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Changes in activity of fast-spiking interneurons of the monkey striatum during reaching at a visual target

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Running Head: Striatal FSIs and the control of movement
Abstract

Recent works highlight the importance of local inhibitory interneurons in regulating the function of the striatum. In particular, fast-spiking interneurons (FSIs), which likely correspond to a subgroup of GABAergic interneurons, have been involved in the control of movement by exerting strong inhibition on striatal output pathways. However, little is known about the exact contribution of these presumed interneurons in movement preparation, initiation, and execution. We recorded the activity of FSIs in the striatum of monkeys as they performed reaching movements to a visual target under two task conditions: one in which the movement target was presented at unsignaled left or right locations, and another in which advance information about target location was available, thus allowing monkeys to react faster. Modulations of FSI activity around the initiation of movement (53% of 55 neurons) consisted mostly of increases reaching maximal firing immediately before or, less frequently, after movement onset. Another subset of FSIs showed decreases in activity during movement execution. Rarely did movement-related changes in FSI firing depend on response direction and movement speed. Modulations of FSI activity occurring relatively early in relation to movement initiation were more influenced by the preparation for movement, compared with those occurring later. Conversely, FSI activity remained unaffected as monkeys were preparing a movement toward a specific location and instead moved to the opposite direction when the trigger occurred. These results provide evidence that changes in activity of presumed GABAergic interneurons of the primate striatum could make distinct contributions to processes involved in movement generation.
New & Noteworthy

We explored the functional contributions of striatal fast-spiking interneurons (FSIs), presumed GABAergic interneurons, to distinct steps of movement generation in monkeys performing a reaching task. The activity of individual FSIs was modulated before and during the movement, consisting mostly of increased in firing rates. Changes in activity also occurred during movement preparation. We interpret this variety of modulation types at different moments of task performance as reflecting differential FSI control over distinct phases of movement.

Keywords
basal ganglia, movement initiation, motor preparation, non-human primate
Introduction

The striatum is composed of a vast majority of projection neurons and several types of interneuron among which GABAergic interneurons constitute a diverse group of cells that can be characterized by expression of different neurochemical markers and basic electrophysiological properties (Tepper et al. 2010; Silberberg and Bolam 2015). These interneurons have been implicated in regulating the information processing within the striatal network. Among them, the fast-spiking inhibitory interneurons (FSIs), that correspond to the parvalbumin-containing group of GABAergic interneurons (Kawaguchi 1993; Mallet et al. 2005; Sharott et al. 2012), have received the most interest. This electrophysiologically identifiable cell type is regarded as the main source of inhibition onto neighboring output neurons, thus shaping the output of the striatum to the downstream nuclei of the basal ganglia (Koós and Tepper 1999; Mallet et al. 2005). Given that these presumed interneurons exert a strong inhibitory effect on the activity of output neurons, it has been suggested that disruption of FSI function can result in the inability to control or inhibit inappropriate motor responses. Experimental studies in animals provide evidence for specific involvement of these interneurons in striatal-based movement disorders, including dystonia (Gernert et al. 2000) and dyskinesia (Gittis et al. 2011). In addition, neuropathological studies in humans have demonstrated reduced density of striatal GABAergic interneurons containing parvalbumin in Tourette syndrome (Kalanithi et al. 2005; Kataoka et al. 2010) and Huntington's disease (Reiner et al. 2013). It is therefore crucial to understand how FSIs contribute to normal behavioral states to gain an insight into their role in the pathophysiology of movement disorders.

Single-neuron recording studies in behaving rats have investigated how striatal FSIs modulate their firing rate during performance in a variety of tasks. The results have shown
different patterns of task-related changes in FSI activity occurring at specific times, such as reaching a goal in the context of spatial navigation tasks (Berke et al. 2004; 2008; Schmitzer-Torbert and Redish 2008) and selecting a particular action in the presence of a choice between movement options (Gage et al. 2010). FSIs have also been shown to be sensitive to prediction errors linked to changes in an action’s expected value (Stalnaker et al. 2012) and to expectation and receipt of rewards (Lansink et al. 2010). It therefore appears that FSIs might make contributions to different aspects of task performance and information processing. In addition, regional differences in the striatum are likely to be important for interpreting task-related changes in FSI activity, with modulations related to actions resulting in rewarded outcomes preferentially occurring in the dorsolateral striatum, assumed to subserve motor functions (Berke 2008; Schmitzer-Torbert and Redish 2008; Gage et al. 2010), whereas changes in FSI activity related to reward outcomes are assumed to be found in dorsomedial (Stalnaker et al. 2012) and ventromedial portions of the striatum (Lansink et al. 2010) which have been proposed to be more involved in cognitive and motivational functions, respectively.

In the primate striatum, electrophysiologically identifiable cell types generally fall into two categories, called phasically active neurons (PANs), assumed to be projection neurons, and tonically active neurons (TANs), thought to be cholinergic interneurons. Recently, recording studies have pointed to the presence of a rarely encountered category displaying spike waveforms and firing rates similar to those described for striatal FSIs in rodents (Adler et al. 2013; Yamada et al. 2016). Knowledge about the functional contributions of FSIs in the striatum of behaving monkeys is however still limited. In a Pavlovian conditioning procedure, in which monkeys were passively exposed to pairings of visual stimuli with different outcomes, striatal FSIs have been reported to display changes in activity consisting of increases in firing rate after presentation of conditioned stimuli and occurrence of outcome (Adler et al. 2013). More recently, a study has emphasized that FSIs are sensitive both to the
actions resulting in rewarded outcomes as well as to the outcomes themselves in monkeys performing arm or eye movement to obtain rewards (Yamada et al. 2016). However, the exact nature of their contribution to behavioral processes carried out in the striatum remains obscure and we still lack an understanding of how this population of presumed GABAergic interneurons participate in distinct steps of movement generation.

