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Modulation of Neuronal Activity in the Monkey Putamen Associated With Changes in the Habitual Order of Sequential Movements

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Deffains M, Legallet E, Apicella P. Modulation of neuronal activity in the monkey putamen associated with changes in the habitual order of sequential movements. *J Neurophysiol* 104: 1355–1369, 2010. First published July 7, 2010; doi:10.1152/jn.00355.2010. The striatum, especially its dorsolateral part, plays a major role in motor skill learning and habit formation, but it is still unclear how this contribution might be mediated at the neuronal level. We recorded single neurons in the posterior putamen of two monkeys performing an overlearned sequence of arm reaching movements to examine whether task-related activities are sensitive to manipulations of the serial order of stimulus-target locations. The monkeys' capacity to learn sequential regularities was assessed by comparing arm movement latencies and saccadic ocular reactions when a fixed repeating sequence was replaced with a random sequence. We examined neurons classified as phasically active projection neurons (PANs) and tonically active presumed cholinergic interneurons (TANs). About one-third of the PANs (35/106, 33%) activated during specific parts of a trial displayed modulations of their level of activation when the sequential structure was changed. This differential activity consisted of either decreases or increases in activity without altering the time period during which task-related activations occurred. In addition, half of the TANs (41/80, 51%) changed their responses to task stimuli with the sequence switch, indicating that the response selectivity of TANs reflects the detection of the context that requires adaptation to changes in the serial order of stimulus presentations. Our findings suggest that task-related changes in activity of projection neurons may be an important factor contributing to the production and adjustment of sequential behavior executed in an automatic fashion, whereas putative interneurons may provide a signal for performance monitoring in specific contexts.

INTRODUCTION

Several lines of evidence suggest that the striatum is an important substrate for the capacity to learn skilled behaviors. Accordingly, lesion experiments in animals have implicated the striatum in procedural learning underlying habit formation and movement automaticity (Graybiel 1998; Mishkin et al. 1984; Packard and Knowlton 2002). Clinical studies in humans showed that patients with striatal dysfunction, such as in Parkinson's disease, are impaired at consolidating stimulus-response associations into habits (Hay et al. 2002; Knowlton et al. 1996) and have difficulty executing learned movements automatically (Wu and Hallett 2005). Functional brain imaging studies in humans have also implicated the striatum in the acquisition and retention of routine behaviors established with extensive practice (Doyon et al. 2009). Further evidence of the role of the striatum in procedural forms of learning comes from single-neuron recording studies in behaving animals that have reported plasticity in the task-related activity of

striatal neurons as performance becomes automatic (Barnes et al. 2005; Carelli et al. 1997; Jog et al. 1999; Kubota et al. 2009; Tang et al. 2007). Despite strong evidence for the involvement of the striatum in the gradual improvement in performance after prolonged practice, the neuronal mechanisms underlying the production of automatized motor behaviors in this structure are unclear.

An important aspect of the role of the striatum in adaptive motor behavior involves recognition of the contributions of different striatal regions to different types of learning. Lesion and electrophysiological studies in rodent have emphasized that the dorsolateral part of the striatum that receives inputs from motor cortical areas constitutes the site for the learning and retention of overlearned skilled behaviors, whereas the dorsomedial part connected to association cortices is critical for acquiring new associations between stimuli and movements and more cognitive action planning (Balleine et al. 2009; Yin and Knowlton 2006). Although few studies in monkeys have investigated the neural processes of specific forms of learning at the level of the striatum, it has been reported that the sensorimotor part of the striatum or posterior putamen in primates, corresponding to the dorsolateral striatum in rodents, contains neurons that are activated when monkeys performed overlearned movement sequences, whereas neurons in the caudate nucleus and adjacent anterior putamen, the homologue of the dorsomedial striatum in rodents, show activations during the planning of novel movement sequences (Miyachi et al. 1997). It has been argued that activation of the posterior putamen reflects the striatal mechanisms that give rise to the expression of skillful sequential behavior once automaticity has been achieved in contrast to more anteriorly located caudate-putamen activation that is implicated in learning new motor sequences. In accord with this suggestion, neuroimaging studies have reported a shift of activity from anterior to posterior regions of the striatum at different stages of motor skill learning, from the initial acquisition of new visuomotor associations to the automatization process during the late learning stage of task performance (Doyon et al. 2003; Floyer-Lea and Matthews 2004; Jueptner et al. 1997; Lehéricy et al. 2005; Poldrack et al. 2005). Hikosaka et al. (1999) have proposed an integrated view of the neuronal representation of declarative and procedural components of motor sequence learning within separate networks of striatal regions and associated frontal cortical areas. However, there is little evidence that the posterior putamen makes a special contribution to the automatization phase of well learned sequential movements. To understand how the striatum controls sequential behaviors that are executed automatically, it is important to examine in more detail neuronal mechanisms of such processes in the region of the striatum thought to be implicated in the production of motor skilled behaviors.

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Single-neuron electrophysiology has stressed that two classes of neurons can be distinguished in the primate striatum. They are called phasically and tonically active neurons (PANs and TANs) and are thought to correspond to projection neurons and one class of interneurons of probably cholinergic nature, respectively. It is known that PANs are activated at different phases in task performance, and modulation of their task-related activity, mostly in the anterior striatum, can be related to the learning of appropriate behavioral reactions to conditioned stimuli (Schultz et al. 2003). On the other hand, TANs display responses to task-relevant events, such as conditioned stimuli and primary rewards, and they may carry signals that are important for reward-related associative learning (Apicella 2002; Graybiel 1998; Kimura et al. 1984). Both types of striatal neurons could be implicated in the formation of motor skills but their respective contribution remains to be elucidated.

Until now, no study has examined the activity of PANs and TANs during automatized task performance and when animals are adapting to a change in task context to exert control over the automatic process. To address this issue, we recorded both types of neurons from the posterior putamen, i. e., the striatal region thought to be central to the learning of habits and motor skills, after the monkeys had been extensively trained on a visuomotor task that involves sequential arm reaching movements, and we examined whether task-related neuronal activities are sensitive to manipulations of the serial order of stimulus-target locations. Our findings indicate that activity of subsets of neurons that belong to the two striatal classes was differentially modulated when a repeating sequence of movements was replaced by a random sequence. This suggests that the posterior putamen could be involved in the production of sequential movements characterized by a certain degree of automatization and in performance adjustments when the serial order was changed.

METHODS

Animals and apparatus

Two male macaque monkeys (*monkeys R and P, Macaca fascicularis*), weighing 7–9 kg, were trained to make arm reaching movements to visual targets to receive a liquid reward. All experimental procedures were in compliance with the National Institutes of

Health's Guide for the Care and Use of Laboratory Animals and the French laws on animal experimentation.

Behavioral procedures

Monkeys were seated in a Plexiglas box and faced a panel containing three metal knobs (10×10 mm), arranged horizontally (right, center, left) and positioned 5 cm apart, at eye level of the animal, and three bicolored light-emitting diodes (LEDs), one above each knob. A resting bar was mounted in the lower part of the panel at waist level. The trial structure is illustrated in Fig. 1. Each trial began with the monkey keeping its hand on the bar. After a period of 1 s, the centrally positioned LED was illuminated with a green light as a cue for the forthcoming trigger stimulus. The cue presentation (500 ms) was followed by a fixed delay of 1 s at the end of which one of the three LEDs was illuminated with a red light. In response to this stimulus, the monkey removed its hand from the bar and touched the target situated below the illuminated LED. Monkeys were rewarded with a drop of fruit juice (0.3 ml) for each correct target contact and the movement-triggering stimulus was extinguished as soon the animal reached the target. After target acquisition, monkeys immediately returned to the bar in preparation for the next trial, which did not begin until the total duration of the current trial (4 s) had elapsed. Trials were presented with an approximately constant intertrial interval of 1 s so that the trigger stimulus was presented every 4 s. The monkey had to release the bar within 500 ms of the appearance of the trigger stimulus and touch the target within 500 ms of releasing the bar. Trials in which the monkey released the bar prematurely or failed to execute the correct response were aborted, and no reward was given.

Before the electrophysiological recordings began, the monkeys were extensively trained (>6 mo) in the task under two sequence conditions: the "repeated sequence" in which the trigger stimuli followed a repeating series of three locations (right-center-left) so that the timing and location of the stimulus were predictable and the "random sequence" in which the location of the trigger stimulus varied pseudorandomly from trial to trial so that the timing of the trigger stimulus was predictable, but its location was not. The repeated and random sequences were conducted in separate blocks of 40–60 trials. The selection of trigger location in the random sequence was balanced such that each target-stimulus was presented ≥ 10 times for a block of 40 trials. There were no external signals that cued the monkey to distinguish between repeated and random movement sequence blocks. Both animals performed the task with the right arm.

