Rhythmically Firing Neostriatal Neurons in Monkey: Activity Patterns During Reaction-Time Hand Movements

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Lebedev, M. A. and R. J. Nelson. Rhythmically firing neostriatal neurons in monkey: activity patterns during reaction-time hand movements. J. Neurophysiol. 82: 1832–1842, 1999. While previous studies have identified rhythmically firing neurons (RFNs) in monkey neostriatum and these rhythmic firing patterns have been shown to evolve in neostriatal tonically active neurons (TANs) after dopamine input depletion, the activity patterns of RFNs during motor behavior are still far from completely understood. We examined the single-unit activity patterns of neostriatal neurons, recorded in awake behaving monkeys during a wrist movement task, for evidence of rhythmic activity. Monkeys made ballistic wrist flexion and extension movements in response to vibrotactile cues. Animals held a steady wrist position for 0.5 to 2.0 s while awaiting the onset of the go-cues (hold period). Although the majority of neostriatal neurons (274/306) did not fire rhythmically, approximately 10% of the neurons (32/306) fired rhythmically at 10–50 Hz during the hold period. Most RFNs (28/32) showed significant activity changes during the time between go-cue presentation and movement onset (premovement activity). One-half of RFNs exhibited premovement activity that differed as a function of movement direction. Only one RFN may have responded to the delivery of a fruit juice reward. Neuronal firing was analyzed using interspike interval distributions, autocorrelations, and serial correlation techniques. These analyses showed that the activity patterns of most RFNs were consistent with an integrate-and-fire model of neuronal rhythm generation. Changes in RFN activity patterns during the premovement interval and intertrial variations in firing frequency could be explained by changes in the general level of excitatory input. These observations are consistent with the firing properties reported for neostriatal cholinergic interneurons. It has been suggested that tonically active neurons may be cholinergic interneurons and that these neurons show changes in activity related to specific aspects of behavioral paradigms, such as rewards. RFNs may constitute a special class of TANs. The results presented here suggest that RFNs may have a role in movement initiation. We speculate that RFNs may modulate the propagation of cortical oscillations via basal ganglia-thalamic-cortical loops.

INTRODUCTION

The phenomenon of synchronous neuronal oscillations has attracted much attention during the last decade (for review, see Engel et al. 1992, 1997; Farber 1998; Funke and Worgotter 1997; Gray 1994; MacKay 1997; Singer 1993; Singer and Gray 1995; Steriade 1993, 1997). Although neuronal oscillations have been extensively studied in cortical areas, oscillatory activity in some subcortical structures, such as neostriatum, is less well understood. The neostriatum may play an important role in regulating oscillatory interactions between cortical areas, because neostriatal neurons receive inputs from numerous cortical areas, and, in turn, ultimately project to the cortex. Thus, these neurons have the capacity to influence cortical activity via basal ganglia-thalamic-cortical loops (Alexander et al. 1986).

In this study, we sought to find rhythmically firing neurons (RFNs) in monkey neostriatum. Networks containing RFNs have been suggested as playing a role in initiating and propagating cortical oscillations (Llinas 1990, 1991). Previously, we demonstrated a subpopulation of monkey somatosensory cortical RFNs by using a task in which monkeys extended or flexed their wrists in response to vibrotactile stimuli (Lebedev and Nelson 1995). In that study, we found that the activity of cortical RFNs is disrupted at the onset of vibrotactile go-cues and/or prior to movement onset. We hypothesized that cortical RFNs may be tonically active inhibitory interneurons (but see a different interpretation in Ahissar and Vaadia 1990). Likewise, if any neostriatal neurons exhibited rhythmic firing during this behavior, these RFNs could be part of an electrophysiologically and morphologically unique subpopulation, for example, neostriatal interneurons.

Several schemes for classifying neostriatal neurons using temporal patterns of discharges have been proposed (Aldridge and Gilman 1991; Anderson 1977; Connor 1970; Crutcher and DeLong 1984; Hikosaka et al. 1989; Kimura et al. 1984; Wilson 1993). Aldridge and Gilman (1991) described a subpopulation of neostriatal neurons with clock-like regular firing patterns recorded in awake, quiescent monkeys. Moreover, exquisitely regular firing patterns of neostriatal neurons were seen in lesion experiments in which neostriatum was depleted of its cortical (Aldridge et al. 1990; Aldridge and Gilman 1991) or dopaminergic nigral inputs (Raz et al. 1996). Despite these studies, the activity patterns of neostriatal RFNs during movement initiation remain poorly understood. To elucidate the functional role of neostriatal RFNs, we analyzed the changes in their activity during the initiation of wrist movements.

