Activity of Pars Reticulata Neurons of Monkey Substantia Nigra in Relation to Motor, Sensory, and Complex Events

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SUMMARY AND CONCLUSIONS

1. The behavioral involvement of neurons in the pars reticulata of the substantia nigra (SNpr) in sensory and motor processes was investigated in order to contribute to the understanding of behavior-related neuronal mechanisms in the basal ganglia, of which the SNpr is a major output station.

2. Electrophysiological properties of SNpr neurons were studied with extracellular recordings from single neurons in monkeys performing in a behavioral GO/NO-GO paradigm, employing significant auditory and visual stimuli, forelimb reaching movements, and mouth movements.

3. Neurons in the SNpr discharged impulses of 0.6 to 1.0 ms in duration, at rates of 23 to 145/s (median 68/s). They contrasted with dopaminergic pars compacta neurons of substantia nigra, which discharged longer impulses at rates below 8/s.

4. Two-thirds of more than 100 quantitatively and statistically evaluated SNpr neurons showed changes with temporal relationships to at least one of the events of the behavioral task. One-fifth of all neurons covaried with more than one event.

5. The largest group of task-related neurons increased or decreased activity with contralateral forelimb movements (46% of all neurons). Most of them covaried with large forward reaching of the arm, and only the minority covaried with distal forearm manipulation. None of the SNpr neurons resembled in their changes the timing of activity of single muscles. Many neurons also showed changes with ipsilateral movements, in a similar fashion as on the contralateral side. Changes began rarely before and mostly with or after onset of muscle activity in prime movers. Changes were quantitatively moderate, rarely exceeding a doubling of discharge rate with increases and reductions by 50% with decreases. Movement relations did not appear to be due to somatosensory input.

6. Some neurons increased or decreased their activity in relation to mouth movements (16% of all neurons). Changes were quantitatively moderate as in the case of movement relationships.

7. Nine percent of all neurons decreased their activity after an acoustic preparatory signal and 9% after a light stimulus indicating the GO or NO-GO situation. Latencies were 50–150 ms (median 90 ms) for the auditory and 80–200 ms (median 120 ms) for the visual responses.

8. Twelve percent of all neurons increased or decreased their activity during the waiting and preparation period, which lay between an initial sensory signal and the permission to move for reward. Changes began mostly in response to an initial preparatory tone or an instructive light stimulus indicating the behavioral situation. A few other neurons were also related to complex behavioral processes by having different relationships to movements under different behavioral conditions.

9. The data suggest that SNpr neurons are related in their activity to components of mammalian behavior dealing with the preparation of behavioral acts, sensory reception, and motor reactions. These relationships are interpreted to be due to neuronal processing in the major input structures, the caudate and putamen, and in the SNpr. The demonstrated behavioral relationships of SNpr neurons are basal ganglia influence other brain structures.

INTRODUCTION

The mammalian basal ganglia receive neuronal information from virtually all areas of primary and associational cortex as well as from some thalamic nuclei. They subserve a variety of sensorimotor and "higher" functions (9). For many years it was believed that most neuronal information is transmitted from the basal ganglia to other brain centers via the globus pallidus. Recently, it has been shown that the pars reticulata of the substantia nigra (SNpr) may represent another major output station (10). Reticulata neurons receive mostly inhibitory afferents from the striatum and the subthalamic nucleus (27, 30, 43) and project to medial parts of ventrolateral and ventroanterior thalamic nuclei, superior colliculus, and nucleus tegmenti pedunculopontis pars compacta (5, 10, 26).

Previous studies have investigated several aspects of the behavioral involvement of SNpr neurons. Neurons of the cat and monkey SNpr were found to be depressed in relation to goal-directed saccadic eye movements and in response to visual and acoustic stimuli (12, 16). Several of the visual and saccadic responses were contingent on memory-related processes (13). In studies dealing with motor-control aspects of cat and monkey basal ganglia, very few SNpr neurons were found to change their activity in relation to distal forelimb movements (7, 37). However, a considerable involvement in movements of the mouth was found (7). Also, SNpr neurons in the cat covaried with phases of locomotion on a treadmill (37). The present work was intended to study SNpr functions in a broader behavioral context without focusing on one particular aspect. If the SNpr represents an output station of the basal ganglia, it would be necessary to know if other information besides that dealing mainly with eye and mouth movements is transmitted to other brain centers. In particular, the conduction of information from SNpr via ventral anterior thalamic areas (5) to the frontal cortex (17) would suggest a larger involvement in processes dealing with the preparation and execution of different motor acts. An additional objective for this investigation was to be able to compare the behaviors of the substantia nigra (SN), the SNpr, and the dopamine cell-containing pars compacta in the same behavioral situation (36). Some of the data have been presented in preliminary form (34, 35).