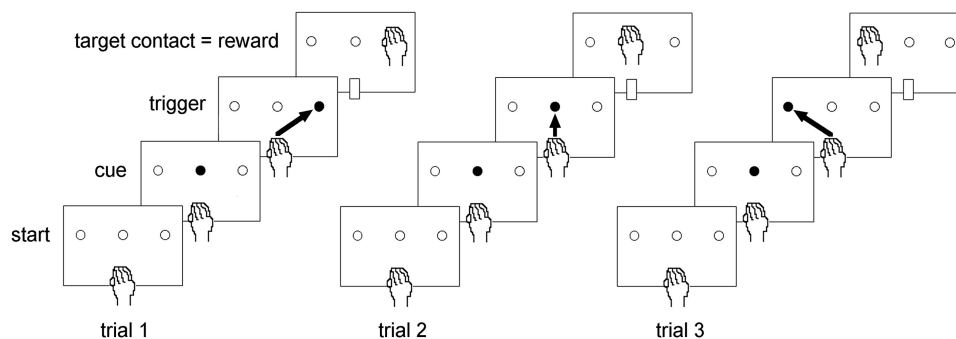


FIG. 1. Temporal sequence of events in the reaching task. Each trial started with the monkey keeping its hand on a resting bar. After a delay of 1 s, a 1st visual stimulus (cue) was presented for 0.5 s at the center of the panel. A 2nd visual stimulus (trigger) was presented 1.5 s after cue onset at 1 of the 3 locations, and the monkey was required to touch the target corresponding to the location of this stimulus. On completion of each correct target contact, a liquid reward was given. After completion of the reach, the monkey moved the hand back to the bar to start the next trial. In the repeated condition, the target followed a repeating sequence from the left to the center, to the right, and back again. In the random condition, target locations were pseudorandomly determined. The temporal sequence of task events remained constant in the two sequence conditions. Each trial had a total duration of 4 s, regardless of the condition. Monkeys were extensively trained to perform the task under both the repeated and random conditions before the recording sessions began.

Surgery

A partial craniotomy was performed under sterile surgical conditions and general anesthesia maintained with pentobarbital sodium (Sanofi, Libourne, France, 35 mg/kg iv) in *monkey R* and isoflurane in *monkey P*. A stainless steel recording chamber was vertically implanted over the left hemisphere with its center stereotaxically directed at the anterior commissure based on the atlas of Szabo and Cowan (1984). In the same surgery session, two pairs of Ag/AgCl electrodes were implanted into the brow ridges for recording eye movements, and two stainless steel cylinders were fixed to the skull with orthopedic bone screws and dental acrylic for subsequent head restraint during neuronal recording sessions. Prophylactic antibiotics (Ampicillin, Bristol-Myer Squibb, Paris, France; 17 mg/kg every 12 h) and analgesics (Tolfedine, Vetoquinol, Lure, France; 2 mg/kg) were injected on the day of surgery and for 5 days after the surgery. The recording chamber was filled with an antibiotic solution and sealed with a removable cap.

Electrophysiological recordings

While the monkeys were performing the task, with head immobilization, extracellular activity of single neurons was recorded with custom-made glass-insulated tungsten microelectrodes. To record from the striatum, a stainless steel guide tube (0.6 mm OD) was lowered below the surface of the dura, the microelectrode was passed inside the guide and was advanced using a manual hydraulic microdrive (MO95, Narishige, Tokyo, Japan). The signal from neuronal activity was amplified 5,000 times, filtered at 0.3–1.5 kHz, and converted to digital pulses through a window discriminator (NeuroLog, Digitimer, Hertfordshire, UK). Presentation of visual stimuli, delivery of reward, and digital pulses from neuronal activity were controlled by a computer using custom-designed software written by E. Legallet. The computer also controlled the measurements of task performance. Electrooculograms (EOGs) were collected during neuronal recordings with the chronically implanted periorbital electrodes. Horizontal components of the eye position were digitized at 200 Hz and stored during each block of trials, concomitantly with neuronal activity, for off-line quantitative analysis of the oculomotor behavior.

The task relationships of neuronal discharges were assessed on-line in the forms of raster dots referenced to different task events (visual stimuli, bar release, target contact), together with analog displays of EOGs. The electrode was advanced to isolate a neuron while the monkey performed the task. This was particularly useful for improving the detection of striatal neurons that have very low spontaneous discharge rates when the monkey is at rest. We ensured that recordings were from only a single neuron by continuously monitoring the waveform of the recorded neuronal impulses on an oscilloscope. The activity of neurons was usually sampled first during a block of trials in the repeated sequence, followed by a block of trials in the random sequence. If the neuron exhibited a change in activity during task performance, we recorded it for ≥ 40 trials. Otherwise, the neuron was considered as unmodulated and the electrode was advanced to search for another neuron. Because we cannot exclude that there were neurons that were modulated for only one sequence condition, we also tested in the random sequence a number of neurons that did not show task-related changes in activity in the repeated sequence. Any neuron exhibiting task-related activity in the repeated sequence was systematically studied in the random sequence. Only the neurons for which the data were collected in both the repeated and random sequences were included in the present study.

Striatal neurons were isolated and identified as PANs or TANs based on several electrophysiological features, such as spike waveform, firing rate, and pattern, and the relationship to specific components of task performance. It is well known that PANs display transient or sustained increases in firing rate occurring in several distinctive forms at specific phases of a task, whereas TANs express

brief decreases in firing rate in response to task stimuli (Apicella 2002).

Data analysis

Performance in both sequence conditions was assessed by calculating the reaction time (RT, time between trigger onset and bar release) and movement time (MT, time between bar release and target contact) of correct responses. The data for each sequence condition were taken from 69 (*monkey R*) and 40 (*monkey P*) blocks of 40–60 trials when neuronal activity was recorded. Trials with excessively short RTs (< 100 ms) were excluded from the averages. Quantitative analysis of EOG data were made off-line by single-trial analysis. The monkey was not required to maintain fixation during the task. For each sequence condition, we calculated the frequency of saccadic eye movements directed toward trigger stimuli and the mean latency of these oculomotor reactions. Anticipatory saccades (latencies < 50 ms) were excluded from the latency analyses. An ANOVA was used for comparison of behavioral variables (RT, MT, saccade latency) between serial orders and stimulus-target locations.

For each neuron, we first determined time course of statistically significant changes in activity by using a sliding time window procedure that has been described previously (Sardo et al. 2000). Briefly, baseline activity was determined in the 1-s period that preceded the onset of the cue, called the “control period.” A test window of 100 ms was moved in steps of 10 ms, starting at the onset of the cue. We then compared activity from the baseline period to activity in the sliding window. Neurons showing a statistically significant difference in activity during ≥ 5 (TANs) or 10 (PANs) consecutive steps (Wilcoxon signed-rank test, $P < 0.05$) were considered as modulated. The latency of a significant change in neuronal activity was defined as the beginning of the first of 5 or 10 consecutive steps showing a significant difference as against the baseline activity during the control period. The duration of a significant change in activity was defined by the first of 5 or 10 consecutive steps in which activity returned to control levels. The magnitude of a change in activity was calculated by counting neuronal impulses during the period with statistically significant change in activity. The number of spikes during this period was normalized by the duration of the period and expressed as the discharge rate in spikes per second. Because a number of PANs showed activations before the cue, we placed, for those neurons, the control period toward a trial epoch where no obvious changes in neuronal activity were seen. Task-related changes in PAN activity were also evaluated in terms of the latency of the peak activity estimated, for each PAN, as the 100-ms interval showing the highest activation in the perievent time histogram referenced to the onset of the trigger stimulus. Peak latency corresponded to the center of this interval.

Differences in proportions of task-related PANs and responding TANs between sequence conditions were assessed by using the χ^2 test. Nonparametric statistical tests were used to determine significant differences between task-related changes in PAN activity occurring during the repeated versus the random sequences (Mann-Whitney U test) or for different spatial locations of the trigger stimulus (Kruskal-Wallis test). TAN response magnitudes were also compared between sequence conditions or stimulus locations with the Mann-Whitney U and Kruskal-Wallis tests, respectively. In all tests, the criterion for statistical significance was set at $P < 0.05$, and for multiple two-sample comparisons, we used the Bonferroni correction method to adjust the chosen significance level according to the number of planned comparisons. For individual neurons, simple linear regression was used to analyze the relationship between magnitudes of task-related changes in PAN activity and behavioral indices of task performance.

In addition to the assessment of activity changes of the individual PANs, we wished to give a description of the overall task-related activity at the population level. To address this, we pooled neuronal

activities across subgroups of PANs activated during specific parts of a trial to yield population activity histograms. For each neuron, a normalized perievent time histogram was obtained by dividing the content of each bin by the number of trials, and the population histogram was obtained by averaging all normalized histograms. To indicate qualitatively how the serial order affected the population activity, we constructed population histograms representing firing rate as a function of time under the two sequence conditions.

Histology

Near the end of neuronal data collection, we made several small electrolytic marking lesions at sites of neuron recording by passing currents through the electrode (20 μ A for 15–20 s, cathodal current). The monkeys were killed with an overdose of pentobarbital and perfused with 0.9% saline followed by a fixative (4% paraformaldehyde, pH 7.4 phosphate buffer) through the heart. Frozen coronal sections were cut at 50 μ m and stained with cresyl violet. The recording sites that had been marked with lesions were identified, and electrode penetrations were reconstructed in serial sections through the striatum to verify the location of recording sites.