METHODS

The behavioral paradigm has been described in detail elsewhere (Lebedev and Nelson 1995; Lebedev et al. 1994). Briefly, each of three adult rhesus monkeys (Macaca mulatta; subjects A, B, and N) sat in an acrylic monkey chair. Each animal’s hand rested on a manipulandum, which was attached to the axle of a torque motor (see Fig. 3F). A torque of 0.07 Nm, which assisted wrist extension and opposed flexion, was applied to the plate. A display consisting of 31 light-emitting diodes (LED) indicating that wrist position was placed 30 cm in front of the monkey at eye level. Each monkey was trained to hold a centered wrist position. After a delay of 0.5, 1.0, 1.5, or 2.0 s (chosen pseudorandomly), monkeys flexed or extended their wrists in...
response to vibration (27, 57, or 127 Hz) of their palms through the manipulandum. Single-unit activity was recorded in neostriatum (Fig. 1) using platinum–iridium microelectrodes (1–2 MΩ at 1.0 kHz) by conventional means (Lebedev and Nelson 1995). Neurons were classified as RFNs following analysis of their activity exhibited during the period of the task during which the monkey held a consistent wrist position and awaited a go-cue. Autocorrelation histograms (ACHs) (Mountcastle et al. 1969, 1990; Perkel et al. 1967; Poggio and Viernstein 1964) were constructed for this epoch. ACHs describe the likelihood of neuronal discharge occurrence after a given discharge. For RFNs, ACHs contain peaks at multiples of the rhythmic period (Ahissar and Vaadia 1990; Karmon and Bergman 1993; Lebedev and Nelson 1995; Perkel et al. 1967; Poggio and Viernstein 1964) (e.g., Fig. 2A, rightmost column). ACHs describe for spike trains using 1-ms bins for epochs of 250 ms and then were normalized so that ACH values represented correlation coefficients (Abeles 1982; Eggermont 1992; Palm et al. 1988). This normalization is preferable since correlation values can be compared across records of single neurons having different firing rates (Palm et al. 1988). ACHs were smoothed using a 10-point Gaussian filter (a conventional procedure for noise reduction; see Aldridge and Gilman 1991; Karmon and Bergman 1993; Raz et al. 1996). The first two ACH peaks and the valley between them were evaluated (Fig. 2A). The first peak was designated as the histogram maximum for the interval 0 to 150 ms. The second peak was designated as the histogram maximum for the interval from 1.5 to 2.5 times the time of the first peak (i.e., the expected second peak time ± one-half of the time of the rhythmic interval). This method facilitated the selection of the most probable (highest) second peak as opposed to smaller peaks, perhaps resulting from noise, for the rhythmically firing neurons. The valley was designated as the minimum histogram value between the first and second peak.

The selection of RFNs was done using K-means cluster analysis (Everitt 1980; Hartigan 1975) implemented in Systat, version 5.2. Standard scores were calculated for two variables: the difference between the magnitudes of the first peak and the valley and the period jitter. Using the peak-to-valley difference rather than the first peak height facilitated the selection of RFNs as opposed to bursty neurons, which we often observed. ACHs for bursty neurons contain prominent initial peaks (Aldridge and Gilman 1991; Wilson and Groves 1981). However, they lack a prominent second peak at twice the interval of the first peak and a valley between the first and the second peaks.

FIG. 1. Locations of the 306 recorded neostriatal neurons demarcated by autocorrelation histogram (ACH) classifications. The recording sites of these neurons are illustrated on two sets of line drawings of the histological reconstructions and on photomicrographs of coronal sections through the basal ganglia from one of the animals. Numbers beneath sections indicate the stereotaxic levels of the sections. Recording sites, in general, were located in the dorsal portion of the putamen, the dorsolateral part of the caudate nucleus, and the cellular bridges between these structures.
K-means clusters were specified because there are four possible combinations of high/low, peak-to-valley differences and high/low jitter values. High peak-to-valley differences and low jitters characterized the cluster containing the RFNs. The whole set of ACHs for the RFNs thus selected is presented in Fig. 2B (rightmost panel) as a surface plot. The units that fell in the other three K-means clusters were grouped together as nonrhythmically firing units. By visual inspection, nonrhythmically firing units were subdivided into the early peak (Fig. 2, leftmost column) and flat ACH types (Fig. 2, middle column). This selection is described in detail in Results. Because the first peak, the second peak, and the valley of the ACH occur at one, two, and one and a half rhythmic cycles, respectively, three estimates of the rhythmic period could be derived from these data: 1) the first peak time, 2) two-thirds of the valley time, and 3) one-half of the second peak time. The average rhythmic period and its jitter were calculated as the mean and the SD, respectively, of these three values. Neuronal activity patterns were further analyzed using interspike interval (ISI) histograms and the patterns’ characteristics: mean, median, and the coefficient of variation (e.g., Aldridge et al. 1990). To evaluate serial dependencies in neuronal spike trains, renewal density histograms (RDHs) were calculated and compared with ACHs. RDH is the ACH calculated after ISIs are randomly shuffled, that is, after the serial structure of the spike sequence is eliminated (Aldridge and Gilman 1991; Lebedev and Nelson 1995; Mountcastle et al. 1969, 1990; Perkel et al. 1967) (see Fig. 2A). In the current implementation, RDHs were calculated after both within-trial and across-trial hold period ISIs were shuffled. Serial dependencies of ISIs were visualized using joint ISI scatterplots, which displayed a given ISI on the x-axis and the subsequent ISI on the y-axis (Rodieck et al. 1962; Siebler et al. 1991; Surmeier and Towe 1987a,b) (Fig. 6, C and D). The relationship between immediately adjacent ISIs was analyzed using coefficients of serial correlation (Perkel et al. 1967; Surmeier and Towe 1987a,b).