METHODS

Four *Macaca fascicularis* monkeys (2.5–4.5 kg) were trained to perform a behavioral task, were implanted with intramuscular electrodes and an electrode base for daily single-cell recordings in SNpr in relation to a behavioral task, and on completion of the experiment, were killed for histological assessment of the recording area. One of these and an additional monkey were used for the recording of task-related eye movements. Many techniques were similar to those previously reported (36).

Animals were seated 3–6 h each weekday in a specially constructed primate chair, which allowed free movements of both arms, limited excursions of the legs, and some postural adjustments. They were released each day into their home cages. Animals were trained to perform in a GO/NO-GO paradigm involving indicative light stimuli, a reaching movement of the forearm towards a small food-containing box, and mouth movements (see Fig. 1, *A–D*). Food rewards consisted of ~1/100 to 1/150th part of an apple. Animals were food deprived on weekdays, their weight being controlled and maintained above 90% of the preexperimental level.

For initiation of a trial, the animal had to keep its hand relaxed on a key. The key was either a telegraph lever, whose mechanical resistance for closing an electrical contact was spring adjusted to the weight of the animal's forearm, or a nonmovable metal bar connected to an electronic frequency-sensing circuit that detected the touch of the animal's hand as a change in electrical capacity. Each trial was initiated by a 100-ms-long tone from a distant sound source of 66 to 67 dB intensity, measured at the head of the animal. This was followed 500 ms later by illumination of either a green or a red light-emitting diode, indicating the GO or the NO-GO situation, respectively. Lights were placed 10 mm above each other at the animal's eye level, one pair on each side, at 19° laterally. While the light stayed on and at 1.5–5 s after the tone, the door of a food-containing box opened with a characteristic low-intensity sound. Food boxes had a frontal opening of 40 × 40 mm and were placed at eye level, at 27° laterally, and at reaching distance of the forearm (250 mm from the animals' shoulders), one on each side. Animals were unable to reach into the box of the opposite side and were prevented from seeing the interior of the box by a

box from below (see Fig. 1, *A–D*). In the GO situation, when the green light was illuminated, the animal released the holding key after door opening, reached into the box, took the food and brought it to the mouth. In the NO-GO situation, when the red light was illuminated, the animal kept the hand on the key after the door had opened, only reaching for food reward a few seconds later when this was presented from a more medially located, uncovered box, or by the experimenter. Lights were extinguished on key release. Premature key release or muscle activity canceled the trial.

Animals performed a full series of trials on the side contralateral to the recording site before being tested ipsilaterally, with both illumination of the lights and arm movement towards the food box occurring on the same side. The unused arm remained unrestrained and relaxed in a flexed posture on a rest in front of the animal, and only minor EMG activities were seen in the shoulder muscles and more proximally, which were unrelated to the activity in the moving arm. GO and NO-GO trials were alternated according to the judgement of the experimenters but were random during the initial testing of each cell.

Behavior was electronically monitored from standard electronic pulses generated with the tone, lights, door opening, and release of the holding key. Phototransistors sensitive to an infrared light beam across the entrance of each food box served to determine the times at which the animal's hand entered and left the box (beginning and end of beam interruption). All behavior-related electrical signals were sampled as bits in parallel by a computer, this being performed on-line at a rate of 1 or 2 kHz (Hewlett-Packard 21MX or Ithaca Intersystems Z80-CPM).

Animals underwent surgery when >90% correct behavioral performance had been obtained. Under deep pentobarbital sodium anesthesia and aseptic conditions, bolts for head fixation and a stereotaxically positioned stainless steel chamber were fixed to the skull to permit vertical access with microelectrodes to both SN. The dura was left intact. Teflon-coated multistranded stainless steel wires (Cooner Wire) were implanted into the extensor digitorum communis (EDC) muscles of both forearms and led subcutaneously to the head. All metal components, including plugs for the muscle electrodes, were imbedded in several layers of dental cement and fixed to the skull with surgical-grade stainless steel screws.