The purpose of this study was to further characterize the functional properties of striatal FSIs by examining their capacity to carry signals related to the preparation, initiation, and execution of target-directed arm movements. Our results indicate that individual FSIs that were not restricted to the motor part of the striatum changed their firing rate around the time of movement initiation, with distinct groups that either increase or decrease their activity, suggesting that they may exert differential control over distinct phases of movement.

Materials and Methods

Behavioral conditions.
The experimental protocol was in compliance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and approved by the Comité d'éthique en Neurosciences INT-Marseille (Protocol A2-10-12). Two adult male rhesus monkeys (Macaca mulatta), F and T, were trained to make visually guided arm-reaching movements to obtain a liquid reward. The behavioral apparatus was similar to that previously described (Deffains et al. 2010). Each monkey was seated in a specially designed restraining box, facing a panel 30 cm from its head. The panel contained two metal knobs (10 x 10 mm) separated by 20 cm horizontally, as well as two light-emitting diodes (two-color green/red LEDs), one above each knob, at eye level of the animal. An unmovable metal bar was mounted at the center of the panel at waist level. The temporal sequence of task events is schematically presented in Figure 1A. The animals kept the hand on the bar until the illumination of a red LED either to
the left or to the right of the panel, the location of the stimulus varying pseudorandomly across trials. In response to this stimulus, monkeys had to reach the target positioned just below the illuminated LED and the movements were rewarded by a small amount of fruit juice (0.3 ml) delivered immediately after touching the target. The monkey then brought the hand back on the bar and waited for the total duration of the trial (6 s) to elapse before the next trial started.

In the standard reaching task condition, called the *uncued condition*, the trigger stimulus occurred at irregular time intervals and in the absence of any predictive cues, so that the monkey could not predict the location of the target on each trial. In order to address the question whether the state of preparation for movement influences neuronal activity, a *cued condition* was also employed in which the monkeys received advance information about the location of the trigger stimulus. In this case, the onset of a green light (duration: 500 ms) presented either to the left or to the right served as a cue for the trigger stimulus which came on 1.5 s later at the same location. In this condition, the cue allowed the animal to prepare arm movement toward a specific location. In another version of the cued condition, called the *unreliable cued condition*, advance information about target location was valid in 80% and invalid in 20% of trials. On valid cued trials, the trigger stimulus was presented at the same location as that indicated previously by the cue, whereas on invalid cued trials, the trigger stimulus was presented on the side opposite to that indicated by the cue. In the latter case, a motor reprogramming process is needed after trigger onset before the correct movement can be executed. This condition was designed to test the monkeys’ ability to inhibit the prepared response and replace it with the other one. Task performance was assessed in terms of reaction time (RT) and movement time (MT) which were the time taken to release the bar in response to trigger onset and to move from the bar to a target, respectively. Both monkeys performed the task with their right arm and the different conditions were presented to the
animals in successive blocks of 30-50 trials, except for the unreliable cued condition which
was run in blocks long enough (about 80 trials) to ensure at least 15 invalid cued trials.

Neuronal recordings.

Data were collected after monkeys were well trained on the three task conditions. After
completion of training, surgery was carried out under gas anesthesia (isoflurane 2.5%) and
sterile conditions to implant a head-holding device and a recording chamber aimed to the left
striatum. We recorded the extracellular activity of single neurons with movable glass-
insulated tungsten microelectrodes over a wide area of the anterior and posterior regions of
the striatum, mostly in the dorsal part of the putamen, some penetrations extending to the
ventral most part of this nucleus. The electrode was driven by a hydraulic microdrive (MO-
95; Narishige, Tokyo, Japan) through a stainless steel guide tube, which was used to penetrate
the dura. The electrode was manually advanced until the spikes generated by a single neuron
were isolated. Neuronal signals were amplified (x 5000), filtered with a band pass of 0.3 to
1.5 kHz, and monitored with oscilloscopes. Single neuron activity was detected on-line by
threshold crossing using a time-amplitude window discriminator (Neurolog; Digitimer), the
waveform of each discriminated spike being monitored continuously during recording. The
resulting standard digital pulses were stored on a computer, together with markers of task
events. Neuronal signals were also digitized and stored on computer (Spike2; Cambridge
Electronic Design, Cambridge, UK) for off-line analysis of spike duration. We classified each
unit as belonging to one of three major classes of neurons in the striatum identified by spike
waveform and firing rate criteria, as described previously (Adler et al. 2013). The relationship
between neuronal activity and behavioral performance was assessed on-line in the form of
rasters aligned on the task events using a custom-designed computer software (LabVIEW,
National Instruments, Dallas, TX). The same software was used to present visual stimuli, to
deliver liquid reward, to measure behavioral parameters, and to collect neuronal data for off-
line analyses. Once a neuron was isolated, we usually first examined its activity in the uncued condition. If the isolation could be maintained satisfactorily, the neuron was then tested in another condition, either the cued or unreliable cued condition, the order of the conditions being counterbalanced across trial blocks.

Data analysis.

The Student’s *t*-test and one-way ANOVAs with Tukey’s honestly significant difference (HSD) post hoc tests were conducted to test for any significant differences in RT and MT between task conditions (uncued, cued) and trial types (valid cued, invalid cued). As regards neuronal activity changes, rasters and perievent time histograms of single-neuron activity aligned to each of the three task events (trigger onset, movement onset, and target contact) were first examined visually and then statistically analyzed. We determined whether neurons showed significant changes in activity occurring at any particular moment during the performance of the reaching task. The baseline firing rate was calculated during the 500 ms preceding the presentation of the first visual stimulus (the trigger stimulus in the uncued condition and the cue in the cued condition), defined as the control period. A test window of 100 ms duration was moved in steps of 10 ms, starting at the presentation of the trigger stimulus, from 300 ms before movement onset, or from 300 ms before target contact. The mean discharge rate in the 100-ms window was compared, at each step, with that in the control period. The onset of a modulation was taken to be the beginning of the first of at least 5 consecutive steps showing a significant difference (*P* < 0.05, Dunn’s test for multiple nonparametric comparisons) as against the baseline activity. The offset of a modulation was defined in the same manner by the first of at least 5 consecutive steps with activity back to control. We then determined the onset and offset of the modulation in the firing of the neuron centered on each task events with 10 ms resolution. In addition, task-related increases in neuronal activity were evaluated in terms of the latency of the peak of activation. To do so,
we divided the trial into successive bins of 10 ms around each of the three task events and we calculated the spike count in each bin. Peak latency was estimated, for each neuron, as the 10-ms interval showing the highest level of activity in the peri-event time histogram referenced to each task event. Based on these measures, we classified FSIs into distinct categories according to the timing of peak activations relative to movement onset.