Changes in activity patterns of RFNs related to task events were analyzed using conventional perievent time histograms (Fig. 3B) and several techniques that helped us to visualize the temporal structure of spike trains. Temporal patterns of activity were visualized using ISI scatterplots (see Lebedev and Nelson 1995) (Fig. 3A). In addition, we used joint perievent time histogram (JPSTH) methods previously developed to analyze dynamic correlations between pairs of neurons (Aertsen et al. 1989; Gerstein and Perkel 1969, 1972). A JPSTH is a two-dimensional plot in which the x- and y-axes represent spike occurrences of the first and second neurons in the pair, respectively. The time is measured in reference to a behavioral task event. In our implementation, both axes represented spike occurrences of the same neuron (Fig. 3E). Thus, these plots represent dynamic autocorrelations rather than cross-correlations. As such, a JPSTH can be thought of as a stack of instantaneous ACHs. The center of each instantaneous ACH is on the JPSTH major diagonal, and the off-center bins are on the line crossing the center, perpendicular to the major diagonal. JPSTHs were
normalized using conventional algorithms, so that their bins (binwidth was 5 ms) represent correlation coefficients (Aertsen et al. 1989). Cumulative sum methods were used to detect the onset of neuronal activity changes (Lebedev and Nelson 1995).

RESULTS

A total of 306 neurons were recorded in the neostriatum. In Fig. 1, the locations of recorded neurons demarcated by ACH classifications are illustrated on sets of line drawings of the histological reconstructions for two animals and on photomicrographs of coronal sections through the basal ganglia for the third animal. Recording sites, in general, were located in the dorsal portion of the putamen, the dorsolateral part of the caudate nucleus, and the cellular bridges between these structures. Of the 306 neurons, 32 (10%) were classified as RFNs. RFNs were observed in the caudate nucleus, bridge, and putamen with equal frequency (Table 1). Nineteen RFNs were tested for peripheral receptive fields (RFs) by manipulating the animal’s forelimb skin surfaces and single joints. Of these RFNs, approximately equal proportions either had no clear RF (10/19, 53%) or responded to the bending of a single joint (8/19, 42%), whereas only one neuron had a cutaneous RF on the hand.

In Fig. 2B (rightmost column), ACHs for the whole population of RFNs are presented as a surface plot. An initial refractory period, at least two ACH peaks and a valley between them can be seen. Rhythmic periods ranged from 17 to 78 ms (across-unit statistics: mean ± SD: 40.5 ± 13.0 ms; median, 40.6 ms). This corresponded to the frequency range 12–58 Hz (mean ± SD: 27.5 ± 9.8 Hz; median, 24.6 Hz). The rhythmic frequency, calculated as the inverse of the rhythmic period, was 1.5 ± 0.1 times higher than the mean firing rate (MFR) for the hold period. This difference between the rhythmic frequency and the MFR indicated the presence of pauses (long ISIs) in otherwise rhythmic spike trains. Because of the pauses, MFR, derived from the spike count, was typically lower than the rhythmic frequency, which was derived from the ACH periodicity. The ISI distributions for RFNs were typically unimodal, with “tails” corresponding to pauses in activity (e.g., Fig. 2A). However, in three cases, clearly bimodal ISI distributions were seen, with the modes corresponding to the rhythmic period and twice its value. ISI counts at these modes were approximately equal. The average rhythmic frequency for these three unique neurons was higher than that for the remainder of the population (51.3 ± 7.7 versus 25.9 ± 9.5 Hz; \( P = 0.0001 \); one-factor ANOVA, Scheffe post hoc test).

Nonrhythmically firing neurons constituted the majority of the sample (274/306, 90%). Using the classification of ACHs, the rhythmic activity of these neurons was divided into four categories: early peak ACH, flat ACH, and single joint and cutaneous RFs. Table 1 summarises the distribution of these categories across the anatomical regions of the neostriatum. The number of neurons classified in each category is shown in parenthesis. The percentage of neurons within each category is given in the column labelled Total.