Intramuscular recordings of electromyographic (EMG) activity from chronically indwelling wires and acute surface EMG recordings from several muscles of the lower and upper arm, shoulder, and trunk were used to assess muscle activity during performance in the paradigm. Together with a closed-circuit video system, they also served to assment during the trial period prior to door opening. Rectified and filtered (10–250 Hz band pass) EMG activity was sampled at 1 or 2 kHz by the computer, following either 12-bit analog-to-digital conversion or in the form of standard digital impulses generated by a Schmitt-Trigger.

Lateral and transverse radiographs of the head were taken with a guide cannula installed on an *X-Y* moveable microstage at a known coordinate in each hemisphere. The distances of the guide cannula to the midline and bony structures were used to establish a reference system with the coordinates of the microstage. Extracellular activity of single cells was recorded with glass-insulated tungsten microelectrodes (exposed tips of 8 to 15 μm length and 2.5 to 4.5 μm diameter), modified after Merrill and Ainsworth (21), which were passed daily inside a rigid guide cannula of 0.6 mm outer diameter into the brain. Parallel electrode tracks were made vertically, roughly in the stereotaxic plane, and conforming to a 1-mm grid. Before investigating the SN, the thalamic area above it was electrophysiologically delineated by recording from the specific face representation in the ventroposteromedial (VPM) thalamus. This was performed under pentobarbital sodium anesthesia 1 wk after implantation and repeated in each recording session in the awake situation. Signals from the microelectrode were conventionally amplified and displayed in full waveform on oscilloscopes. Neuronal discharges were converted into standard digital pulses by passage through an adjustable Schmitt-Trigger and fed to an electronic counter and the computer (sampling rate 1 or 2 kHz).

The relationships of neuronal discharges and EMG activity to the digital behavioral events of the paradigm were assessed in each trial on line in the form of dot displays and perievent time histograms on a computer video screen, referenced to up to four different events. All data were stored uncondensed on computer disks. Off-line computing facilities permitted statistical evaluation of changes in discharge rate by using the two-tailed Wilcoxon matched-pairs signed-rank test on single-trial data. For this test, the series of single-trial dot displays for a given neuron in the same behavioral situation (GO vs. NO-GO on the same side) were visually inspected for apparent modulations in relation to a behavioral event. Two time epochs of equal length were chosen, one before and one after occurrence of the event, constant in position and length for all trials of a given neuron. The number of impulses in the time periods of each trial were considered as a pair and subjected to the test. Differences only become statistically significant when changes occur reproducibly in the same direction in virtually every trial. Mean changes of >25% from background discharge rate during the whole epochs of comparison and at $P < 0.01$ were considered to show a temporal ring any causalities.

Horizontal and vertical eye movements were monitored in relation to arm movement toward the food box in two monkeys, one of which was also used for SNpr cell recordings. This was done by fine-wire electrodes inserted bitemporally, or by chronically implanted Ag-AgCl electrodes in the lateral, superior, and inferior canthi of the orbitae (3). Eye-movement signals were 12-bit analog-to-digital converted at a rate of 1 kHz, displayed during experiments, and stored uncondensed on computer disks. The occurrence of eye movements and their temporal relationships to door opening and arm movement were determined from single-trial analysis using a movable cursor on a computer screen.

During the last sessions with each animal, several small electrolytic marking lesions were placed immediately after recording from a neuron in the SN and above in the same track by passing a small negative current through the microelectrode (5–10 μA for 5–20 s), thereby producing distinct patterns of vertically oriented histological marks in each hemisphere. Animals were deeply anesthetized and conventionally perfused with formaldehyde through the heart. Guide cannulas were inserted into the brain at known coordinates of the implant system in order to delineate the general area of recording. The tissue was cut in 50-μm thick serial coronal sections on a cryotome and stained with cresyl violet. Recording positions in individual microelectrode tracks were reconstructed using the distances to the electrolytic lesions in the same or a parallel neighboring track up to 1.5 mm away. In one of the animals, electrode positions were only reconstructed by their distance to the inferior border of the specific somatosensory face representation in VPM thalamus through which the electrode passed in each track, by tissue marks from passage of the guide cannula and the microelectrode, and by the presence of typical electrical activity from nigral pars compacta neurons in the vicinity.

Data in this report are only presented from those neurons whose positions were histologically located within the SN and which were recorded in the absence of variations of impulse height caused by pulsations or other local tissue movements. All neurons were tested with at least 10 trials in the GO situation contralateral to the recording electrode, and all their data were stored on computer disks and submitted to the Wilcoxon test in relation to several behavioral events.