For each neuron, we also used a 300 ms window immediately before or after movement onset to test for dependence of neuronal activity on target location (left, right), condition (uncued, cued), and trial type (valid cued, invalid cued), on the basis of mean firing rate measured in these windows. The assessment of statistical differences between conditions was performed with the use of multivariate analysis of variance (MANOVA). Further details on data analysis are given at the appropriate place in the Results section. To examine the temporal relation between changes in FSI activity and behavioral indices of task performance (i.e., RT and MT), we made a correlation analysis on a trial-by-trial basis (Pearson’s correlation, P < 0.05).

In addition to the quantitative assessment of task-related activities of individual FSIs, we pooled activities across samples of neurons modulated during particular task conditions and trial types and made population activity curves. For each neuron, a normalized peri-event time histogram was obtained by dividing the content of each bin by the number of trials and the population activity was obtained by averaging all normalized histograms referenced to a particular task event. Data were analyzed using custom-written MATLAB (version R2015b, Mathworks, Natick, MA) scripts and statistical analyses were performed using JMP software (version 11, Chicago, IL).

Reconstruction of recording sites.

Histological confirmation of recording sites is available in monkey T, but not in monkey F who is still participating in another study. Consequently, for this latter animal, approximated
recording sites were determined from the neuronal activity characteristic to the striatum and
electrophysiologic features of neighboring structures, including the external and internal
segments of the globus pallidus, the internal capsule, and the anterior commissure. In monkey
T, the block of tissue including striatal recording sites was cut into frontal sections of 40 μm
thickness on a freezing microtome and every section was stained with cresyl violet. Based on
the location of recording sites identified histologically in this animal, FSIs were recorded
between 4 mm anterior and 8 mm posterior to the anterior commissure, over the entire lateral
to medial extent of the nucleus, including some penetrations in the most ventral portion of the
precommissural putamen. Based on the known topographic organization of cortical
projections, the region of the striatum explored in the present study is divided into a “motor”
region, which predominantly includes the postcommissural part of the dorsal putamen, and an
“associative” region, which includes mainly the precommissural part of the dorsal caudate
nucleus and putamen. The most ventral region of the precommissural part of the caudate
nucleus and putamen is usually referred to the "limbic" striatum (Haber and McFarland 1999).

Results

Behavior

As shown in Figure 1B, both monkeys had significantly shorter RTs in the cued condition
than in the uncued condition (Student’s t-test, P < 0.01), suggesting that the presence of prior
information on location of the trigger stimulus prepared the animals for directing their
movement at a specific target leading therefore to a shortening of RT. There was also a
significant effect of condition on MTs in monkey T (P < 0.01) and monkey F (P < 0.05) with
the MT in cued trials being shorter than the MT in uncued trials. Both monkeys continued to
perform well the task in the unreliable cued condition (above 90% correct) when unexpected
invalid cued trials occurred in the course of a block, namely they reached the target accurately
regardless of whether the cue was a valid predictor or not of target location. Figure 1C shows the mean RTs and MTs for invalid cued trials and for trials immediately preceding and immediately following them. In both monkeys, RTs for invalid cued trials were significantly longer than for trials with a valid cue (one-way ANOVA, monkey F: F (2, 594) = 143.59, P < 0.01; monkey T: F (2, 702) = 124.45, P < 0.01), reflecting a slowness of movement initiation when responding to the trigger stimulus which did not match the cued location. There was also a significant effect of trial type on MT in monkey F (F (2, 594) = 12.19, P < 0.01) who took longer to reach the target in invalid than in valid cued trials, whereas such a difference was less evident in monkey T (F (2, 702) = 3.33, P < 0.05).

Neuronal data

We recorded from 701 neurons in two monkeys (413 and 288 neurons requiring 19 and 11 recording months in animals F and T, respectively). Of the 701 neurons, 83 (12%) were judged to be FSIs and we report here on 64 of them (32 in each monkey) that were recorded in at least one of the three task conditions employed in this study. Table 1 gives an overview of the number of recorded FSIs in the different task conditions and the mean number of trials per recording. Consistent with prior studies (Adler et al. 2013; Yamada et al. 2016), FSIs were rarely encountered in awake monkeys compared to PANs and TANs. Despite their relative scarcity, FSIs were clearly distinguishable from the other neuron types (i.e., PANs and TANs) usually recorded in the monkey striatum. The patterns of neuronal discharge in the striatum of our monkeys (Figure 1D) were similar to those described previously (Adler et al. 2013; Yamada et al. 2016). The FSIs had a mean baseline firing rate of 12.8 ± 8.9 spikes/s (n=64), which is higher than that of the TANs (5.7 ± 2.2, n=85) or PANs (1.4 ± 1.8, n=87). The duration of spikes of the FSIs, defined as the time interval between the first negative and second positive peaks of the spike, was generally shorter (424 ± 57 μs, n=63) than that of the TANs (919 ± 198 μs, n=80) and PANs (754 ± 80 μs, n=80) (Wilcoxon's rank sum test, P <
0.01). These spike waveform and firing rate characteristics are very similar to those of FSIs recorded in the rodent striatum which have been closely associated with PV-containing GABAergic interneurons.