### Table 1. Recorded neurons

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total (n = 306)</th>
<th>Putamen (n = 166)</th>
<th>Caudate Nucleus (n = 91)</th>
<th>Bridge (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhythmically firing</td>
<td>19/166 (11)</td>
<td>8/91 (9)</td>
<td>5/49 (10)</td>
<td>13/49 (27)</td>
</tr>
<tr>
<td>RF tested</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Single joint RF</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8/19 (42)</td>
</tr>
<tr>
<td>Cutaneous RF</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1/19 (5)</td>
</tr>
<tr>
<td>No clear RF</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0/10/19 (53)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
dridge and Gilman (1991), these were subdivided qualitatively into the early peak ACH (84/306, 28%) and flat ACH (190/306, 62%) types (Table 1, Fig. 2). Early peak ACH neurons can be described as bursty neurons (Wilson and Groves 1981; Wilson 1993). For these neurons, ACHs contained initial elevations that stabilized over ~150 ms, a time corresponding to the characteristic burst duration. In agreement with the results of Aldridge and Gilman (1991), the initial peaks were lower in RDHs than in ACHs. Thus, these activity patterns essentially depended on the sequential arrangement of ISIs into groups of short ISIs (bursts) and longer ISIs (interburst intervals). None of the selected RFNs had these qualitative features of bursty firing patterns. Some of the ACHs classified as flat had initial rising parts of peculiar shapes that often changed after spike shuffling. Examples of such shapes previously have been reported (e.g., see Fig. 2B in Raz et al. 1996). Because these shapes were difficult to classify, we did not subdivide the flat ACH type any further.

ISI distribution characteristics—mean and median ISI and the coefficient of variation of ISIs—have been used to classify neostriatal neurons (Aldridge and Gilman 1991). The neuronal groups selected in our study differed as to these characteristics (Table 2). Most notably, RFNs had the lowest coefficients of variation of ISIs, whereas the early peak ACH neurons had the highest. In addition, for early peak ACH neurons, mean and median ISIs were markedly different (mean ISI ~1.5 times the median ISI), whereas this difference was less for RFNs (mean ISI ~1.05 times the median ISI). These results are consistent with the regularity of the ISIs for RFNs and the greater variability of the ISIs for the early peak ACH neurons and flat ACH neurons.

Neostriatal neurons often are classified using MFR as one of the criteria, the measurements of MFR being made during the periods when an animal is not engaged in task performance (Aosaki et al. 1994, 1995; Raz et al. 1996). Because, in these experiments, it was difficult to produce a state of “no motor behavior” without disrupting the overall task performance, neuronal activity outside of task performance was not recorded. Therefore, direct comparison of the findings below to those of previous studies cannot be done with absolute certainty. However, it is worth noting that the types of neurons selected by us were significantly different in their MFRs (Table 2). RFNs had the highest MFRs during the hold period of the task (~17 spikes/s), whereas the early peak ACH neurons had the lowest (~11 spikes/s). MFRs of the majority of RFNs were >10 spikes/s (30/32, 94%), whereas the corresponding proportion for the early peak ACH neurons (39/84, 46%) was significantly less (P < 0.0001; chi-squared test). The flat ACH neurons exhibited a wide distribution of MFRs spanning the MFR ranges of the RFNs and the early peak ACH neurons and averaging ~15 spikes/s. MFRs were not statistically different as a function of the neuron’s location (putamen, caudate nucleus, or bridge).

Figure 3 illustrates some common characteristics of the firing patterns of RFNs during the behavioral task performance. During the epoch in which the animal actively held its hand steady and awaited the vibratory go-cue, the ISIs of this neuron were distributed around a central value of ~50 ms. This neuron’s firing rate increased prior to the onset of extension movements (Fig. 3B) and was not changed prior to flexion movements (not shown). As can be seen from the ISI scatterplot (Fig. 3A), ISI distribution center gradually shifted in correspondence to the firing rate change. JPSTH analysis (Fig. 3E) indicated that the peak-valley-peak pattern in the autocorrelogram persisted as the neuron’s firing rate increased. Rhythmic frequency at a given time can be determined by drawing a line perpendicular to the major diagonal of the JPSTH (see Methods) and estimating the distance between the peaks along this line. By this methods of visual inspection of the JPSTH, it can be seen that the rhythmic frequency of this neuron increased from ~20 to ~80 Hz. We term this type of rhythmic firing change a regular transition because it resembles the firing pattern of cortical regular spiking neurons (McCormick et al. 1985). These latter neurons exhibit very regular, rhythmic discharges on depolarization with constant intracellular currents and rhythmic frequency increases with larger current. During regular transitions, a neuron’s rhythmic frequency is linearly related to its firing rate. For RFNs, we observed a linear relationship between the MFR and the rhythmic frequency. These characteristics, compared during the hold period of the task, showed a highly significant correlation (Pearson correlation coefficient = 0.75; P < 0.0001). Elsewhere, a different type of transition has been reported for premotor cortical neurons (Lebedev and Wise 1998). For these neurons, the rhythmic frequency remained nearly constant during firing rate changes in individual units and also across units. Moreover, transitions from rhythmic to nonrhythmic firing patterns have been described for primary somatosensory cortical RFNs (Lebedev and Nelson 1995). For 26 of 32 (93%) RFNs, the rhythmic patterns of activity appeared to undergo regular transitions, as judged by visual inspection of ISI scattergrams and JPSTHs. Eight examples of these ISI plots with gradual shifts