RESULTS

General

The sequence of behavioral events during performance in the GO situation is shown in Fig. 1, *A–D*, together with the activity of some of the basic types of modulations of SNpr cell activity seen in this paradigm (Fig. 1, *G–K*).

Activity in one of the prime mover muscles, the extensor digitorum communis (EDC) is shown in Fig. 1*E* in direct relation to the behavioral events of the paradigm. EMG activity in this muscle began at a median time (50th percentile) of ~250 ms after door opening and at medians of ~120–160 ms before key release. Peaks of activity occurred before key release as well as before and during hand manipulation inside the food box. Further details of EMG recordings in the GO and NO-GO situations are shown in Fig. 2. Muscles related to lifting of the hand (EDC) and the forearm (biceps) became active shortly before key release in both situations. Forearm flexor muscles were predominantly active during hand manipulation inside the food box (between "enter" and "leave" box). Muscles of the dorsum and upper trunk show very little involvement in the individual movements of the task, as exemplified in Fig. 2 by recordings from the latissimus dorsi. Muscle activity was qualitatively similar in the GO and NO-GO situations. There were no consistent changes in EMG activity following the instructive light signals or during the waiting period within each trial.

The timing of mouth movements was indirectly assessed by single-cell recordings in the anterior VPM thalamic nucleus (Fig. 1*F*). Input from different somatosensory receptive fields showed that mouth movements already began while the hand manipulated a piece of food inside the box and reached a peak 200–1,000 ms after leaving the box. They then continued at lesser intensity, in a variable manner, for another few seconds. The timing and magnitude of VPM cell activation were used for assessing the relationships of SNpr cells to mouth movements.

Neurons of the SNpr spontaneously discharged initially negative impulses of 0.6 to 1.0 ms duration at rates between 23 and 145/s (median 68/s; Fig. 3) when the animal sat quietly while not performing in the task. Impulses of SNpr neurons were thus distinctively shorter and showed a much higher rate than those of dopamine neurons of the pars compacta of SN (32, 36). A few neurons with typical reticulata characteristics were found in or near pars compacta cell nests within SN and were considered to be SNpr neurons.

