley et al., 2011).

FSIs were found to display high sensitivity to cortical input and probably integrate information from diverse cortical areas (Parthasarathy and Graybiel, 1997). However, although FSIs are coupled by gap junctions (Kita et al., 1990; Koos and Tepper, 1999), studied in rodents have shown that their activity was highly individualized (Berke, 2008; Schmitzer-Torbert and Redish, 2008). I also found that primate FSI activity, unlike that of TANs and dopamine neurons, was uncorrelated and unsynchronized (chapter IV). Furthermore, FSIs form powerful perisomatic synapses onto MSNs, as their firing can delay or even block spike generation in spiny cells (Tepper et al., 2008). GABAergic FSIs may provide strong feed-forward inhibition that shapes the
firing patterns of MSNs (Planert et al., 2010; Gittis et al., 2010). Nonetheless, recent findings show that this inhibition does not functionally control the precise spike timing of MSNs (Gage et al., 2010). In line with these publications I did not find FSIs to display direct mono-synaptic inhibition of the MSNs. However, unlike the flat CCHs of MSN-TAN pairs, the MSN-FSI CCHs displayed an asymmetrical broad peak. These results suggest that proximal MSNs and FSIs receive similar cortical input and that FSI feed-forward inhibition expands the dynamical range of afferent inputs to which the MSNs can respond (Pouille et al., 2009; Gittis et al., 2010). My results suggest that the various BG modulators play a different role in modulating MSN activity and striatal information processing. Whereas the highly synchronized TANs (and possibly dopamine neurons) are likely to have a widespread influence via volume conductance, the less synchronized FSIs appear to be more involved in spatially constrained feed-forward information processing in the striatal network.

**Challenges to classic views of the BG**

**Classic direct and indirect BG model**

According to the classic model of the BG (Albin et al., 1989; DeLong, 1990) information is transmitted through direct and indirect pathways from the striatum and into the output nuclei of the BG. In the direct pathway, striatal neurons project directly to the GPi/SNr, whereas in the indirect pathway they project via the GPe and STN. When the system is activated by the firing of corticostriatal glutamatergic neurons, striatal GABAergic neurons discharge. The release of GABA from striatal MSNs giving rise to the direct pathway leads to inhibition of GPi/SNr (output neurons of the BG) and thus the disinhibition of the targets of the BG. The release of GABA from striatal MSNs giving rise to the indirect pathway leads to the opposite effect. GPe cells are inhibited and thus BG output neurons (GPi/ SNr) increase their firing and inhibit the targets of the BG by two mechanisms. First, the loss of inhibitory input to the excitatory neurons of the STN leads to increased activity and hence increased excitation of the GPi/SNr neurons. The second is a direct disinhibitory effect by the inhibited GPe cells on the GPi neurons (Bolam et al., 2000). Dopamine increases the excitability of striatal D1 direct pathway projecting neurons and decreases the excitability of D2 indirect projecting neurons (Gerfen et al., 1990; Surmeier et al., 2007). Therefore, when the system is activated, this model predicts a reciprocal relationship between GPe and GPi. However, studies have found
that there is a more complex organization of BG pathways than previously suggested. For example, GPe sends feedback innervations to the striatum (Bolam et al., 2000; Tepper et al., 2004), direct pathway MSNs which project to the GPI/SNr send collaterals to the GPe (Levesque and Parent, 2005; Nadjar et al., 2006) and cortical neurons send direct projections to the STN (the "hyper-direct" pathway) indicating that like the striatum, the STN is an input stage of the BG (Feger et al., 1994; Nambu et al., 2002). My results also point to a complex organization of the BG. In the transition between the "eye open" and "eye closed" states I found (Adler et al., 2010) similar changes in GPe and GPi firing rates, strengthening the evidence against GPe-GPi anatomical reciprocity and revealing that the functional efficacy of the GPe to GPi inhibitory connections is weak. A possible explanation for the similar changes in both pallidal segments could be a common pathway of innervations to both areas. Striatal MSNs and/or STN projections could serve as possible candidates. My results do not overturn the classic direct/indirect model of the BG; however they demonstrate the complexity of the network which might appear to be organized differently in different spontaneous, behavioral and pathological states.

**Classic division of the striatum**

Based on connectivity, histology and functional evidence the striatum has been segregated into the dorsal and ventral sub regions (Heimer et al., 1982; Haber, 2003). The dorsal striatum has been further segregated into the dorsolateral (putamen) and dorsomedial (caudate) sub regions. All afferent projections of the striatum terminate in a functional, topographic manner (Parent, 1986). However, there is no cortical, amygdaloid, thalamic, or dopaminergic afferent which definitively distinguishes between dorsal and ventral striatum (Berendse et al., 1992; Bolam et al., 2000). Furthermore, behavioral data indicate similarities between the dorsal and ventral striatal regions and therefore a mediolateral-oriented functional striatal gradient has been proposed (Voorn et al., 2004). I found that the correlation structure of the putamen sub region was different from that of the caudate and VS sub regions. My results therefore sustain a functional dorsolateral vs. medioventral segregation of the striatum, rather than the classical dorsal/ventral distinction.