RESULTS

Behavior

The percent correct reaching performance of the monkeys was as high (>95%) in the random sequence as in the repeated sequence. Table 1 shows the mean RTs and MTs for the two monkeys. ANOVA revealed a significant main effect of the spatial location of the trigger stimulus [*monkey R*: $F(2,234) = 19.82$, $P < 0.0001$; *monkey P*: $F(2,391) = 33.09$, $P < 0.0001$] and serial order [*monkey R*: $F(1,234) = 8.34$, $P < 0.004$; *monkey P*: $F(1,391) = 17.56$, $P < 0.0001$] on movement latencies, so that monkeys had longer RTs when the stimulus was located on the left, i.e., in the hemispace contralateral to the moving arm, than when it was presented at the center or on the right. The RTs of both monkeys were also longer in the random sequence (173 \pm 15 and 246 \pm 28 ms in *monkeys R* and *P*, respectively, average of all 3 trigger locations) than in the repeated sequence (168 \pm 16 and 234 \pm 31 ms in *monkeys R* and *P*, respectively), but there was no interaction between location and order regarding RTs [*monkey R*: $F(2,234) = 0.25$, $P > 0.05$; *monkey P*: $F(2,391) = 0.06$, $P > 0.05$]. The analysis also showed that movements made in response to stimuli the location of which was contralateral to the moving arm were associated with longer MTs than those made in response to other stimulus locations [*monkey R*: $F(2,234) = 103.42$, $P < 0.0001$; *monkey P*: $F(2,391) = 18.75$, $P < 0.0001$], but differences in MT between repeated and random sequences were not significant [*monkey R*: $F(1,234) = 0.46$, $P > 0.05$; *monkey P*: $F(1,391) = 0.64$, $P > 0.05$]. There was no inter-

action between location and order regarding MTs [*monkey R*: $F(2,234) = 0.24$, $P > 0.05$; *monkey P*: $F(2,391) = 0.26$, $P > 0.05$].

Figure 2A shows examples of eye movement records. We classified eye movements into two types, predictive and reactive. They were considered to be predictive if eye positions were already on target before the trigger stimulus appeared or if saccades to the trigger onset occurred with excessively short latencies (<50 ms). Eye movements reactions to the trigger with latencies ranging from 50 to 250 ms were considered to be reactive. The average frequency of reactive and predictive eye movements and the mean latency of saccadic eye movements in the two sequence conditions are illustrated in Fig. 2B. Predictive eye movements were more frequent in the repeated sequence than in the random sequence in *monkey R* (60 and 38% in the repeated and random sequences, respectively; $\chi^2 = 48.78$, $df = 1$, $P < 0.01$) and to a lesser extent in *monkey P* (30 and 24% in the repeated and random sequences, respectively; $\chi^2 = 4.84$, $df = 1$, $P < 0.05$), suggesting that both animals showed an increased tendency to orient their gaze before stimulus presentation when it followed the repeating sequence. The mean latencies of saccades to the trigger onset were significantly different between sequence conditions in *monkey P* [$F(1,581) = 7.37$, $P < 0.007$], the repeated sequence being associated with the shortest saccade latencies, but not in *monkey R* [$F(1,522) = 0.50$, $P > 0.05$]. On the other hand, in both animals, there was a significant effect of location [*monkey P*: $F(2,581) = 37.11$, $P < 0.0001$; *monkey R*: $F(2,522) = 105.05$, $P < 0.0001$], the latency being longer with leftward than rightward saccades. A significant interaction between location and order was seen in *monkey R* [$F(2,522) = 3.31$, $P < 0.05$] but not in *monkey P* [$F(2,581) = 1.09$, $P > 0.05$].

In summary, the behavioral results show that extensive training with a fixed repeating sequence of movements, as opposed to a random order, influenced monkeys' task performance. In both animals, arm movement latencies were shorter when stimulus-target location was predictable on repeating sequence trials. On the other hand, the speed of movement from the resting bar to target was not affected by the serial order of stimulus presentations. Also in both monkeys, the repeated sequence appears to involve more predictive eye movements than the random sequence, and, at least in *monkey P*, the repeated sequence was associated with the shortest latencies of saccades. Overall these data suggest that task performance was dependent on the sequential features of the trigger stimuli and corresponding movements, the initiation of movement being presumably more automatic in the repeated sequence than in the random sequence.

TABLE 1. Task performance for the repeated and random movement sequence conditions

Task Condition	Reaction Time						Movement Time					
	Repeated			Random			Repeated			Random		
	Left	Center	Right	Left	Center	Right	Left	Center	Right	Left	Center	Right
<i>Monkey R</i>	177 \pm 17	161 \pm 13	166 \pm 14	181 \pm 15	168 \pm 14	172 \pm 15	176 \pm 18	166 \pm 15	141 \pm 22	174 \pm 15	166 \pm 14	138 \pm 14
<i>Monkey P</i>	251 \pm 28	230 \pm 28	223 \pm 31	261 \pm 26	241 \pm 26	236 \pm 27	313 \pm 49	306 \pm 42	280 \pm 47	320 \pm 47	305 \pm 43	285 \pm 47

Values of arm reaction and movement times are means \pm SD in ms. Mean reaction and movement times were calculated by averaging 40 (*monkey R*) and 69 (*monkey P*) trial blocks in each task condition and for each stimulus-target location.

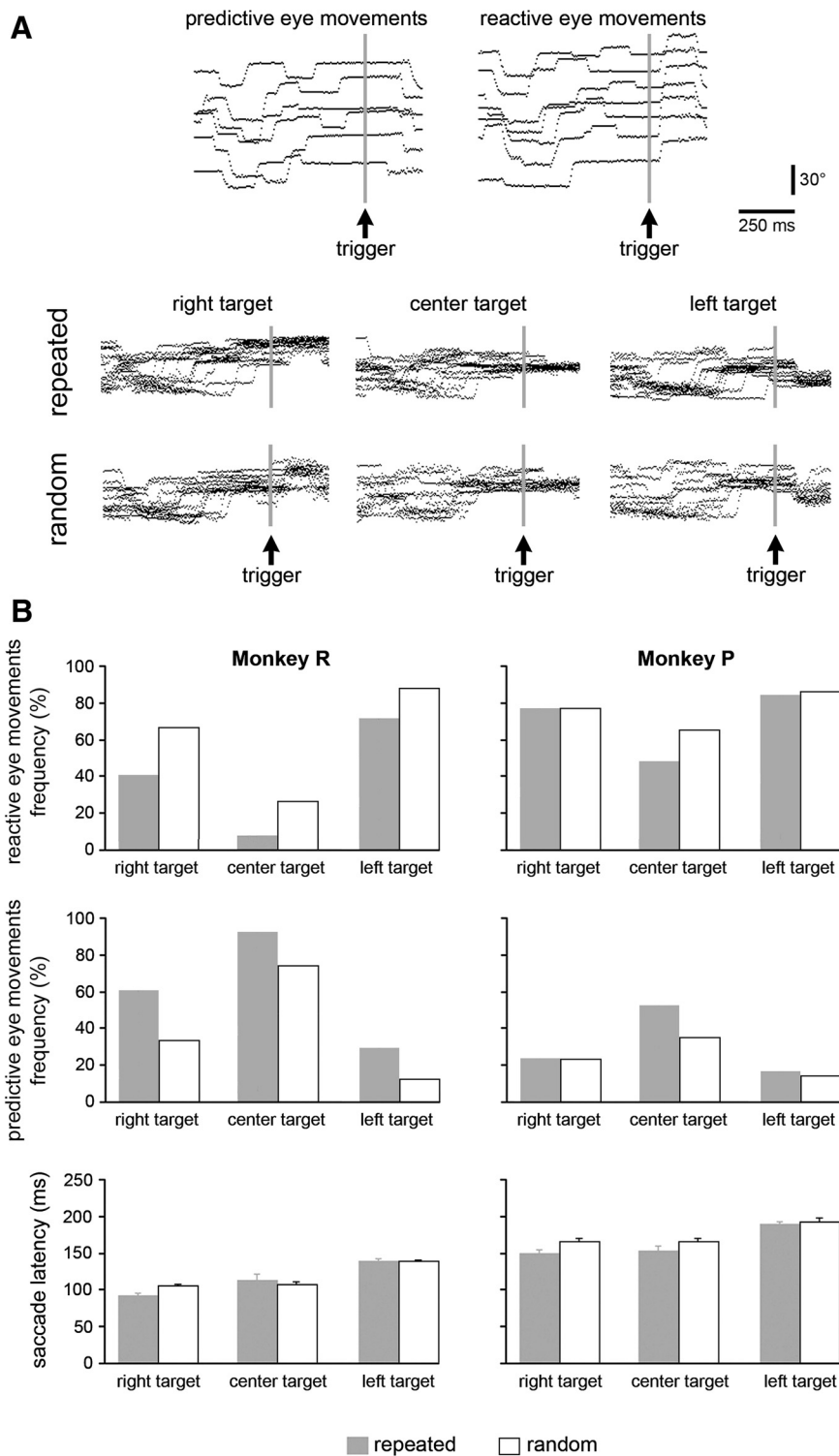


FIG. 2. Eye movements under the two task conditions. *A*: each *top* display shows examples of traces of horizontal components of eye movements aligned on the onset of the trigger stimulus. Eye movements were defined as predictive when they started before the trigger onset or with excessively short latencies (<50 ms) after the trigger and as reactive when they started after the trigger onset with latencies between 50 and 250 ms. Each *bottom* display shows ~ 20 superimposed traces of eye position for each of the 3 locations of the trigger stimulus in each sequence condition. Upward deflections indicate saccades toward the right. *B*: frequencies and latencies of eye movements for the 3 locations of the trigger stimulus in the 2 task conditions. Frequencies denote the occurrence of reactive and predictive eye movements in percentage of trials (total numbers of trials: *monkey R*: 561 and 504 in the repeated and random conditions, respectively; *monkey P*: 420 and 454 in the repeated and random conditions, respectively). Values of saccade latency were calculated from the total numbers of reactive eye movements and are given as means \pm SE.