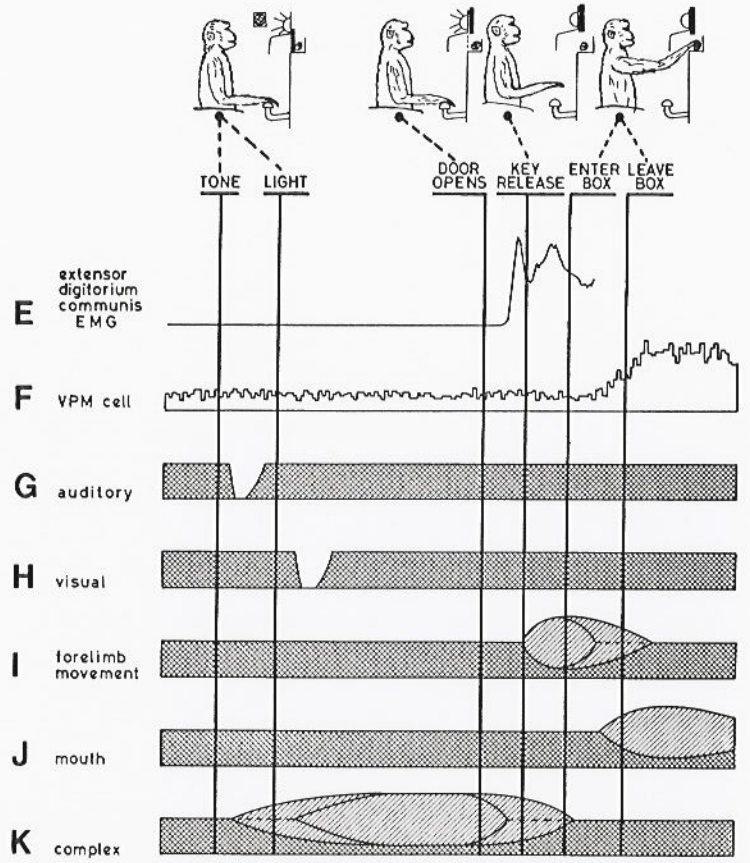

FIG. 1. An overview of the behavioral paradigm (*A–D*), 2 biological markers (*E, F*), and the observed types of behavioral relationships of SNpr cells (*G–K*). *A–D* show performance in the GO situation: While the animal kept its hand on the lever with its muscles relaxed (*E*), a tone and then a light appeared (*A*). After 1.5–5.0 s, the door of a food-containing box at eye level in front of the animal opened (*B*). The animal released the key (*C*) and reached forward to collect a piece of food (~0.8 g of apple) (*D*). After leaving the food box ("leave box"), the animal brought the reward to the mouth. *E* shows the averaged EMG activity from 576 arm movements on the right-hand side during performance in the paradigm on 5 days. Recordings in *E* were obtained from chronically indwelling multistranded Teflon-coated stainless steel wires in one of the earliest active muscles, the extensor digitorum communis of the forearm. Averages in *E* are referenced to onset of movement (key release). EMG reaction time in *E* had a median of 246 ms, the delay time between EMG onset and key release being 146 ms. *F* shows a perievent time histogram of the discharges from a VPM thalamic cell, referenced to "leave door," at a bin width of 20 ms and with 20 trials. This cell had an intraoral receptive field. *G–K* show schematics of the five principal types of changes seen in SNpr neurons in relation to the tested behavioral events. Only depressions of activity were seen with auditory (*G*) and visual (*H*) related cells. With forelimb movement (*I*), mouth (*J*), and complex (*K*) cells, both increases and decreases in activity were seen. Schematics *G–K* show means of changes, except in *I* and *K* where maximal and minimal time courses are indicated.

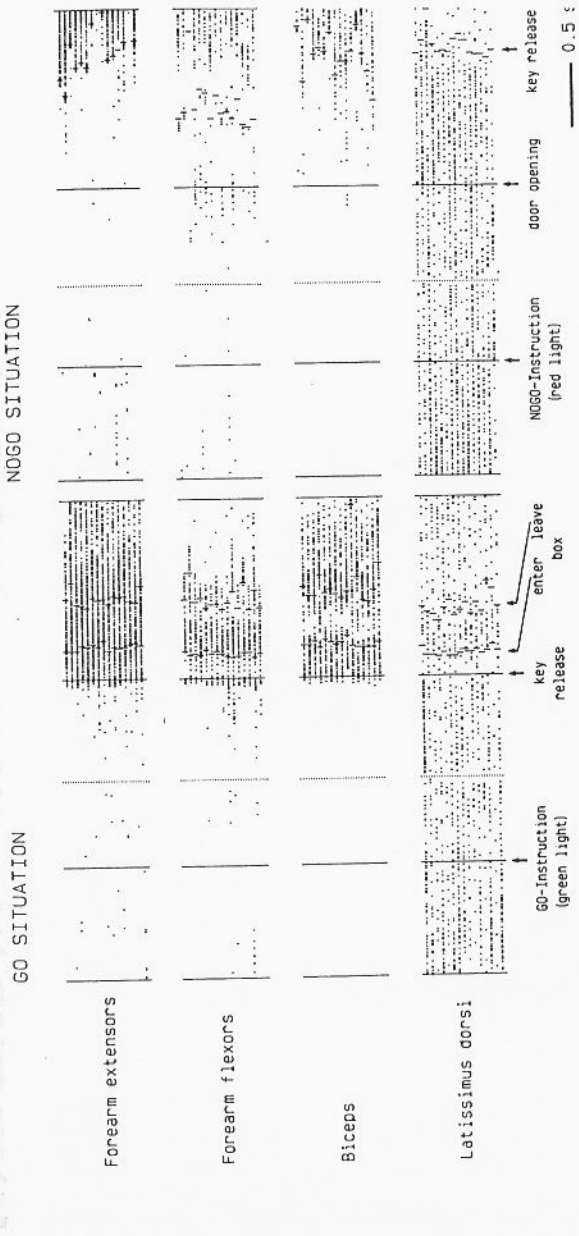

FIG. 2. Electromyographic activity from different arm and trunk muscles of a monkey performing in GO and NO-GO situations of the task. Data are shown as dot disp in which each *dot* represents the moment at which the rectified EMG surpasses a preset Schmitt-Trigger level. Trigger level for the latissimus dorsi was set very low to re trace modulations. Trials are regrouped according to the behavioral situation (GO/NO-GO). *Vertical lines* above "GO-Instruction" and "NO-GO-Instruction" indicate c of the green and red lights, respectively. "Key release" indicates movement onset of the arm. The hand began and ended interrupting the photobeam across the box entr at "enter" and "leave box." Opening of the covered door is indicated by "door opening" in the NO-GO situation. In the GO situation, this event immediately triggered re of the key and is not shown. Time axes are split at *vertical interrupted lines* because of variable intervals between respective behavioral events. All dot displays are short by ~1 s at these lines.