We first examined temporal profiles of activity changes for each neuron recorded in the uncued condition (n=55) using the sliding time window procedure (see Materials and methods), starting at the onset of the trigger stimulus and at 300 ms before the movement onset and the target contact. Here we focused on FSI activity just before and during movement for analysis, the modulations at a later stage of the task (i.e., reward period) being addressed in another report. We found that 53% (29 of 55) of neurons (11 and 18 in monkeys F and T, respectively) showed statistically significant changes in activity in relation to movement initiation (Dunn’s-test, P < 0.05). Among these, 17 neurons showed increases in activity, 9 neurons showed decreases in activity, and 3 neurons had increases followed by decreases. Figure 2 shows the activity profile for each of the 29 modulated FSIs, sorted according to the latency of the peak of activation for neurons that showed increased activity and to the earliest time of significant modulation for neurons that showed decreased activity only. As it can be seen, increases in activity were more prominent before the onset of movement, with neurons reaching their peak activation just after the presentation of the trigger stimulus (3 neurons) or within a 300 ms window immediately preceding movement onset (11 neurons). Other neurons reached their peak activation within a 300 ms window immediately following movement onset (6 neurons). We classified the neurons that showed increases in activity into two groups according to which of the two 300 ms windows was the one in which the peak activation occurred. Neurons having peak activation time prior to or following the initiation of arm movement were classified as early increase-type and late increase-type, respectively. All neurons displaying decreased activity only were modulated after movement onset and were included in a decrease-type category. Figure 3A shows
example neurons from each category. To give an overview of the modulations at the population level, averaged activities of all FSIs of each of the three categories are illustrated in Figure 3B. Neurons with no modulation in relation to movement initiation are also shown for comparison.

We next examined whether FSIs were sensitive to the target location and/or the direction of the arm movement. We performed this analysis by comparing the spike counts in the 300 ms window before (early increase-type neurons) or after the movement onset (late increase-type and decrease-type neurons) between leftward and rightward movements, for each modulated neuron in our sample. Of the 29 modulated FSIs, 7 (24%) showed activity that differed significantly between the two directions of movement (Wilcoxon’s test, P < 0.05), of which one half showed stronger activity associated with movements directed to the side ipsilateral to the moving arm and the other half with movements directed to the contralateral side (Figure 4A). Although FSI modulations dependent on the direction of movement were found in all three categories of neurons, they seem to be most frequent in early increase-type neurons, but the sample sizes were too small to allow for adequate statistical analysis. We also examined whether FSI activity was concerned with the fluctuations of reaching performance. For each modulated neuron, we calculated the trial-by-trial correlation (Pearson’s r) between movement parameters (RT and MT) and FSI activity, using the same time windows defined before. Across the sample of studied neurons, 17% (5 of 29) was sensitive to the RT and 17% (5 of 29) to the MT, and there was no trend toward a more pronounced relationship between FSI modulation and motor variables in one category of neuron than another (Figure 4B), significant correlations being either in the same or opposite direction (i.e., the FSI activity increased or decreased as RT or MT increased). In sum, we have provided evidence for FSIs increasing their firing rates around the initiation of movements, most of them having their peak activations that preceded movement onset
whereas a comparatively lower number of FSIs had peak activations that followed movement onset. We also found neurons that had opposite changes in activity (i.e., decreases in firing rate) after movement onset. It therefore appears that striatal FSI can display a variety of patterns of modulation as the monkeys initiated reaching movements in response to the trigger stimulus.

Effect of motor preparation on FSI activity

In order to determine whether the activity of FSIs was influenced by the presence of spatial cues that allowed the monkeys to prepare themselves to react more quickly to the trigger stimulus, the activity of 32 neurons was tested in the cued condition (14 and 18 in monkeys F and T, respectively). In this condition, short-lasting increases in FSI activity in response to the cue onset were seen in 4 neurons, whereas 2 other neurons exhibited increases in activity which began at 200-410 ms before the trigger and terminated in close temporal relation to its presentation, one neuron being activated both after cue onset and before trigger. Among FSIs that were tested, none showed sustained changes in activity throughout the whole delay period (i.e., the 1.5-s interval between cue and trigger presentations). Figure 5A (left part) shows an example of early increase-type neuron displaying a phasic increase in activity in response to the trigger stimulus in the uncued condition. When tested in the cued condition, the same neuron responded to the cue, while the trigger stimulus became ineffective for eliciting a response, as though the sensitivity was shifted from the trigger to the cue containing information about the upcoming direction of movement. Figure 5A (right part) shows an example of late increase-type neuron with a phasic increase in activity after the trigger stimulus on both uncued and cued trials, the activation being well aligned to movement onset in the two conditions.

To give an overview of changes in activity of the whole sample of neurons tested in the two task conditions, we averaged separately the activity from early increase-type (n=11)
and late-increase type neurons (n=5). As can be observed in Figure 5B (left part), when early increase-type neurons were grouped together, a brief activation in response to the cue was visible at the population level. Also, the population level analysis for this category revealed that the magnitude of activation in response to the trigger stimulus was affected by the task condition, being higher in uncued trials than in cued trials (MANOVA during the 500 ms period after the trigger onset, P < 0.05). On the other hand, the activity across the sample of late increase-type neurons was not significantly modulated during the cue period and the magnitude of activation after the trigger onset was not significantly different between the two conditions (MANOVA during the 500 ms period after the trigger onset, P > 0.05) (Figure 5B, right part).