Neurons

We recorded 117 PANs (26 and 91 in *monkeys P* and *R*, respectively) and 80 TANs (65 and 15 in *monkeys P* and *R*, respectively). The recording sites were confirmed histologically in the two monkeys. TANs were easily discernible from the PANs by their comparatively irregular tonic firing, long-duration extracellular spike waveforms, and typical pause responses to task stimuli (Apicella 2002).

PAN activations

We studied 106 PANs (21 and 85 in *monkeys P* and *R*, respectively) showing activations at different phases in task performance while animals performed the repeating sequence of movements. Figure 3 illustrates temporal profiles of all neurons with task-related activity in the repeated sequence. We classified the vast majority of these neurons into the following four categories: *cue*: activation preceded or began just after

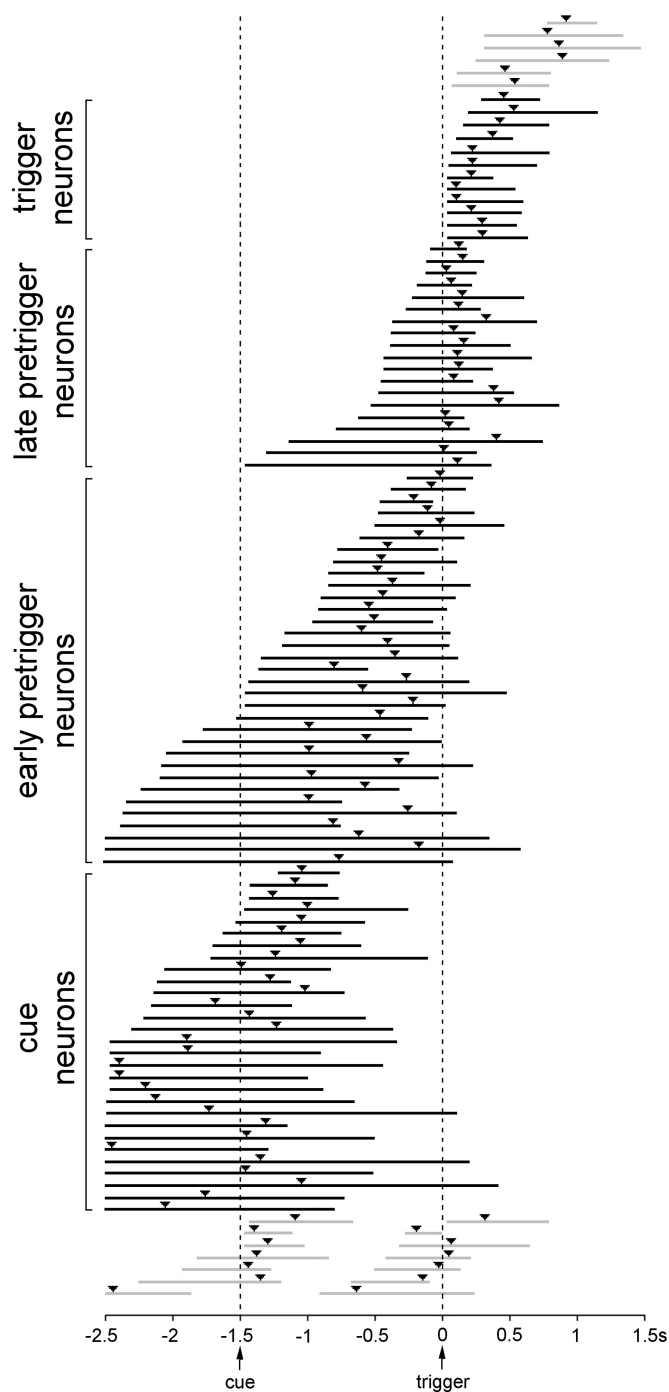


FIG. 3. Time courses of activation of all task-related phasically active projection neurons (PANs, $n = 106$) in the repeated sequence condition. Each horizontal line represents the period with a statistically significant increase in activity for a single neuron. The small triangles above lines indicate the onset time of the peak activation. Task-related activations are grouped into 4 main categories according to their temporal characteristics, i. e., onset of activation and timing of the peak. In each category, lines are rank-ordered according to the earliest time of significant modulation. The gray lines correspond to neurons that do not belong to the 4 categories ($n = 13$). The presentation of the cue and the onset of the trigger stimulus are marked by vertical dotted lines.

onset of the cue and peaked before its offset ($n = 29$); *early pretrigger*: activation began before the onset of the trigger stimulus and peaked between the offset of the cue and the onset of the trigger stimulus ($n = 33$); *late pretrigger*: activation

began before the onset of the trigger stimulus and peaked after the trigger onset ($n = 19$); and *trigger*: activation began and peaked soon after the onset of the trigger stimulus, these phasic activations being time-locked to the reaching movements ($n = 12$). An additional phasic component very close to or after trigger onset was observed in two neurons of the late pretrigger category. Only three neurons were phasically activated following cue presentation. We also identified six other neurons (Fig. 3, *top*) that showed a transient activation at the end of the trial, after the reaching movement was completed, without clear relationship to the movement itself. Another seven neurons (Fig. 3, *bottom*) exhibited separate activations occurring during the period preceding the cue and during the period prior to or soon after the trigger onset, suggesting a convergence of activations that were related to two distinct task periods. For these seven neurons, the first activation was of the cue type and the second activation of the early pretrigger ($n = 4$), late pretrigger ($n = 2$), or trigger type ($n = 1$). We focus here on the four main categories of task-related neurons ($n = 93$).

Although the putamen was explored at the level posterior to the anterior commissure, an area known as containing PANs related to movements of specific body parts, it was unlikely that such relations could account for most of the presently reported activations except for trigger neurons that were active during the reaching movement. In particular, sustained activations preceding cue or trigger onset were observed while no overt movement occurred during these time periods, the monkeys remaining motionless waiting for one or the other visual stimulus. Also the activity of PANs was not systematically modulated at the end of a trial by the delivery of reward or the return movement of the hand back to the initial position to start the next trial. With regard to orofacial activity, it is known that PANs related to the preparation and execution of licking movements are located primarily in the ventromedial portion of the posterior putamen, which was rarely targeted by our electrode tracks. Finally, the presently reported activations could not be simply explained on the basis of some aspect of oculomotor behavior because PANs that are related to saccadic eye movements or particular ocular fixation patterns are located in the body and head of the caudate nucleus and not in the putamen. These arguments suggest that most activations we have observed did not reflect a motor aspect in any simple manner.

Effect of the spatial location of the trigger stimulus

To determine whether spatially distinct presentation of trigger stimuli and/or reaching toward different targets influenced the task-related PAN activity, we performed a Kruskal-Wallis test with the factor trigger location on the period of statistically significant activations in each neuron (see METHODS). Among the 93 neurons of the four categories defined in the preceding text, 23 (25%) showed activations that differed according to the location of the trigger stimulus and/or direction of the movement. There were significantly higher frequencies of spatially sensitive PANs in the trigger category than in the early pretrigger ($\chi^2 = 9.72$, $df = 2$, $P < 0.01$) and cue categories ($\chi^2 = 5.12$, $df = 2$, $P < 0.05$). Trigger location modulation was also significantly more prevalent in the late pretrigger category than in the early pretrigger category ($\chi^2 = 6.02$, $df = 2$, $P < 0.05$). It therefore appears that the sensitivity

to the spatial features of stimuli and associated movements was preferentially seen in neurons with activations occurring in the late part of the task. We defined each neuron's preferred stimulus location as the location that was associated with the largest increase in discharge rate (post hoc Mann-Whitney *U* tests with Bonferroni's correction for multiple comparisons). Among the 23 PANs showing a spatial preference, the activation was greater when the trigger stimulus was presented on the left side in 10 neurons, at the center in 5 neurons, or on the right side in 8 neurons.

Effect of task performance

Changes in movement speed were considered as a potential confound for our study because it is known that the activity of neurons in the posterior putamen can be related to kinematic variables, such as movement direction and velocity (Crutcher and Alexander 1990; Crutcher and DeLong 1984). To determine whether task-related PAN activity reflected movement parameters, a linear regression analysis was made for each neuron. As shown in Table 2, the relationship between PAN activation and RT was significant for a relatively low number of neurons, indicating that the task-related activity did not vary markedly with increasing RT ($\chi^2 = 0.68$, $df = 3$, $P > 0.05$). On the other hand, neurons tended to have more frequent significant effects for the MT ($\chi^2 = 8.06$, $df = 3$, $P < 0.05$) with late pretrigger and trigger neurons being more frequently influenced by MTs than were early pretrigger neurons. Despite these significant effects, there was a weak correlation between PAN activation and MT as indicated by relatively low correlation coefficients ($r < 0.64$). This suggests that the changes in task-related activity did not appear to be directly related to the performance differences.