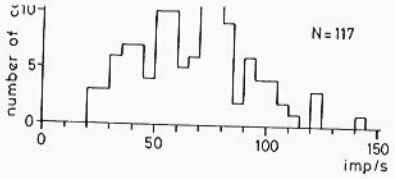

FIG. 3. Histogram of spontaneous discharge frequencies of behaviorally fully tested pars reticulata neurons of monkey substantia nigra.

A limited number of cells discharging short impulses (0.5–0.8 ms) at low rates (0–15/s) are not included in the present sample because they differed in these characteristics from the typical pars compacta or SNpr neurons, as found in this and a previous study (36). Some of these cells showed strong phasic changes in relation to arm movements that were quantitatively much stronger than those of typical SNpr neurons. Data from an apparently similar type of neuron in the SNpr were recently reported (24). Monophasic discharges of short duration (0.1–0.3 ms), which were difficult to maintain, were assumed to originate from axons (11) and were also rejected.

Of 117 SNpr neurons that were fully tested in all parameters, 74 covaried with at least one of the different events of the paradigm. Changes in these neurons surpassed 25% against background activity and were reproducible from trial to trial, as indicated by a $P < 0.01$ in the Wilcoxon test. Five types of changes were seen (Table 1; Fig. 1, *G–K*): *1*) 10 neurons were depressed in their activity following the auditory signal; *2*) 10 neurons were depressed following the illumination of the instructive green or red light, or both lights; *3*) 54 neurons were activated or depressed in relation to contralateral forelimb movements in the GO situation; *4*) 19 neurons were activated or depressed during chewing; *5*) 14 neurons were activated or depressed during the waiting period between an initial sensory stimulus and the availability of reward. Of the 74 modulated neurons, 56 covaried with only one behavioral event, while the remaining 18 showed multiple relationships (see Table 1 for details). In a few forelimb-movement-related neurons both activations and depressions were

The occurrence of eye movements was investigated in order to delineate a participation of oculomotor mechanisms in the observed changes of SNpr activity. Eye movements occurred very consistently in the form of one horizontal saccade between door opening of the food box (Fig. 4*A*) and onset of arm movement (Fig. 4*C*). Most saccades preceded the onset of EDC EMG activity (Fig. 4*B*), which itself preceded key release (Fig. 4*D*). Fixation was on the food box until after the hand had left it, and then smaller and irregular saccades occurred during the hand movement towards the mouth and thereafter. Vertical eye movements occurred less consistently and at lower amplitude during the trial-on period until the food was collected. This may have been due to the fact that lights and the food box were installed at eye level. Changes of neuronal activity between door opening and key release therefore suggest a relation to horizontal saccades. This was observed in the form of reductions of activity in about 50 additional SNpr cells, which were not used for further testing in the paradigm. A typical example is shown in Fig. 5.

Histological location of neurons

For reasons of simplicity and in accordance with the current anatomical literature, the substantia nigra (SN) was subdivided into the pars compacta, which contains mostly dopaminergic neurons, and the nondopaminergic pars reticulata (SNpr). Although it is often difficult to draw clear boundaries between the two subdivisions in the monkey, the SNpr lies mostly ventral in the anterior parts of SN and is increasingly disrupted by compacta cell nests more posteriorly. Histological reconstructions, aided by marker lesions, were used to localize the positions of individual neurons within the boundaries of SN (Fig. 6, *A* and *B*). More than 40 additional neurons with impulse forms and discharge rates similar to SNpr neurons were found dorsally to the SN and are not included in the sample of cells. The distribution of the different types of changes within the SNpr was assessed by subdividing the SN into three rostrocaudal parts [the borders being at A6.5, A7.8, A9.1, and A10.5 according to the atlas of Shanta et al. (38)] and into three mediolateral parts. Sampling of neurons was biased