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Firing Rate During Hold Period (BKG; spikes/s)</th>
<th>Mean Interspike Interval During BKG (μsI; ms)</th>
<th>Median ISI During BKG (Median ISI; ms)</th>
<th>Coefficient of Variation During BKG</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFNs (N = 32)*</td>
<td>17.4 ± 5.6 (3)</td>
<td>42.6 ± 13.6 (2.3)</td>
<td>40.5 ± 11.7 (NS)</td>
<td>.34 ± .013 (2.3)</td>
</tr>
<tr>
<td>Flat ACH (N = 190)*</td>
<td>15.4 ± 10.8 (3)</td>
<td>59.6 ± 50.1 (1)</td>
<td>48.7 ± 38.7 (NS)</td>
<td>.59 ± .016 (1.3)</td>
</tr>
<tr>
<td>Early peak ACH (N = 84)*</td>
<td>11.4 ± 8.6 (1.2)</td>
<td>66.4 ± 42.1 (1)</td>
<td>42.7 ± 22.8 (NS)</td>
<td>.89 ± .019 (1.2)</td>
</tr>
</tbody>
</table>

Values are means ± SD; numbers in parentheses indicate statistically significant differences in values from entries corresponding to numbers (factorial ANOVA with Games/Howell post hoc test; P < 0.05). NS, not significant. * One entry per neuron (records from movement trials in each of two directions combined).
in ISI distribution centers are provided in Fig. 4, along with the locations at which the neurons showing these regular transitions were recorded. For one of the illustrated neurons (neuron 8), note a transition from a bimodal to a unimodal ISI distribution along with an increase in rhythmic frequency ~200 ms prior to movement onset. Transitions from bimodal to unimodal ISI distributions also were observed for two other neurons. Transitions to nonrhythmic activity patterns were seen for only 2 of 32 (7%) neurons.

Modulations of activity related to the onset of wrist movement and its direction were common for RFNs (Fig. 5). For the majority of RFNs (28/32, 88%), the earliest changes in activity preceded movement onsets. When cases involving flexion and extension movements were grouped together, activity changes occurred at 159 ± 83 ms prior to movement onset, that is, 180 ± 68 ms after go-cue onset. The timing of premovement activity changes was not significantly different as a function of ACH classification (Table 3) nor was the sign of the premovement activity change relative to the activity exhibited during the hold period (Table 4). These results for timing of activity changes are in correspondence to the results of a previous analysis done for a portion of the non-RFNs (Gardiner and Nelson 1992). Thus, RFNs did not exhibit any unique properties in the timing of their task-related activity. However, the degree of firing rate modulation in RFNs was different from the other unit types. Firing rate modulations during premovement activity were expressed as the ratio of the MFR during 100 ms after activity onset to that during 100 ms before activity onset. This ratio was on average less for RFNs than for the early peak and flat ACH neurons for cases of rate increase (Table 3). No significant difference was found for cases of exhibiting MFR decrease.

Neostriatal neurons with high background firing rates often exhibit responses to reward delivery (Aosaki et al. 1994, 1995; Kimura et al. 1984). Aligning the activity records on reward revealed that there was as much activity surrounding the reward onset as there was around the movement onset (Fig. 5). However, no consistent activity change aligned with the reward was found for the population of RFNs. Moreover, the activity patterns commonly depended on the direction of wrist movement. It should be noted that, at the time of reward delivery, the wrist movement continued (see position traces in Fig. 3, C and D). Thus, the reward-related activity modulations, if present, could be superimposed on the movement-related activity, making the former difficult to discriminate. In only one instance was there any evidence that a neuron changed firing rate immediately after the delivery of the fruit juice reward. Rasters for this neuron showed one or two spikes in some trials that were aligned with reward delivery and occurred at a latency of about 25 ms.