Effect of the change in serial order

To test for the sensitivity of task-related PANs to a change in the serial order of stimulus presentations, we compared the level of activation between the repeated and random sequences, using a Mann-Whitney *U* test with the factor order on the period of activation in each neuron. Of the 106 PANs showing task-related activity in the repeated sequence, all except 1 were always activated during the same time period when tested in the random sequence. We also tested 11 neurons that did not show significant changes in activity in the repeated sequence, and none of these neurons became activated when tested in the random sequence. A total of 35 neurons had activation levels that were significantly different when passing

from the repeated to the random sequences, consisting of either increases or decreases. We focus on those neurons with differential activation for repeated and random sequences that belong to the four categories of activation ($n = 26$). Although the total number of PANs tested in *monkey P* was relatively small, there were no significant differences between animals (χ^2 test, $P > 0.05$) in the fraction of neurons of these four categories showing a sequence-dependent effect (*monkey P*: 3/18 neurons, 17%; *monkey R*: 23/85 neurons, 27%). The proportions of neurons that showed statistically significant effects for the order are shown in Fig. 4A. The fraction of trigger neurons (5/12, 42%) and early pretrigger neurons (11/33, 33%) influenced by order was slightly higher compared with cue neurons (6/29, 21%) and late pretrigger neurons (4/19, 21%), but none of these differences in proportion reached statistical significance (χ^2 test, $P > 0.05$). Among the 26 neurons showing differential activation, 14 displayed an increase and 12 a decrease in their level of activation after the switch in serial order. The proportions of neurons that increased their activation and those with decreasing activation were not statistically different among the four categories of PANs (χ^2 test, $P > 0.05$) except for trigger neurons in which activity was always greater in the repeated sequence than in the random sequence. It therefore appears that the sequence in which monkeys know the location of the trigger stimulus was not systematically associated with higher or lower level of activation than the condition in which trigger location was not predictable. Figure 4B illustrates the activity of two neurons (*top* and *middle*) showing a sequence-related modulation in activity, while another neuron (*bottom*) was activated in a similar manner in both sequence conditions. In Fig. 5, we summarize the magnitudes of changes in task-related activity of individual neurons as a function of sequence condition for the four PAN categories. The graph reveals that neurons of the cue and early pretrigger categories showed the strongest differences in their level of activation when the repeated sequence was replaced by a random sequence.

To further examine whether temporal characteristics of the PAN activations were sensitive to the switch in sequence condition, we compared activation parameter measures (latency, duration, and peak timing) between repeated and random conditions for the four categories of task-related PANs. As shown in Table 3, none of these activation parameters was altered when the order of stimuli and movements changed except for the timing of peak activation of cue neurons that occurred significantly earlier in the repeated sequence than in the random sequence.

We also compared the proportions of task-related PANs with a spatial preference between the two sequence conditions. When tested in the random sequence, 13 of 92 (14%) neurons showed some degree of selectivity for the location of the trigger stimulus and/or direction of the movement. Among the 13 PANs showing a spatial preference, the activation was greater when the trigger stimulus was presented on the left side in 6 neurons, at the center in 3 neurons, or on the right side in 4 neurons. There were significantly higher frequencies of spatially sensitive PANs in the repeated sequence than in the random sequence ($\chi^2 = 4.20$, $ddl = 1$, $P < 0.05$). As shown in Fig. 6, the change in sequence condition affected the strength of spatial preference among PAN categories. In particular, cue and early pretrigger neurons lost their spatial preference when

TABLE 2. Numbers and percentages of PANs with a significant effect of task performance

PAN Category	<i>N</i>	RT	<i>r</i>	MT	<i>r</i>
Cue	29	3 (10%)	0.37–0.59	4 (14%)	0.28–0.42
Early pretrigger	33	4 (12%)	0.36–0.57	2 (6%)	0.40–0.41
Late pretrigger	19	1 (5%)	0.43	6 (32%)	0.35–0.64
Trigger	12	1 (8%)	0.42	4 (33%)	0.38–0.64

Values correspond to number and percentage of the neurons that had task-related activations in which the slopes of regression line were significant ($P < 0.05$). *N*, number of neurons in each phasically active projection neuron (PAN) category; *r*, correlation coefficient.

tested in the random sequence, and there was only a tendency for late pre- and trigger neurons with a spatial preference to be found in lower and higher numbers, respectively (χ^2 test, $P > 0.05$).

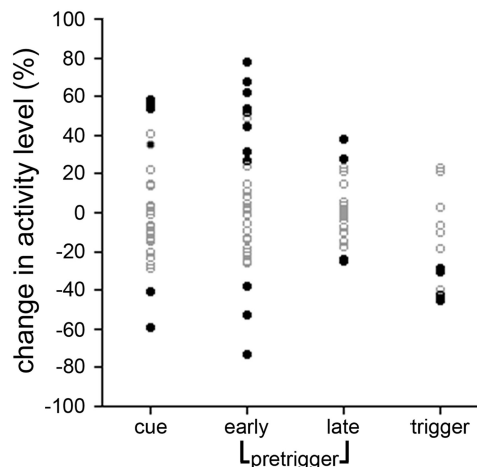
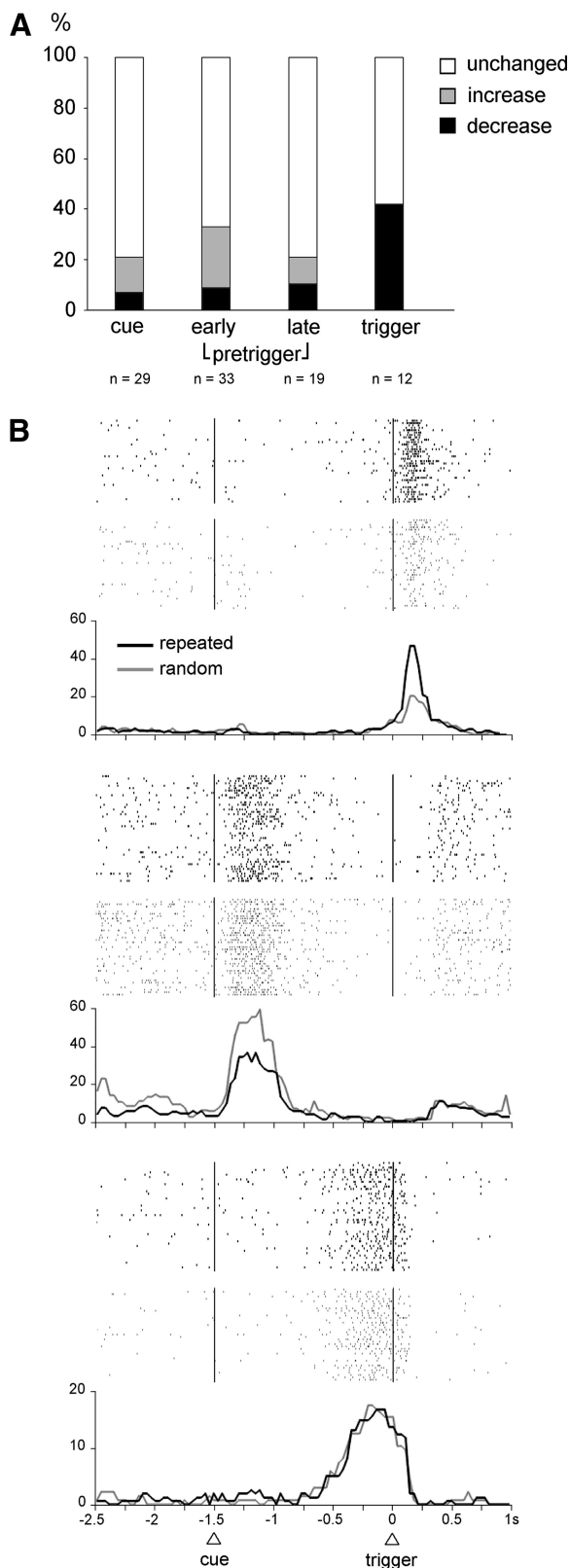


FIG. 5. Sequence modulation of task-related PAN activity. Magnitudes of changes are indicated as increases or decreases in percentage above or below the level of activation measured in the repeated condition. ●, PANs with a significant change in activity; ○, PANs with an insignificant change in activity. Each value is the mean change in activity when passing from the repeated to the random sequence condition for a neuron. One outlying value was removed from this graph.

Population activity

Population histograms comparing activity of the four categories of task-related PANs in the repeated and random sequences are shown in Fig. 7. For each of the categories, neurons were divided into those decreasing, those increasing, and those maintaining their level of activation when the sequence was changed. Despite the small numbers of neurons, the population activity of certain subgroups showed a slight difference in their temporal characteristics according to the serial order. For example, the average activity of cue, early pretrigger and late pretrigger neurons of the decrease type (*middle*) seemed to start earlier in the repeated sequence compared with that in the random condition, whereas the average activity of the neurons of the increase type started approximately at the same time, regardless of the serial order (*right*).

TAN responses

As previously reported, TANs exhibited homogeneous phasic decreases in tonic firing in response to task stimuli that distinguish them from the diversity of task-related activations seen in PANs (Apicella 2002). Of 80 TANs recorded in the repeated sequence, 26 (33%) responded exclusively to the trigger stimulus, 5 (6%) responded exclusively to the cue, 17 (21%) responded to both stimuli, and 32 (40%) were unrespon-

FIG. 4. Influence of the serial order of stimulus presentations on task-related PAN activity. *A*: relative proportions of PANs with a condition-dependent change in activity. Differentially active PANs show decreasing or increasing task-related activity when passing from the repeated to the random conditions. *B*: activity of 3 example PANs tested in both conditions. Data are shown as corresponding raster displays and periventric histograms aligned on the onset of the trigger stimulus. Each line of dots in the raster displays corresponds to the activity during 1 trial, and each dot represents a single spike. The original sequence of trials is preserved downward. The superimposed average firing rate for repeated and random trials is plotted as black and gray curves, respectively. Bin width for histograms: 35 ms. Histogram data were smoothed by a 3-point median filter. Vertical scale denotes impulses/s.