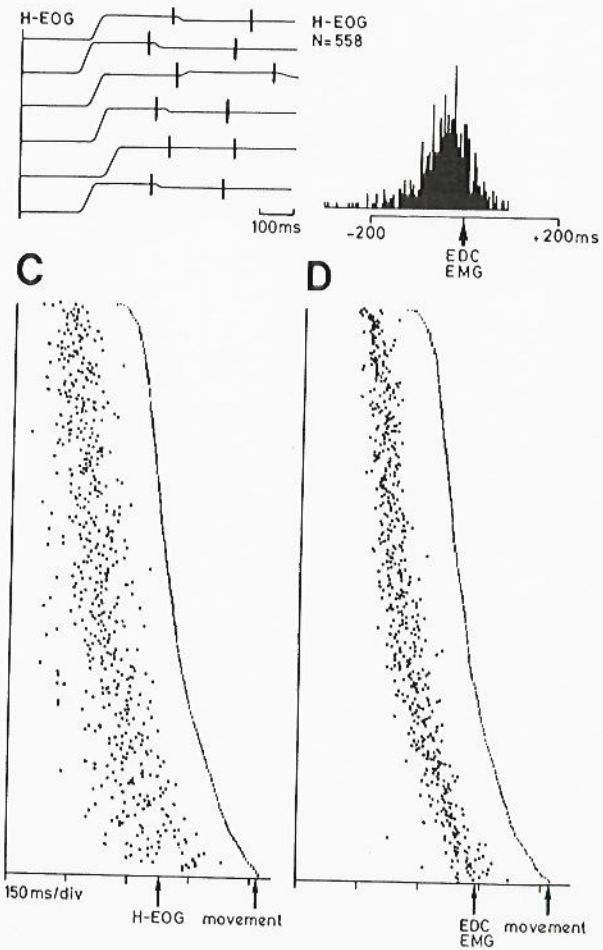

FIG. 4. Horizontal eye movements (H-EOG) in relation to different events of the behavioral paradigm in a monkey used for single cell recordings in SNpr. *A*: specimen records of eye movements following door opening (*vertical full line* to the *left*). *First* and *second short vertical lines* denote key release and entering of the food box, respectively. *B*: the relation of H-EOGs to onset of EMG activity in EDC muscle of the arm. The histogram shows time intervals between the two events as assessed from single-trial analysis. *C*: the occurrence of H-EOG following door opening (*left vertical line*) and before key release ("movement," *short vertical lines* to the *right*). *D*: the onset of EDC-EMG activity between door opening (*left* line) and key release (*short lines* to the *right*). Individual trials in *C* and *D* are regrouped according to increasing reaction times.

towards intermediate and lateral parts of SNpr, since we oriented electrode tracks after the landmark VPM thalamus in all animals. Mouth-movement-related, auditory, and complex cells appeared to be predominantly located in the rostrolateral two thirds of SNpr,

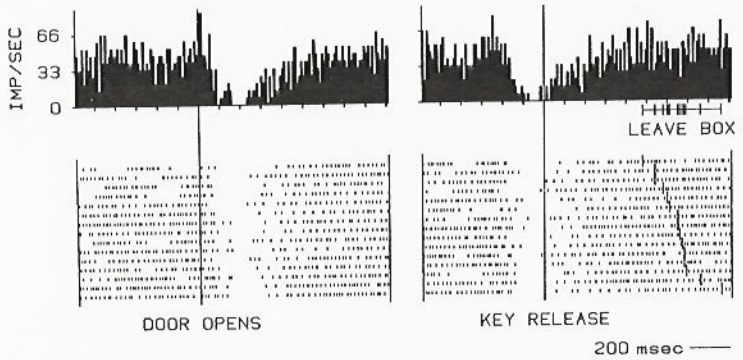

FIG. 5. Reduced activity of an SNpr neuron during the time of horizontal saccade between opening of the food box door and onset of movement ("key release"). In this and all following figures, dot displays are shown below perievent time histograms, each *dot* representing one discharge of the cell under study. Histograms are always composed of those impulses that are shown as dots below. The relative distance of each dot to the behavioral event (in this figure "door opens" and "key release") corresponds to the real-time interval. Each *line* shows the discharges during performance in 1 trial, the natural sequence of trials being preserved downwards, except where noted. In this figure, the trials in the right part were rank-ordered according to the time interval between key release and the hand leaving the food box ("leave box," taken as the last interruption of the photobeam across the box entrance). Occurrence of the latter event is indicated by longer lines below the histogram and within the dot display. The shown data indicate that there was no relation of cell activity to the total duration of movement. Bin width is 10 ms; *short lines* below histograms indicate 10 bins.

whereas motor and visual cells did not show any preferential distributions. Cells without relationships to any of the behavioral parameters were found more often in the posterior third than elsewhere in SNpr.

Changes related to forelimb movements

Neurons were classified as forelimb movement related according to their changes in the GO situation. Of 117 fully tested SNpr neurons, 27 increased and 27 decreased their activity in relation to contralateral forelimb movements. Increases and decreases were seen with neurons of all ranges of spontaneous discharge rate. Table 1 shows that about one-third of these neurons also showed relationships to one or more of the other behavioral events.