**Functional organization of the striatum into cell assemblies**

The striatum is a key element in BG anatomy and functionality. It is the primary recipient of dopaminergic innervations of all the BG nuclei (Haber et al., 2011), it is
the locus of experience-dependent synaptic plasticity (Reynolds et al., 2001; Surmeier et al., 2007) and it is the most intensively studied BG nucleus (Surmeier et al., 2011). Despite the abundance of research and the influential segregation of the striatum into functional sub circuits, how information is processed within the striatal network and how this leads to observed behavior is still open to debate. Recently, a growing body of evidence has been put forward to support a functional organization of the striatal network into cell assemblies (Berke et al., 2004; Costa et al., 2006; Carrillo-Reid et al., 2008). These studies suggest the formation of neuronal assemblies, or dynamically changing functional correlations within the striatal MSN network devolved to particular types of computation. The neuronal connections underlying the activity in cell assemblies are presumably acquired through experience-dependent synaptic plasticity and are not hard wired or innate. Theoretically, the striatal network possesses many of the components required to generate this kind of activity (Surmeier et al., 2011). The efficacy of the cortico-striatal synapse undergoes modulations according to Hebbian learning rules (Reynolds and Wickens, 2002). Furthermore, the dense local axon collateral system found between spiny cells (Tepper et al., 2004; Tepper et al., 2007) is necessary to establish synchronized neuronal pools. Third, striatal interneurons create feed-forward networks of connections and appear to be able to synchronize MSN activity (Planert et al., 2010; Gittis et al., 2010). Finally, the striatum is able to sustain autonomous network activity via its interneurons (Berke et al., 2004; Ding et al., 2010; Tepper et al., 2010). However, it is difficult to demonstrate the existence of cell assemblies physiologically in vivo. The functional evidence alluding to its formation can arise indirectly from experience-dependent correlated, synchronized activity of the neurons and/or from co-activation of the neurons in a stereotyped way. In my research I found a functional reorganization of the MSNs during performance of a well-practiced task in a cue (reward vs. aversive and neutral) and location (putamen vs. caudate and VS) specific manner. Specifically, I found that putamen MSN pairs displayed increased correlations following cue presentation that could not be accounted for by the changes in discharge rate (JPSTH analysis, Adler et al., 2013). Furthermore, I found a strong linear relationship in the putamen between the signal and noise correlation measures (Adler et al., 2013). Together these results suggest dynamic changes in the strength of the input to putamen MSNs during over-trained behavior, both from a common source (e.g. dopaminergic, thalamic, cortical) and/or recurrent connections and based on valence.
Additionally, I found putamen MSNs were organized into stereotyped response groups which were sequentially co-activated and changed dynamically along the trial (Adler et al., 2012). My results thus imply a model of functional interconnected striatal sub networks which share common inputs. These support the notion of information processing in the striatum in a dynamic network of cell assemblies. Finally, changes in dopamine levels are associated with alterations in the coordinated activity of neurons in the BG (Goldberg et al., 2002; Goldberg et al., 2004; Costa et al., 2006) and with BG pathology. Therefore studying striatal information processing and synchronicity is crucial to understanding BG function and dysfunction.

**Concluding remarks**

Over the years many different models have attempted to provide a framework for understanding basal ganglia (BG) physiology and pathology. Early models of the BG dealt mainly with the feed-forward network of connections along the BG by describing the direct and indirect pathways in the classic "box- and- arrow" model, or by trying to answer questions of convergence vs. parallel processing of information. Additional anatomical and physiological results led the focus to action selection and reinforcement learning models. However, experimental data keep challenging these models, by demonstrating that BG anatomy, connectivity and functionality are complex. In my Ph.D. I therefore deliberately did not focus on a single element but rather studied different nuclei in the BG, different cell populations and different behavioral states. My results suggest that in an automated habitual state information efficiently converges from synchronized cell assemblies in the putamen to the GPe (and BG downstream structures). Future studies will help reveal whether similar activity patterns emerge in the caudate and/or VS under additional conditions.
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The task. The measurement of the activity of the cells in the GPe was comprised of two activity response groups which overlapped during their activity and which showed sustained activity at the single cell level.

Results support a functional convergence, not just an anatomical one, of the response clusters from the striatum to the cells in the GPe. Finally, the anatomical models of the basal ganglia predict reciprocal firing nature between the GPe and GPi, but physiological research indicates a similarity in the transient responses of neurons in those nuclei which calls into question those conclusions. I found that neurons in both populations of the pallidum (GPe and GPi) showed a decrease in firing rate in the situation of "eyes closed" accompanied by high CV values of firing time intervals (ISI).

Moreover, the changes in firing pattern in both nuclei were systematic and began before closing the eyes. Therefore, changes in firing pattern in the GPe and GPi were compatible.

All results suggest that in a well-regulated state, the basal ganglia organize themselves into clusters of cells which are connected to each other in the putamen and encode the ongoing behavior and thus ensure a smooth transition to the extended areas of the basal ganglia.
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