TABLE 3. Comparisons of task-related activations between repeated and random conditions

PAN Category	N	Repeated			Random		
		Latency	Duration	Peak Timing	Latency	Duration	Peak Timing
Cue	29	-2143 ± 88	1521 ± 114	-1539 ± 86	-2080 ± 80	1437 ± 126	-1296 ± 91
Early pretrigger	32	-1368 ± 125	1367 ± 116	-482 ± 50	-1304 ± 128	1287 ± 117	-564 ± 100
Late pretrigger	19	-505 ± 91	905 ± 108	151 ± 30	-477 ± 92	883 ± 106	122 ± 37
Trigger	12	101 ± 23	559 ± 47	287 ± 39	115 ± 23	505 ± 46	327 ± 34

All values are referenced to trigger onset and are given as means ± SE. N, number of neurons in each PAN category.

sive to stimuli. We examined the sensitivity of TANs to the spatial location of the trigger stimulus and found that none of the neurons showed responses that reflect selectivity for the target location, and there were also no significant differences in the magnitudes of TAN responses among the three locations (Kruskal-Wallis, $P > 0.05$, data not shown).

We found that the overall responsiveness of TANs was not significantly affected, in terms of fraction of responsive TANs (χ^2 test, $P > 0.05$, data not shown) and magnitude of responses (Wilcoxon test, $P > 0.05$, data not shown), when passing from the repeated to the random sequences. However, even if the responsiveness of TANs remained approximately the same, it is noteworthy that a number of neurons changed their response selectivity when the serial order of target-stimulus presentations was changed. For example, among 26 TANs responding exclusively to the trigger in the repeated sequence, 12 (46%) maintained this selective response and 14 (54%) changed their responsiveness when tested in the random sequence. Also 14 of the 32 TANs (44%) that were unresponsive in the repeated sequence started responding in the random sequence with various degrees of selectivity. Comparison across TAN classes of the relative frequencies of neurons is shown in Fig. 8A. It therefore appears that some responding TANs adjusted their selectivity while other unresponsive TANs became responsive when tested in the random sequence. One example of a TAN that changed its response selectivity is shown in Fig. 8B. This neuron responded only to the trigger stimulus in the repeated sequence and displayed an additional response to the cue in the random sequence.

In summary, these findings indicate that the sensitivity of TANs to a change in the serial features of the trigger stimuli and corresponding reaching movements relied on an adjustment in the degree to which TANs are selectively responsive to

one or the other stimulus without altering their overall responsiveness to task stimuli.

Recording sites

As shown in Fig. 9, the vast majority of recorded neurons were located in a region extending from the level of the anterior commissure to the most posterior regions of the putamen. We did not find any difference in the distribution of the different classes of task-related PANs and responsive TANs over the part of the putamen sampled. Also no striking differences in the anatomical distribution of PANs and TANs showing a sensitivity to the change in sequence condition appear in our data. We attempted to separate the postcommisural putamen into two parts based on the known distribution patterns of cortical inputs along the mediolateral extent of this nucleus with primary motor cortex and supplementary motor area projecting mainly to the lateral and medial parts, respectively (Nambu et al. 2000; Takada et al. 1998). Of the 35 PANs with sequence-dependent activations, 17 were considered to be located within the lateral part and 18 within the medial part. The frequency of PANs with a sensitivity to condition appeared somewhat weaker in lateral (17 of 60 neurons, 28%) than in medial parts (18 of 46 neurons, 39%), but this difference was not statistically significant ($\chi^2 = 1.37$, $df = 1$, $P > 0.05$). There was also a trend toward an increase in the percentage of TANs with sequence-dependent responses located within the medial part of the posterior putamen (30 of 56 neurons, 54%), relative to the lateral part (11 of 24 neurons, 46%), but again this difference was not statistically significant ($\chi^2 = 0.40$, $df = 1$, $P > 0.05$).

DISCUSSION

In the present study, we targeted our recordings to the posterior putamen, which is considered as the region of the primate striatum involved in the production of motor skilled behaviors acquired through repeated practice. The task that we employed involved visually triggered reaching movements toward spatially distinct targets, and both monkeys showed evidence of having learned the serial order of stimuli and movements, resulting in a higher degree of automatization of task performance in the repeated sequence than in the random sequence. We then compared the activity of projection neurons and of putative interneurons when the stimuli and movements followed a fixed sequence or were randomly selected. We observed that a group of PANs decreased or increased their level of task-related activation without altering their temporal profile of activation, whereas other PANs maintained their level of task-related activation regardless of the sequence

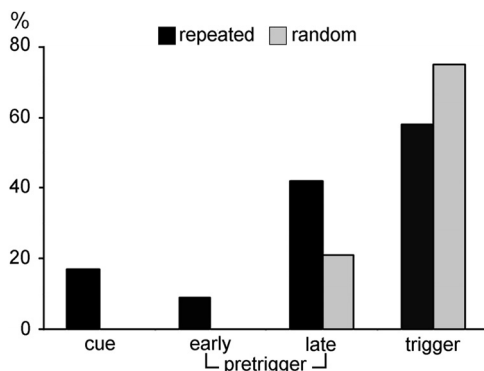


FIG. 6. Bar plots indicating proportions of task-related PANs with spatial preference in the two task conditions. Ordinate indicates percentage of neurons in each of the 4 categories of task-related PANs.

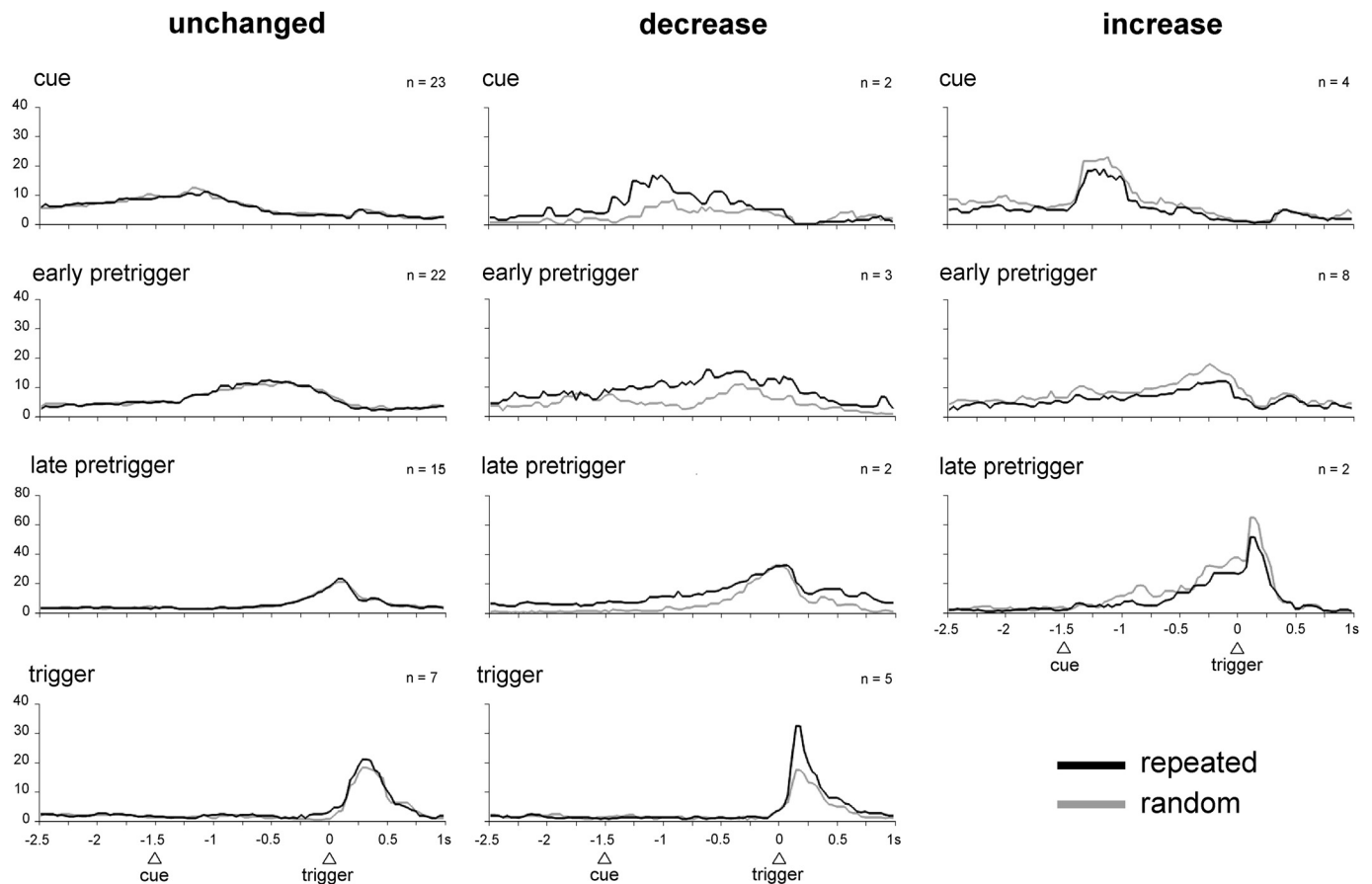


FIG. 7. Population activities for each of the 4 categories of task-related PANs. Each panel shows the averaged activity for repeated (black) and random trials (gray) for a subset of PANs aligned on stimulus presentation. *Left*: histograms obtained from only task-related PANs in which activity remained the same, regardless of the condition (“unchanged”); *middle*: histograms obtained from only task-related PANs showing decreasing activity in the random condition, relative to the repeated condition (“decrease”); *right*: histograms obtained from only task-related PANs showing increasing activity in the random condition, relative to the repeated condition (“increase”). Histogram data were smoothed by a 3-point median filter. Vertical scale denotes impulses/s.

condition. In addition, response properties of TANs varied markedly by considering the selectivity of the responses of individual neurons to one or the other stimulus when the repeated sequence was replaced with a random sequence. These findings suggest that PANs and TANs in the posterior putamen can be differentiated by their distinct involvement in the production of motor routines that mediate the procedural learning associated with extensive practice of the same sequence of movements.