Serial dependencies in the spike trains of RFNs were analyzed to examine the possibility that rhythmic activity patterns in these neurons were evoked by an external source, for example, a rhythmic drive from the cortex. In the case of an external rhythmic drive, negative serial correlation of ISIs may occur (Lebedev and Nelson 1995, 1996; Lebedev and Wise 1998; Surmeier and Towe 1987a,b). The joint ISI scattergrams for RFNs typically contained a cluster of dots along the major diagonal, indicative of positive serial correlation (Fig. 6C). For the total set of RFNs, the mean of the coefficients of serial correlation was 0.164 (SD, 0.105). In addition, 30 of 32 (94%) of the coefficients of serial correlation were positive. These positive serial correlations appeared related to changes in ISI distributions across trials (Fig. 6, A and B). Thus, the following analysis was conducted. The mean of a trial’s ISIs was subtracted from each of that trial’s ISIs. The resultant joint ISI plots did not exhibit features of positive correlation (Fig. 6D). The serial correlation coefficients calculated for these normalized data were, on the average, negative (mean ± SD: 0.120 ± 0.085). We conclude, therefore that for short epochs (exhibiting intratrial variations), ISIs were negatively correlated. That is, a short ISI was likely to be followed by a longer ISI in a given trial. For longer epochs (exhibiting intertrial variations), ISIs could be described as positively correlated in the sense that short ISIs were grouped with short ISIs and long ISIs with long ISIs in their corresponding trials.

DISCUSSION

This study of the activity patterns of single neostriatal neurons recorded in awake, behaving monkeys showed that slightly over 10% of neurons exhibited sustained rhythmic firing when monkeys actively held against a load awaiting a go-cue. This rhythmic firing was often modulated ~160 ms prior to movement onset. This modulation occurs approximately in the middle of the reaction time period that averaged 337 ± 73 ms. The rhythmic frequency changes accompanied task-related changes in the firing rate. This pattern of rhythmic frequency transition was termed a regular transition because it resembles firing frequency changes in cortical regular spiking neurons injected with intracellular currents (McCormick et al. 1985). The simplest model of such transition is the integrate-and-fire model in which rhythmic frequency increases with increases in input strength (Segundo et al. 1968; Softky and Koch 1993). Also consistent with an integrate-and-fire model is our observation that mean ISIs drift somewhat from trial to trial. Mean ISI drifts can be explained sufficiently by changes in the level of excitatory inputs to integrate-and-fire neurons.

Certain lines of evidence suggest that RFNs may be interneurons, a heterogeneous cell type (for review, see Kawaguchi et al. 1995). It is commonly believed that the principal neurons of neostriatum, the medium spiny neurons, are unlikely to exhibit regular firing because of their electrophysiological properties and the characteristics of their cortical inputs (for review, see Wilson 1993). It has been demonstrated that, in urethane-anesthetized rats, medium spiny neurons typically fire in bursts (Stern et al. 1997; Wilson 1993; Wilson and Groves 1981). Bursty firing patterns have been described for the majority of neostriatal neurons, presumably medium spiny neurons, recorded in awake monkeys (Aldridge and Gilman 1991). Our analysis, which could depict bursts shorter than 250 ms, indicated such was the case for ~28% of the neuronal sample. Bursty neurons had the highest degree of task-related modulation of firing rate. These neurons are likely to be principal neurons. Neostriatal interneurons, unlike principal neurons, may be better suited electrophysiologically for generating rhythmic patterns of activity. Cholinergic interneurons, for instance, have prominent afterhyperpolarizations (Bennett and Wilson 1998; Wilson et al. 1990). Thus, given a steady excitatory input, cholinergic interneurons tend to fire rhythmically in an integrate-and-fire mode. In addition, the proportion of rhythmically firing neurons that we saw is roughly consistent
FIG. 4.  A: interspike intervals (ISI) scattergrams for 8 of the 28 rhythmically firing neurons that showed smooth transitions in ISI distributions as a function of time relative to onset of wrist movement. The left column shows the records of four neurons that decreased their firing rates before movement; the right column shows four examples of records from neurons with firing rate increases.  B: line drawings of coronal sections at stereotaxic levels indicated by the lower numbers in each, along with the recording locations for the examples shown in A.
TABLE 3. Characteristics of activity for neurons having premovement activity changes, by groups