To address the influence of motor preparation more quantitatively, we compared, for each neuron, the magnitude of activity in the uncued and cued conditions. We performed this analysis on averaged spike counts during the same 300 ms intervals defined before. If the activity change was significantly larger (MANOVA during the 300 ms period after the trigger onset, P < 0.05) in one condition than the other, the neuron was judged to be modulated by the task condition. A total of 13 of the 20 movement-related neurons (65%) showed statistically significant differences in their modulations between the two conditions. As shown in Figure 6A, it was found that neurons displaying a preferential sensitivity for either uncued or cued condition occurred more frequently in the early increase-type category than other categories (early increase-type: 73% (8 of 11) 3 and 5 in monkeys F and T, respectively; late increase-type: 60% (3 of 5) all in monkey T; decrease-type 50% (2 of 4) both in monkey F). Figure 6B shows the superimposed averaged activity for the FSIs tested in the two conditions, separately for the three categories of neurons. For the early increase-type category, the two curves were virtually identical during the period immediately preceding the onset of the trigger stimulus with a rise in activity starting just after the trigger onset and reaching a peak before the
movement onset, the level of activation during the cued trials being significantly less than
during uncued trials, as mentioned before (MANOVA during the 300 ms period after the
trigger onset, P < 0.05). To estimate the timing of when FSI activity in uncued trials differed
from that in the cued trials, we performed time-series analysis for every 50-ms window
moved in 10-ms steps over the period from 300 ms before to 300 ms after the trigger and
movement onsets. Statistical analyses revealed that differences between uncued and cued
trials became significant (MANOVA, P < 0.05) 160 ms after the trigger presentation and were
maintained until 60 ms before movement onset (Fig. 6B). For the late increase-type category,
the population average showed a gradual increment for both cued and uncued trials which
reached a peak very close to movement onset. There was an extensive overlap between the
two curves, without significant differences in the level of activation between the two
conditions (MANOVA, P > 0.05). Although the small number of decrease-type neurons tested
in both conditions makes analysis of the data less reliable, the average activity was very
similar between uncued and cued trials for this category of neurons.

Finally, we further investigated the impact of motor preparation on FSI activity by
testing the monkeys on the unreliable cued condition, in which a prepared action was
unexpectedly replaced by the other movement option when the trigger occurred. As
mentioned in the behavioral results, both monkeys took longer to respond to the trigger
stimulus in invalid cued trials than in valid cued trials. This condition thus appeared well
suited for examining whether an increase in demands for response initiation would elicit
differences in FSI activity. A statistical analysis using the same two 300 ms windows as
previously defined was performed to examine, for each neuron in our sample, whether the
activity differed as a function of trial types. This comparison indicated that the activity of only
4 neurons of the 45 movement-modulated neurons (9%) varied significantly depending on the
trial type, these few neurons being assigned to all three categories. Among 58 neurons that
were tested in the unreliable cued condition, 45 (78%) showed significant changes in activity surrounding the initiation of movement. Of these, 17, 12, and 16 were categorized as early increase-type, late increase-type, and decrease-type, respectively, based on the temporal profiles of their activity changes in the valid cued trials. The activity modulation occurred in relation to any of the three classes of FSIs we have categorized (Figure 7A) (6% (1/17) monkey T; 8% (1/12) monkey F; 13% (2/16) monkey T). We further examined FSI activity across the population. Figure 7B shows that FSI activity averaged over all neurons of each category overlapped almost completely in the two types of trials.

In sum, FSI activity did not vary markedly during the preparatory period preceding the initiation of the movement. Specifically, the level of activity of early increase-type neurons appeared to be influenced by motor preparation, thus indicating that cognitive demands, and not just movement initiation, might be encoded by changes in FSI activity. The similarity of the level of changes in FSI activity in the valid and invalid cued trials seen at the population level indicates that increased latency of movement initiation was not associated with altered FSI activity when an action should be re-specified at the time the trigger became available for selection.

Recording positions

Finally, we took into account the functional specialization within the striatum and examined whether the responses of FSIs could be related to their location in the ventral and dorsal striatal regions. Recording sites were verified histologically in monkey T (Figure 8A), while we confirmed that our recordings were in the striatum based on characteristic patterns of neuronal activity in monkey F. The majority of the neurons were recorded in the putamen, some of them being in the rostroventral putamen, which was identified as part of the ventral striatum (see Material and Methods). Figure 8B illustrates the distribution of the neurons we recorded. Among 64 neurons, 50 were considered to be located within the dorsal part (28 and
The three categories of FSIs were present in equal proportions and showed a considerable overlap in their distributions throughout the anteroposterior extent of the striatum explored. In particular, we found that FSIs with movement-related activity were not only restricted to the posterior putamen, which subserves motor functions, but were also found in the anterior putamen, including its ventral part. Early increase-type and late increase-type neurons were found in the dorsal (14 of 64 neurons; 22%) and ventral striatum (8 of 64 neurons; 13%). The frequency of neurons with a sensitivity to movement failed to vary significantly between the two regions ($\chi^2 = 0.411, \text{df} = 1, P > 0.05$).

Discussion

This study provides the first detailed analysis of the potential contribution of fast-spiking interneurons (FSIs) in the monkey striatum to information processing during movement preparation, initiation, and execution. Our analysis of changes in FSI activity highlighted the following points. First, the variety of changes in FSI activity, in terms of timing relative to movement initiation and the direction of these changes, suggests that these presumed interneurons do not provide a homogeneous signal at a particular moment during reaching task performance. Second, modulations of FSI activity occurring relatively early in relation to movement initiation all consisted of increases in firing rate, whereas those that followed the onset of movement consisted of either increases or decreases in firing rate. Third, increases in FSI activity reaching a peak before movement onset appear to be more influenced by the state of motor preparation, compared with those which peaked after movement onset. Fourth, movement-related changes in FSI firing were not influenced by an unexpected change in target location when monkeys were preparing a movement toward a specific location and instead elicited a movement in the opposite direction. We interpret the variety of these
modulation types as reflecting a contribution of presumed GABAergic interneurons to
different aspects of motor performance through an inhibitory influence on striatal output
circuits.