Automatized task performance

Both monkeys were trained for several months on the repeated and random sequences of the task. This resulted in shorter latencies of arm movement reactions and higher percentages of predictive eye movements when stimulus locations follow a fixed repeating sequence as opposed to a random order, indicating that monkeys have acquired information about the serial order of stimulus-target locations. It has been previously reported in monkeys that an increased tendency for eye movements to occur in an anticipatory manner after extensive sequential task training may serve as an index of a skillful and automatic performance (Miyashita et al. 1996). Target-directed hand movements occurring before the trigger presentation became also much more frequent in monkeys

performing repeating sequences of movements at a stage in which sequential movements can be automatically executed (Matsuzaka et al. 2007). Although it is not possible in our experiments to unambiguously characterize a repeating sequence of movements as automatic, we refer to the notion that automaticity is associated with a speedy performance due to faster processing and reduced attentional demands (Fitts 1964; Logan 1988). In our task, both monkeys showed evidence of having learned the sequential feature of the stimuli and movements to produce quicker, presumably more automatic responses on the basis of predictable stimulus locations. Our protocol was comparable with the serial reaction time (SRT) task used in human subjects (Nissen and Bullemer 1987) in which learning of sequential regularities was assessed by a speedy performance when stimulus locations follow a fixed repeating sequence as opposed to a random order. Few studies have explored procedural motor learning in the monkey using SRT protocols, and they have led to inconsistent results, including reports of little or no change in task performance when conditions switch from repeating to random movement sequences (Lee and Quessy 2003; Matsumoto et al. 1999; Nixon and Passingham 2000; Procyk et al. 2000). The reason for these somewhat disparate results might be related to differences in task designs, duration of motor sequence training, sequence length, and temporal proximity of successive stimu-

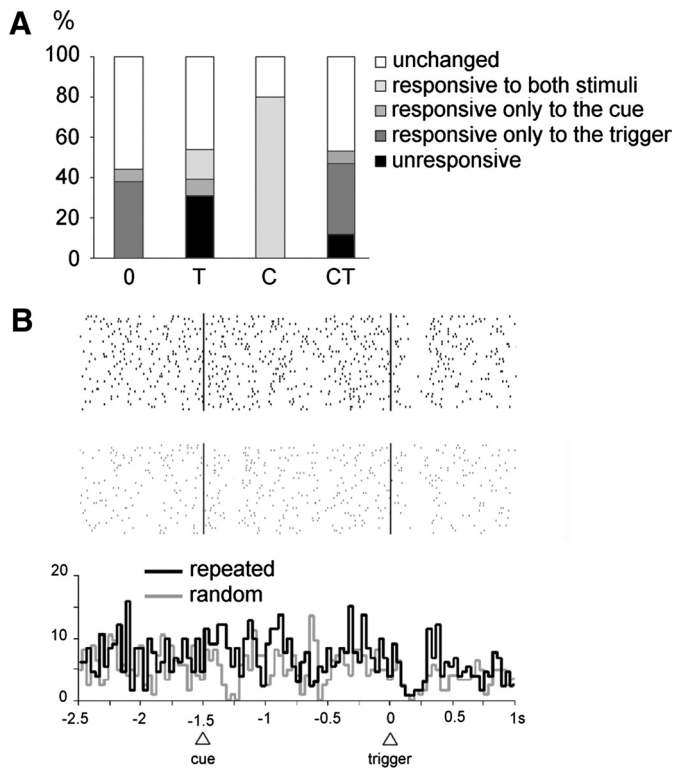


FIG. 8. Influence of the serial order of stimulus presentations on the responsiveness of tonically active neurons (TANs). *A*: proportions of TANs maintaining or not their response to the cue and/or the trigger stimulus when tested in the random condition. TANs were classified into 4 types depending on their responsiveness in the repeated condition. U, unresponsive; T, responses only to the trigger stimulus; C, responses only to the cue; CT, responses to both stimuli. *B*: an example of TAN which remained responsive to the trigger stimulus, regardless of the condition (black), and started responding to the cue in the random condition (gray). Same conventions as in Fig. 6*B*. Bin width for histograms: 35 ms.

lus presentations. In our experiments, both monkeys displayed a RT increase when a random sequence replaced the repeated one, indicating that they were able to use serial order information to start the movement more quickly. An important aspect of the task design used here was that we presented a cue at the beginning of each trial, and it seems possible that the presence of this temporal reference for the ensuing motor reaction may have improved the degree of sequence learning by making the elements of the sequence more predictable in time, thus reducing RT in a rather automatic fashion. This suggestion is supported by the data from human SRT literature showing that the temporal organization of serial sensorimotor events is crucial for automatizing sequential movements (Dominey 1998; Stadler 1995).

Neuronal correlates of automaticity in the posterior putamen

We found that PANs in the posterior putamen were activated during specific parts of the task when overtrained monkeys produce a repeating sequence of movements. This is consistent with a previous report in monkeys trained on a visuomotor sequential task showing that performance of highly practiced movement sequences appears to activate preferentially PANs in the posterior putamen, whereas PANs activated during trial-and-error sequence learning are more frequent in the

anterior striatum (Miyachi et al. 2002). Interestingly, most of the task-related changes in activity consisted of sustained activations that preceded task events, thought to reflect processes relating to the preparation of movement or expectation of a specific task-relevant stimulus (Alexander and Crutcher 1990; Apicella et al. 1992; Hikosaka et al. 1989). We surprisingly found a relatively small number of PANs phasically activated during the arm movement. It is well known that PANs in the posterior putamen are frequently activated during movements, whereas PANs with preparatory or expectation-related activations are more frequent in the anterior striatum (Alexander and Crutcher 1990; Crutcher and Alexander 1990; Kimura 1990; Miyachi et al. 1997; Romo et al. 1992; Schultz and Romo 1992). Also neurons that fired more to one stimulus-target location than the other were rarely observed in the present study, whereas previous studies have found that the direction of movement is an important determinant of PAN activity in the posterior putamen (Crutcher and Alexander 1990; Crutcher and DeLong 1984). It could be argued that with extensive practice, PANs show less movement-related activity and more activity linked to anticipatory aspects of task performance. Studies in rats have shown that many neurons in the dorsolateral striatum—the rodent homologue of the posterior putamen in primates—display gradual decreases in movement-related activity with overtrained instrumental behaviors (Carelli et al. 1997; Tang et al. 2007). It is possible that performance of a highly practiced movement sequence ultimately leads to a reduced number of PANs involved in the control of movements.

Our results show that task-related changes in PAN activity were distributed across the entire trial duration after extensive training. These results contrast with data from previous studies in behaving rodents showing that dorsolateral striatal neurons show task-related activities that may reflect stereotyped performance acquired with overtraining. In particular, these studies have highlighted dynamic changes in activity of PANs during the early and late phases of motor skill learning with task-related activations preferentially occurring at the start and end of T-maze overtrained performance (Barnes et al. 2005; Jog et al. 1999; Kubota et al. 2009). This reorganization of activity patterns has been interpreted as an influence on striatal processing involved in the development of automaticity. In our study, the continued presence of task-related PAN activities across the entire trial duration may have been the result of the task design, which allowed the continuous responding to target stimuli in the same trial block without introducing pauses into the animal's performance.

Another class of striatal neurons reported in the present study, referred to as TANs and thought to be cholinergic interneurons, displayed responses to the cue and/or the trigger stimulus when the movement sequence was repeatedly performed. There are two novel findings here regarding the sensitivity of TANs to task-relevant events. First, our data show that the proportion of TANs responding to the trigger stimulus (54%) was higher than that in a previous study (36–40%) using a much simpler reaching task in which stimulus-target location never varied (Sardo et al. 2000). Also the fraction of TANs responding to the cue markedly differs between the two studies (29% in the present study vs. 56–60% in Sardo et al.). These differences are probably due to the fact that our SRT task provides a higher degree of uncertainty as to the location of the trigger stimulus compared with a simple