<table>
<thead>
<tr>
<th></th>
<th>Mean Firing Rate During Hold Period (BKG; spikes/s)</th>
<th>Coefficient of Variation During BKG</th>
<th>Time of Activity Change Relative to Movement Onset (ms)</th>
<th>Mean Firing Rate for 100 ms Before Activity Change</th>
<th>Mean Firing Rate for 100 ms After Activity Change</th>
<th>Ratio of Mean Firing Rates (After/Before)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RFNs (26/32)</td>
<td>22.5 ± 7.2</td>
<td>0.34 ± 0.12</td>
<td>-128.1 ± 69.8</td>
<td>27.0 ± 9.5</td>
<td>34.6 ± 14.9</td>
<td>1.3 ± 0.33</td>
</tr>
<tr>
<td>2. Flat ACH (181/242)</td>
<td>17.1 ± 13.1</td>
<td>0.53 ± 0.16</td>
<td>-171.9 ± 116.4</td>
<td>23.0 ± 15.5</td>
<td>34.9 ± 22.8</td>
<td>2.1 ± 2.9</td>
</tr>
<tr>
<td>3. Early peak ACH</td>
<td>12.9 ± 10.3 (1,2,4,5)</td>
<td>0.70 ± 0.19 (1,2,4,5)</td>
<td>-172.0 ± 94.2</td>
<td>18.4 ± 13.3</td>
<td>38.4 ± 28.3</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(NS)</td>
<td>(NS)</td>
<td>(1)</td>
<td>(4,5,6)</td>
<td>(4,5,6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1,3,4,6)</td>
<td>(NS)</td>
<td>(6)</td>
<td>(4,5,6)</td>
<td>(1,4,5,6)</td>
</tr>
<tr>
<td>4. RFNs (24/32)</td>
<td>22.4 ± 7.7 (3)</td>
<td>0.27 ± 0.11 (2,3,5,6)</td>
<td>-192.0 ± 86.0</td>
<td>20.0 ± 7.1</td>
<td>14.0 ± 7.2</td>
<td>0.68 ± 0.24</td>
</tr>
<tr>
<td>5. Flat ACH (102/138)</td>
<td>20.9 ± 13.6 (3)</td>
<td>0.50 ± 0.14 (1,3,4,6)</td>
<td>-176.0 ± 109.6</td>
<td>18.5 ± 11.6</td>
<td>12.6 ± 11.0</td>
<td>0.63 ± 0.26</td>
</tr>
<tr>
<td>6. Early Peak ACH</td>
<td>18.4 ± 10.1 (NS)</td>
<td>0.69 ± 0.11 (1,2,4,5)</td>
<td>-200.5 ± 97.0</td>
<td>15.4 ± 9.0</td>
<td>9.9 ± 8.3</td>
<td>0.61 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SD; numbers in parentheses indicate statistically significant differences in values from entries corresponding to numbers (factorial ANOVA with Games/Howell post hoc test; P < 0.05). NS, not significant. * One entry per neuron for values from movement trials in each of two directions.

FIG. 5. Surface-plot representations of the peri-event time histograms for the whole population of rhythmically firing neurons (n = 32). A: flexion trials. Centering is on movement onset. B: extension trials. Centering is on movement onset. C: flexion trials. Centering is on issuance of the pulse that activated the reward solenoid. D: extension trials. Centering is on reward onset. The histograms were ranked by mean firing rate during the hold period in A. For panels B, C, and D, the same order is kept.
with the proportion of neostriatal cells thought to be aspiny interneurons (Graveland and DiFiglia 1985; Kemp and Powell 1971).

The regular transitions in RFN firing, which were associated with wrist movements, correspond well to the properties of cholinergic interneurons described by Wilson et al. (1990) (also see Bennett and Wilson 1998). These authors studied cholinergic interneurons in vivo in urethane-anesthetized rats. Although spontaneous rhythmic firing was not observed under these conditions, it was predicted that cholinergic interneurons may fire rhythmically if they are depolarized so that their firing is determined by spike afterhyperpolarization. A rhythmic firing frequency of \(~16\) Hz was predicted. This frequency corresponds to the lower frequency range of RFNs. The same authors evoked rhythmic firing in cholinergic interneurons by injecting current pulses. They saw firing frequencies of 15 to 100 Hz that were linearly dependent on the amount of injected current, i.e., regular transitions. This frequency range corresponds well to the frequencies that we saw for RFNs during execution of the behavioral task in our study. Moreover, the negative serial correlations of ISIs in individual trials may be related to the spike frequency adaptation in cholinergic interneurons (Bennett and Wilson 1998; Wilson et al. 1990). We conclude therefore that there is reasonable evidence to suggest that RFNs and cholinergic interneurons may be the same type of cell.

Could the rhythmic firing patterns in RFNs be evoked by an external rhythmic drive, for example, by rhythmic cortical input? Elsewhere (Lebedev and Nelson 1995, 1996) we have discussed the features of serial correlations of ISIs that could indicate external rhythmic drive. Recently these features have been demonstrated for premotor cortex neurons (Lebedev and Wise 1998). One of these features, negative serial correlation within trials, was indeed found for RFNs, but only after the normalization procedure, which corrected for ISI drifts across trials. As discussed above, this negative serial correlation could be due spike frequency adaptation. Other features such as diagonal bands in the joint ISI scatterplot and multimodal ISI distribution typically were not seen. In addition, cortical oscillations wax and wane (Murthy and Fetz 1992; Sanes and Donoghue 1993), whereas the activity of RFNs was sustained during the hold period of the task and was modulated in