Characterization of FSIs in the monkey striatum

In line with previous single-neuron recording studies in the striatum of behaving monkeys
(Adler et al. 2013; Yamada et al. 2016), we found that a particular class of neurons can be
identified as FSIs on the basis of spike waveform and firing rate criteria. In particular, they
are clearly distinguishable from the two other electrophysiologically determined types of
neurons in the primate striatum, i.e., PANs and TANs (Kimura et al. 1984; Aosaki et al. 1994;
Apicella 2002). It is noteworthy that studies of the functional properties of striatal FSIs,
particularly in behaving monkeys, have been limited due to the difficulty in targeting these
rarely encountered neurons for recordings. In the present study, the proportion of FSIs that we
detected (12%) is higher than reported in previous studies (~3-5%) (Adler et al. 2013;
Yamada et al. 2016) possibly because we paid special attention to the detection of these
neurons. Studies in freely moving rats have reported that the proportions of striatal FSIs may
vary from 11% (Gage et al. 2010) to 33% (Stalnaker et al. 2012), highlighting the fact that
extracellular recordings can be subjected to considerable sampling biases. Putative FSIs in the
monkey striatum have firing properties resembling those of FSIs described in the rodent
striatum, which are thought to correspond to the parvalbumin-containing group of
GABAergic interneurons and are the source of strong inhibitory influences on striatal output
pathways (Koós and Tepper 1999; Gittis et al. 2010). However, given the variety of striatal
GABAergic interneurons that have not been characterized in vivo, we cannot rule out that
neurons classified electrophysiologically into FSIs include, at least in part, one or more other
classes of interneurons.
The role of FSIs in the initiation and execution of movement

Although there is considerable interest in the role of FSIs in regulating the excitability of striatal output pathways, the exact nature of their contribution to movement generation remains unclear. Accordingly, the present study was designed to determine how FSIs modulate their firing in monkeys performing a reaching task that allows to distinguish between initiation and execution of movements. We found that the modulation of FSI firing rate were heterogeneous, with some neurons modulated at the presentation of the trigger stimulus and others being modulated later during the execution of movement. Most modulations of FSI firing around the time of movement initiation consisted of increases, whereas other FSIs changed their firing in the opposite direction. The variety of these modulation types may indicate the presence of distinct FSI subgroups involved in the processing of different movement-related information.

There are only a few studies in behaving monkeys that have reported changes in FSI activity consisting in phasic increases in activity after cue presentation and outcome delivery in a Pavlovian conditioning protocol (Adler et al. 2013) or more complex changes related to actions and outcomes in instrumental conditioning paradigms (Yamada et al. 2016). A comparatively large number of studies in rats trained in a variety of behavioral procedures have shown that FSIs can display changes in activity at certain phases of task performance, such as stimulus presentation, initiation of movement, position in space, and outcome delivery (Berke 2008; Schmitzer-Torbert and Redish 2008; Gage et al. 2010; Lansink et al. 2010; Stalnaker et al. 2012). It therefore appears that FSIs do not modify their activity in a homogeneous manner during task performance in behaving animals, suggesting that they are involved in different processes depending on the task demands. As discussed further below, variations in the location of the recording sites could also account for differences in task-related activities of FSIs in the striatum.
Although the sample of FSIs recorded in the present study was heterogeneous in terms of the timing of activity changes and the direction of these changes (i.e., increased or decreased in firing rates), the limited diversity of FSI modulations around the time of movement contrasted with the wide variety of task-related activations observed in output neurons of the striatum (i.e., PANs). Numerous studies in behaving monkeys have reported that PANs display transient or sustained increases in firing rate occurring in several distinctive forms during the initiation and execution of actions (Kimura 1990; Crutcher and Alexander 1990). PAN activations may also reflect representational processes, such as preparation of movement, expectation of a trigger stimulus for movement (Alexander and Crutcher 1990; Apicella et al. 1992), or the motivational value of reward obtained at trial end (Hollerman et al. 1998; Hassani et al. 2001; Cromwell and Schultz 2003).

In rats performing a nose-poke task with a choice between two movement options, a relatively uniform increase in FSI activity has been reported at the time of the initiation of movement, with most activations reaching their peak just after choice execution (Gage et al. 2010). These findings have led to the hypothesis that changes in FSI activity constitute signals for selecting between competing actions, by suppressing unwanted action commands to facilitate the selection of the appropriate response. Our task design did not allow us to examine whether FSI activity can be preferentially modulated in the presence of a choice requirement, an issue which remains to be investigated with a task designed specifically to add demands for decision making.

We found that the target location and/or the direction of movement associated with reaching influenced the firing rate changes of about one-third of the FSIs (24%), with no systematic preference for one against the other movement direction. This modest selectivity for target location is somewhat different from the study of Gage et al. (2010) showing that a large majority of FSIs (78%) displayed directional selectivity with either ipsilateral or
contralateral movements. It is possible that we recorded from fewer direction selective FSIs because our task design did not require sufficient demands on response selection. In addition, we found only few FSIs whose magnitude of changes in activity was correlated with latency or duration of the reaching movement, indicating that the firing level of FSIs may not be particularly predictive of variability in the monkey’s performance speed.

The role of FSIs in the preparation for movement

Until now, studies of the role of striatal FSIs have not examined in detail their possible involvement in the preparation of movements and the expectation of task events. In the present study, we tested if FSI activity was sensitive to the preparation component of the task when the monkeys could improve the speed of their movements by using advance information about target location. We found that 65 % of the recorded FSIs exhibited transient changes in firing during the cue-trigger interval. Furthermore, these changes were mostly observed in the subset of FSIs that we classified as early increase-type, whereas late increase-type neurons did not change the magnitude of their movement-related activity, with or without motor preparation. Although the functional implications of these distinct groups of FSIs remain to be determined, our findings suggest that perhaps the FSIs are functionally more heterogeneous than currently appreciated, with the activity of early increase-type neurons time-locked to the trigger onset being more influenced by the state of preparation for movement, compared to late increase-type neurons. Specifically, modulation by predictive cues indicates that cognitive processes, and not just the process of movement initiation, might influence FSI activity.