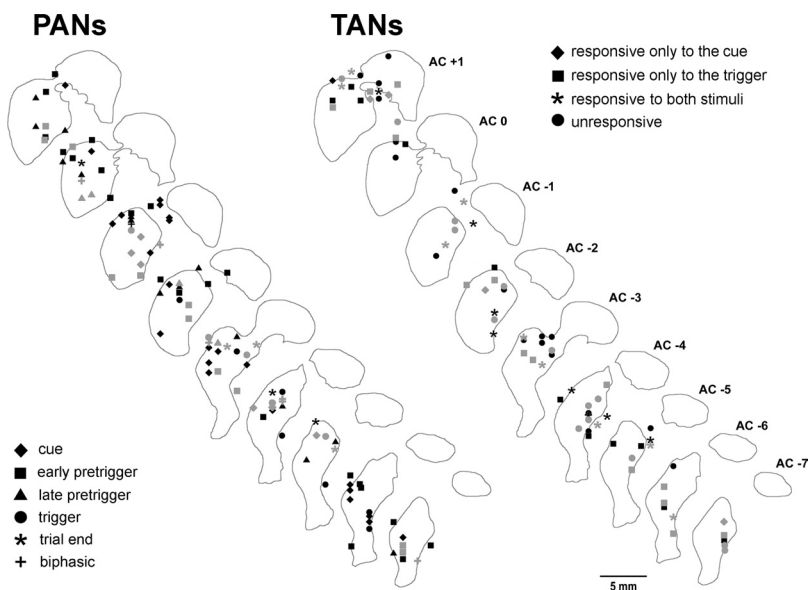


FIG. 9. Histological reconstruction of striatal neurons tested in the 2 conditions. Recording locations of all PANs (*left*) and TANs (*right*) from monkeys *R* and *P* are superimposed on coronal sections of the striatum. AC -7 to +1, levels posterior and anterior to the anterior commissure. Recording sites that yielded condition-dependent changes in activity are indicated with gray symbols.

reaction time task even if repeated movements in the same sequence allow the monkey to predict forthcoming trigger locations. In previous work, we demonstrated that TAN responses were strongly affected by the temporal predictability of task events with decreased responsiveness for high temporal predictability (Sardo et al. 2000). It therefore appears that the sensitivity of TANs is dependent on both the spatial and temporal aspects of stimulus prediction. Second, in contrast to previous studies showing that TANs may have responses that differed according to the location of the trigger stimulus and/or the direction of movement (Ravel et al. 2006; Shimo and Hikosaka 2001), we found that TANs appeared to be completely insensitive to the spatial location of the trigger stimulus in the current task. The question remains whether the lack of any spatial effect is due to the fact that the monkey was required to select one out of three serially ordered movements. In this regard, the response selectivity of TANs may vary as a function of task context (Lee et al. 2006; Ravel et al. 2006; Shimo and Hikosaka 2001; Yamada et al. 2003).

Influence of the serial order of stimulus-target locations on the task-related activity of PANs

We examined if PAN activations were influenced by changing the serial order of stimulus presentations. The results showed that a substantial number of PANs did not modify their activations in relation to the switch in movement sequence condition, whereas a subset of PANs were differentially activated. Importantly these sequence-dependent changes in PAN activity were expressed as modulations in the level of activation, while maintaining the temporal characteristics of the patterns of activity associated with task performance. We found only one neuron that was activated exclusively in the repeated sequence, and none of the neurons that did not show task-related changes in activity in the repeated sequence became activated in the random sequence. It is noteworthy that the task-related activity modulation occurred in relation to any of the four classes of PANs we have categorized, suggesting that these neurons continued to function in relation to different phases in task performance, possibly reflecting maintenance of

movement automaticity and changes in task-related activity found in our study might contribute to the behavioral adjustment when it is necessary to reestablish control over the automatized task performance.

Our results show that the number of PANs increasing their activity is similar to that of PANs with decreasing activity except for movement neurons, which always displayed higher activation in the repeated sequence than in the random sequence. These data suggest that the transition from automatic to controlled task performance has a more homogeneous influence on PAN activity related to the execution of movement than other behavioral components, such as preparation for and expectation of sensorimotor aspects of task performance. One possible interpretation of the opposite changes in activation observed among PANs in this study is that they reflect the relative activity of two functionally opposite pathways, the so-called "direct" and "indirect" pathways, one facilitating movement initiation and the other mediating movement suppression (Albin et al. 1989; Mink 1996). Although there is debate regarding the segregation between these two pathways (Bar-Gad et al. 2003), it could be speculated that reduced activity in one pathway and enhanced activity in the other one may lead to the inhibition or activation of the basal ganglia output structures, which in turn control movement selection and execution. This process may play an important role in the generation or suppression of competing motor responses when monkeys switched from an automatic action to a more controlled action that involves concurrent processing of inappropriate and required motor commands.

In the present study, we show that the striatal mechanisms involved in establishing control over an automated task performance are located in the sensorimotor part of the striatum or posterior putamen in primates. This result is in agreement with the findings of a recent study of Kubota et al. (2009), who have examined how the activity of PANs in the dorsolateral striatum changes as mice learned a new stimulus-response association in a previously well-practiced T-maze task. Although task-related activity patterns were relatively unaffected by the switch in sequence condition, a group of neurons showed changes in activity that are likely to reflect the changes in the

processing of information when task contingencies are altered. Our data support the suggestion made by these authors that this part of the striatum is not only central to the learning of habits and motor skills but also contains neuronal mechanisms that allow animals to flexibly adjust behavioral performance to a changing serial order of stimuli and movements.

As pointed out in METHODS, as soon a neuron was isolated, we first recorded its activity while the monkeys were performing the repeating sequence of movements and then shifted to the random sequence, suggesting that the order of testing could potentially introduced a bias in our analysis. However, both animals received extensive training sessions with the two sequence conditions before neuronal recordings started, and we continuously used one or the other sequence condition when searching for a neuron, the change in serial order being not indicated by any external signals. It therefore appears unlikely that time order is a confounding factor in our experimental design.

Finally, the possibility that sequence-dependent modulation of PAN activity may have been related to slowness of movement initiation that occurred with the switch in serial order cannot completely be ruled out in our study. However, we did not find a clear evidence for a relationship between the level of task-related activity and the monkey's performance speed, suggesting that differential activity may reflect the changes in the processing of information about the serial order of stimulus-target locations, independently of changes in task performance. On the other hand, decreased predictability of the location of the trigger stimulus is supposed to demand greater attention, and it is possible that the task-related activity modulation can be explained by at least partially differences in attentional motor control. In the repeated sequence condition, information about the target location was available, and therefore the attentional requirement was supposed to be minimized, resulting in a high degree of automatization in comparison with the random sequence condition. Monkeys exhibiting less stereotyped behavioral responses when sequential regularities were altered, and this might plausibly correspond to attentional modulation of striatal activity. However, the reported task-related changes in PAN activity were weaker or stronger with the switch in sequence condition, suggesting that the differential activities are not in a simple way related to different levels of attention. More information must be added to our data to determine to what degree striatal activity is dependent on attentional demands of the task.

Influence of the serial order of stimulus-target locations on the responses of TANs to task stimuli

We show here that the overall responsiveness of TANs to the cue and/or the trigger stimulus was maintained relatively constant when the monkeys switched from the repeated to the random sequences as was the fraction of unresponsive TANs. What changed was the response selectivity of individual TANs for one or the other stimulus. In approximately half the cases, the responsive TANs changed their response selectivity or lost their responses after the switch, whereas other TANs that were not responsive in the repeated sequence started responding with various degrees of selectivity in the random sequence. It therefore appears that the stimulus selective tuning functions of TANs are highly dynamic and may discriminate between different context established by the serial order of stimulus

presentations. The reconfiguration of TAN responses might favor more flexible behavior in a changing task context, particularly when monkeys are adapting to a serial order in which task stimuli are processed in a less automatic manner. These observations agree with recent data that emphasize the context dependency of TAN responses (Lee et al. 2006; Ravel et al. 2006; Shimo and Hikosaka 2001; Yamada et al. 2004). Previous studies have shown that TANs exhibit a range of response properties from stimulus features, such as spatial location and motivational value (Apicella 2007), to the detection of an error in the prediction of reward (Apicella et al. 2009; Joshua et al. 2008). This capacity to carry multiple signals may be critical for the detection of the task context.

Role of the striatum in movement automaticity

Functional brain imaging studies in humans have suggested that the posterior putamen is part of a brain network involved in the long-term storage of skilled movements, particularly during the later automatization phase of motor skill learning (Doyon et al. 2003; Floyer-Lea and Matthews 2004; Jueptner et al. 1997; Lehéricy et al. 2005; Poldrack et al. 2005). Patterns of increasing activation have been reported in both the primary motor cortex and putamen after extensive training in sequential tasks, suggesting that this cortico-striatal network becomes more active after a motor sequence is practiced extensively. Our findings indicate that two separate populations of neurons in the posterior putamen function with some degree of dependence to process information on the sequence of actions during habitual task performance. The question therefore arises as to what potential roles might PANs and TANs be playing in automatization of movement sequences and reestablishment of control over this automatized sequential behavior? We found that the activation of the majority of task-related PANs was independent of the serial order of stimuli and movements, suggesting that these neurons could be involved in the processing of invariant features of the task, such as the temporal structure of the task, which remained the same regardless of whether the movement sequence was repeated or not. In addition, a subset of PANs recorded from the same striatal region might contribute to performance adjustments when animals switched from automatic to controlled action. In this group of PANs, changes in activation level are likely to reflect the changes in the processing of information about the serial order of stimuli and movements. Another plastic change that occurred with this type of behavioral switching concerns the reconfiguration of the response selectivity of TANs. It is still unclear how such TAN signals are used to interact with task-related PAN activity for adapting behavior to changing sequence conditions. It is assumed that the TANs, presumed cholinergic interneurons, may impact the sensitivity of PANs to cortical inputs by modulating their state of excitability (Akins et al. 1990; Calabresi et al. 2000). This may reflect a potential influence on striatal processing involved in suppressing inappropriate automatic motor commands, while facilitating nonhabitual movements, then leading to performance adjustments. A disturbance of this mechanism in various movement disorders linked to dysfunction of the striatum can lead to an impaired ability to flexibly respond to changes in the environment.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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