<table>
<thead>
<tr>
<th>Recorded Neurons</th>
<th>Putamen (n = 19)</th>
<th>Caudate Nucleus (n = 8)</th>
<th>Bridge (n = 5)</th>
<th>Total (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant premovement activity changes</td>
<td>17/19 (89)</td>
<td>7/8 (88)</td>
<td>4/5 (80)</td>
<td>28/32 (88)</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>2/17 (12)</td>
<td>2/7 (29)</td>
<td>1/4 (25)</td>
<td>5/28 (18)</td>
</tr>
<tr>
<td>Directional</td>
<td>6/17 (35)</td>
<td>5/7 (71)</td>
<td>0/4 (0)</td>
<td>11/28 (39)</td>
</tr>
<tr>
<td>Nondirectional</td>
<td>9/17 (53)</td>
<td>0/7 (0)</td>
<td>3/4 (75)</td>
<td>12/28 (43)</td>
</tr>
<tr>
<td>Significant activity changes with movement*</td>
<td>6/19 (32)</td>
<td>3/8 (38)</td>
<td>0/8 (0)</td>
<td>9/32 (28)</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>1/6 (16)</td>
<td>1/3 (33)</td>
<td>—</td>
<td>2/9 (22)</td>
</tr>
<tr>
<td>Directional</td>
<td>4/6 (67)</td>
<td>2/3 (67)</td>
<td>—</td>
<td>6/9 (67)</td>
</tr>
<tr>
<td>Nondirectional</td>
<td>1/6 (16)</td>
<td>0/3 (0)</td>
<td>—</td>
<td>1/9 (22)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. * As defined in Gardiner and Nelson (1992).
association with wrist movements. By contrast, in premotor oscillatory neurons, rhythmic frequency often remains constant during task-related firing rate modulations (Lebedev and Wise 1998). Thus, there is not sufficient evidence to suggest an external rhythmic drive to RFNs. However, it is possible that rhythmic inputs, for example, cortical inputs, could modulate the activity of RFNs.

In a series of studies conducted by Kimura and colleagues (Aosaki et al. 1994, 1995; Kimura et al. 1984), it was suggested that tonically active neurons (TAN) in the neostriatum are cholinergic interneurons. How do RFNs compare with TANs? TANs are identified by their high firing rates (2–10 spikes/s), when compared with the rest of population (e.g., Aosaki et al. 1994). They discharge tonically but nonrhythmically. Firing rates of neostriatal neurons in the present task (~15 spikes/s) were somewhat higher than those previously reported. The higher firing rates in this task are probably related to the requirement to actively maintain hand position against a load of 0.07 Nm. In the tasks implemented by others, monkeys often sat quietly (Aldridge and Gilman 1991) or sat quietly and awaited a reward (Aosaki et al. 1994). It is possible that, under conditions of increased task-related excitatory input, TANs could begin to fire rhythmically like RFNs (Bennett and Wilson 1998; Wilson et al. 1990). We, however, did not observe nor document clear differences in the extracellular action potential duration of RFNs compared with non-RFNs, although longer action potential duration is a part of the classical definition of TANs (Crutcher and DeLong 1984; Kimura et al. 1984). Thus, the relationship between RFNs and TANs awaits further study.

Does the rhythmic pattern of activity of RFNs have a functional role? An intriguing possibility is that RFNs could regulate oscillations in other groups of neostriatal neurons. Indeed, cholinergic interneurons are strategically located at the borders of neostriatal compartments (patch and matrix) and influence the activity of neurons in both (Kawaguchi 1993; Kubota and Kawaguchi 1993). Given that cortical neuronal populations periodically oscillate, the activity of neostriatal neurons could become entrained to these oscillations. The activity of RFNs may be modulated during these oscillatory episodes, especially if the evoked oscillation frequency and RFN frequency are similar. In addition, RFNs could interact with the evoked oscillations in medium spiny neurons, for example, by a phase-locked loop mechanism (Ahissar and Vaadia 1990). This hypothesis has yet to be tested. Raz et al. (1996) reported that groups of TANs may fire at a high level of synchrony—a potentially powerful mechanism for regulating network activity. Moreover, after dopamine depletion, TANs exhibit synchronous oscillations at ~16 Hz (Raz et al. 1996). Thus, the oscillations in TANs (and possibly RFNs) appear to be under dopaminergic control.

Other classes of neostriatal neurons may have contributed to the present RFN sample. Bimodal distributions of ISIs observed for some of the higher firing rate RFNs (also see Aosaki et al. 1995) do not fit the integrate-and-fire pattern of activity, which presumably is characteristic for cholinergic interneurons (Kawaguchi 1993; Kubota and Kawaguchi 1993). These neurons may have been some other type of neostriatal interneurons.

Recent reports suggest that TANs in the neostriatum develop responses to relevant parts of behavioral tasks after some acquisition period and maintain these associations even after long lapses in task performance (Aosaki et al. 1994, 1995). Most notably, TANs are thought to develop responses associated with sensory stimuli coupled to rewards. In our experiments, we were not able to demonstrate reward-related responses except for one of the RFNs. However, premovement changes in activity were observed for the majority of RFNs. Moreover, these premovement activity changes occurred at approximately the same time as the premovement activity changes in other neostriatal neurons in this task (Gardiner and Nelson 1992). We suggest that RFNs have a role in movement initiation. Whether or not RFNs alter their firing patterns during behavioral conditioning requires further study.

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