Some FSIs showed a transient activation (less than 500 ms in duration) toward the end of the delay period of the task, just before trigger presentation, but none of them displayed activity changes that were maintained for a longer duration through the cue-trigger interval. This suggests that even if the preparatory period may introduce additional demands for
behavioral inhibition (i.e., active withholding of movement until trigger onset), sustained changes in FSI activity did not appear to be necessary for the behavioral expression of that preparation. On the other hand, short-lasting activations immediately preceding the trigger onset might represent a FSI signal in adjusting the movement preparation to the initiation of the subsequent movement. In the monkey putamen, it is relatively common to find striatal PANs displaying sustained activations that are thought to reflect movement preparation and/or stimulus expectation during delayed-response tasks (Alexander and Crutcher 1990; Apicella et al. 1992), while other PANs are phasically activated during movement initiation and execution (Kimura 1990; Crutcher and Alexander 1990). Different populations of PANs are therefore activated in sequence during the delay period and during the motor components of the task. It is possible that the observed changes in FSI activity may be required to facilitate the transition between subsets of PANs representing distinct steps of movement generation.

To further examine the influence of motor preparation on FSI activity, we also tested monkeys on a condition in which they were preparing a movement toward a specific location and instead must move in the opposite direction when the trigger occurred. Both monkeys slowed down in the trials where the cue was invalid, indicating that the recalibration of the movement direction places increased demands on the timing of movement initiation. We then examined whether this movement recalibration was accompanied by a change in FSI activity. We found that very few FSIs were differentially modulated on invalid and valid cued trials, despite the fact that the stimulus–response remapping had a strong impact on task performance. This finding indicates that the firing level of FSIs around movement initiation was not correlated with how quickly the monkey responds to the trigger stimulus, further confirming that FSI activity was not directly correlated with changes in the monkey’s performance. However, in our task design, the switch in movement direction occurred when the prepared movement was not yet initiated and it is possible that FSIs are critical for the
behavioral adjustment when the decision on the movement direction has to be replaced by another after the movement onset.

Functional properties of FSIs and regional specializations of the striatum

Based on evidence of regional specializations of information processing within the striatum, we examined the possibility that the functional properties of FSIs could be related to their location in different parts of the striatum. Neuronal recording in behaving rats have outlined differences in the way striatal FSIs are modulated during task performance according to their anatomical location, namely FSIs in the dorsolateral striatum may be important for controlling motor functions (Gage et al. 2010), whereas those in the dorsomedial and ventromedial striatum may contribute to outcome processing (Stalnaker et al. 2012; Lansink et al. 2010). In our study, no difference was found in the distribution of movement-related FSIs over the extent of the putamen sampled, encompassing motor putamen caudal to the anterior commissure and associative putamen rostral to it. This appears to confirm findings from previous recording studies in behaving monkeys (Adler et al. 2013; Yamada et al. 2016) in which FSI modulations related to task events were evenly distributed throughout the explored striatum. Interestingly, we found that a subset of FSIs located in the most ventral part of the anterior putamen, which is to say in limbic striatum, also exhibited movement-related activity. This differs from PANs recorded in the ventral striatum which have been reported to be specialized in processing motivational information (Apicella et al. 1991). However, given the low neuron number obtained in the present study, it is premature to draw any firm conclusions concerning a variation of functional properties of FSIs that may be related to the regional specializations of the striatum. In addition, it remains to be determined, in our study, whether the impact of reward on FSI activity may be particularly strong in the ventral striatum, as has been observed in behaving rats (Lansink et al. 2010).
How FSI signals are used by the striatal output pathways that control action?

It is thought that FSIs form a local inhibitory circuit that targets selectively the output of the striatum to downstream targets (Szydlowski et al. 2013). Increases in FSI activity are supposed to lead to inhibition of striatal output pathways and therefore suppression of movements by decreasing inhibition of basal ganglia output structures. One can assume that the reverse may occur in the case of decreases in FSI activity, resulting in facilitation of movements. This suggests that the two types of FSI modulations described in the present study might be differently involved in movement generation, one suppressing and the other facilitating movements. Our findings have shown that increases in FSI activity can occur sufficiently early to be involved in preparation and initiation of movements. It is possible that this type of FSI modulation may reflect an enhanced inhibitory level necessary for the expression of the correct response by preventing inappropriate motor commands from being selected. On the other hand, we observed a prevalence of decreases in FSI activity after the onset of movement, suggesting that these modulations may play a role in ongoing motor performance. This may be associated with a release of output neurons from inhibition and therefore facilitation of motor commands. This late FSI signal may be related to the inhibition of competing responses when the action has already been initiated. It is still an open question whether increased and decreased in FSI firing rates might reflect differential influences on functionally distinct output pathways thought to exert opposing effects on motor behavior (Gittis et al. 2010; Planert et al. 2008).

In conclusion, evidence in favor of prominent involvement of striatal FSIs in motor control has come from inactivation studies in animals (Gernert et al. 2000; Gittis et al. 2011; Burguière et al. 2013; Xu et al. 2016), as well as from clinical studies in humans (Kalanithi et al. 2005; Kataoka et al. 2010; Reiner et al. 2013). Our results are the first demonstration of modulations of activity from individual FSIs during the successive stages of movement.
generation, reflecting contributions to different aspects of motor performance, rather than an exclusive involvement in movement initiation. Although it is still unclear what features are precisely encoded by these changes, it is conceivable that disturbed FSI signaling might interfere with action selection, resulting in an impaired ability to inhibit inappropriate behaviors.

Author contributions

P.A. designed research, K.M. and P.A. performed research, analyzed data, and wrote the paper.

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Figure Legends

Figure 1. Behavioral task conditions, reaching performance, and classification of striatal neurons. A. The monkey kept the hand on a bar while waiting for the presentation of a visual stimulus (red light) on either the right or the left side of a panel in front of it. In response to this signal, the animal was required to release the bar and reach for the target located below the light, which it had to touch to receive a liquid reward. There were three task conditions with the same basic structure: (1) an uncued condition, in which the trigger stimulus occurred at irregular time intervals and in the absence of any predictive cues; (2) a cued condition, in which a visual stimulus (green light) gave advance information regarding the location of the forthcoming trigger presented 1.5 s later; (3) an unreliable cued condition in which the specification of trigger location was invalid on a small percentage of trials (invalid cued trials). In the example shown, a rightward movement is cued and the trigger stimulus is presented on the opposite side thereby eliciting a leftward movement. The three conditions were tested in separate blocks of trials. B. Reaction times and movement times for the uncued and cued conditions. Values are means ± SEM (numbers of trials: 283-317 for monkey F, 238-403 for monkey T). **P<0.01, *P<0.05. C. Reaction times and movement times from the invalid cued trials and the valid cued trials immediately before and after the invalid ones. Each value was obtained by calculating the mean ± SEM (199 and 235 trials of each type for monkey F and monkey T, respectively). **P<0.01, *P<0.05. D. Comparison of the electrophysiological characteristics of the three main types of neurons found in the striatum of the awake macaque monkey. Left part Sample spike trains from each category of neuron. Middle part Mean waveforms for one representative striatal neuron of each category. ptp, peak-to-peak, i.e., time elapsed between the first negative peak and the following positive peak. Right part Scatter plots of mean spike waveform durations (x-axis, firing rate
(logarithmic scale); y-axis, peak-to-peak time). Each dot represents a single neuron colored according to its category.

Figure 2. Time courses of change in FSI activity around the initiation of reaching movement. The scheme shows the temporal profile of activity changes for all FSIs tested in the uncued condition (n = 29), separately referenced to trigger onset (left), movement onset (middle) and target contact (right). Each horizontal line represents the duration of statistically significant changes in activity for a single neuron. Red and blue indicate neuronal activity above and below the baseline firing rate, respectively. The small losanges on red lines indicate the onset time of the peak activation. Lines are ordered according to the timing of the peak for neurons with increased activity and to the timing of the onset of modulation for neurons with decreased activity only.

Figure 3. FSI activity during the initiation and execution of reaching movements. A. Activity of three example neurons with significant changes in activity around the initiation of movements. Data are shown as corresponding raster displays and perivent histograms aligned on the onsets of the trigger stimulus (left) and the movement (right). Each dot in the raster displays represents a single spike, and each line of dots corresponds to the activity during one trial. Trials were ordered off-line according to the latency of movement. Trigger and movement onsets are indicated by red and gray markers in raster displays, respectively. Binwidth for histograms, 20 ms. B. Population activities for each of the 3 categories of movement-related FSIs. Colored curves depict mean activity (± SEM) for the three categories of FSIs: early increase-type (n=14), late increase-type (n=6), and late decrease-type (n=9). FSIs that were not activated during movement initiation are indicated for comparison (n=26). Shading indicates SEM. Activity is aligned to the onset of the trigger (left) and movement (right), which are marked by vertical dashed lines.
Figure 4. FSI activity in relation to the location of the target stimulus and motor variables. A. Changes in FSI activity as a function of target location (left or right). Significant differences (P<0.05) are indicated by black lines. B. Correlation of movement latency and duration with changes in FSI activity. Black dots indicate the data with a significant r value (P<0.05). RT, reaction time; MT, movement time.

Figure 5. Effect of movement preparation on FSI activity. A. Comparison of the activity of two individual neurons during uncued and cued trials. Conventions are the same as in Fig. 3A, except that the superimposed average firing rate for the two types of trial is plotted as colored curves smoothed by a three-point median filter. B. Time courses of the population activity preceding the trigger onset under the uncued and cued conditions for early increase-type neurons (n=11) and late increase-type neurons (n=5) recorded in both conditions.

Figure 6. Comparison of FSI activity in the presence or absence of movement preparation. A. Relative proportions of neurons with statistically significant differences in movement-related activity between the uncued and cued conditions. The percentages are calculated from the total number of recorded neurons of each category. n, number of neurons. B. Population average activity included all FSIs recorded in the two task conditions. Each panel shows the averaged activity for uncued and cued trials for a category of FSIs aligned on trigger presentation and movement onset. The *orange horizontal lines* below the curves indicate the periods when neuronal activity during uncued trials differed significantly from that during cued trials.

Figure 7. Comparison of FSI activity during invalid and valid trials. A. Relative proportions of neurons with statistically significant differences in movement-related activity between the invalid and valid trials. The percentages are calculated from the total number of recorded neurons of each category. n, number of neurons. B. Same conventions as in Fig. 3B.
Figure 8. Recording positions of FSIs. **A.** Coronal section stained with cresyl violet at the level of the striatum, showing traces of electrode paths above the putamen. **B.** Sagittal view of positions of recorded neurons plotted along the reconstructed electrode penetrations. Each dot corresponds to the position of a single FSI. For each neuron, the anteroposterior location (zero indicates the approximate level of the anterior commissure) is plotted against the depth of the electrode at which it was recorded. The recording depth was measured from a zero reference point as determined by using microdrive depth readings. Color and gray dots indicate neurons that were or were not modulated during movement, respectively. The dashed line indicates the approximate boundary between dorsal and ventral striatum. Cd, caudate nucleus; Put, putamen; GPe, globus pallidus external segment; GPi, globus pallidus internal segment.
Table 1. Numbers of recorded neurons and recorded trials in the different task conditions

<table>
<thead>
<tr>
<th>task conditions</th>
<th>N</th>
<th>increase</th>
<th>decrease</th>
<th>number of recorded trials</th>
<th>range of recorded trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>early</td>
<td>late</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uncued</td>
<td>55</td>
<td>14</td>
<td>6</td>
<td>9</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>cued</td>
<td>32</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>34 ± 8</td>
</tr>
<tr>
<td>unreliable cued</td>
<td>58</td>
<td>17</td>
<td>12</td>
<td>16</td>
<td>43 ± 11</td>
</tr>
</tbody>
</table>

N: total number of neurons recorded in each task condition; "increase" and "decrease" denote neurons that are activated and depressed around the time of movement, respectively; "early" and "late" denote neurons whose activations peaked before and after movement onset, respectively; number of recorded trials per condition is mean ± SD.
A

early increase-type  late increase-type  decrease-type

6%  8%  13%

n=17  n=12  n=16

B

early increase-type

late increase-type

decrease-type

valid cued trials
invalid cued trials

trigger

movement onset