Most forelimb-movement-related SNpr neurons covaried with large forward reaching of the arm. Changes continued in several neurons during hand manipulation inside the food box and during arm movement towards the mouth (Table 2; Figs. 7 and 8; left part). Ten movement-related SNpr neurons showed changes only during distal forearm movements when the animal handled a food morsel inside the box (Fig. 9), which was accompanied by EMG activity in forearm flexors and extensors (Fig. 2). With the possible exception of these hand-manipulation-related neurons, we have seen no changes in SNpr neurons which resembled the timing of EMG activity in single muscles.

In the NO-GO situation, forelimb movements occurred later than in the GO situation, after a waiting period of 1 to several seconds after door opening. Of 35 neurons covarying with forelimb movements in the GO situation, 21 also showed qualitatively and quantitatively similar movement relationships in the NO-GO situation (see Figs. 7 and 8, middle parts). Four neurons showed an inverse relationship, i.e., a decrease of activity during movements in the NO-GO and an increase in the GO situation (two cases), or the opposite. The 10 remaining neurons did not significantly covary with movements in the NO-GO situation.

Ipsilateral movements were tested with 25 SNpr neurons that showed contralateral movement-related changes. Only five neurons did not covary ipsilaterally. Thirteen neurons showed qualitatively and quantitatively symmetrical changes (see Figs. 7 and 8, right parts), but their onset relative to the onset of movement was often delayed by a few tens of milliseconds. Asymmetrical relationships were

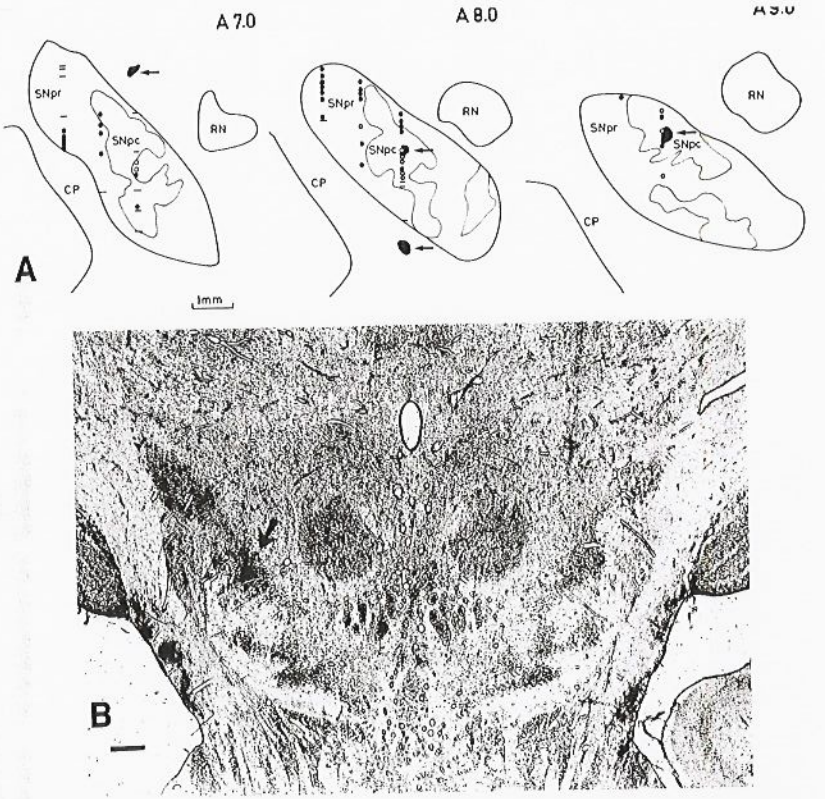

FIG. 6. *A*: histological reconstructions of cell positions in the substantia nigra from 1 monkey. Coronal sections are shown with their approximate anteroposterior levels in millimeters according to the atlas of Shanta et al. (Ref. 38). *Closed circles*, SNpr type neuron; *open circles*, pars compacta type neuron; *horizontal line*, unclassified neuron not belonging to either of the two main subgroups; *arrows*, lesions performed directly after cell recording; SNpr, pars reticulata; SNpc, pars compacta of substantia nigra; RN, red nucleus; CP, cerebral peduncle. *B*: photomicrograph of a coronal section through the ventroanterior midbrain, with a lesion marking the position of a recorded neuron in the substantia nigra (*arrow*). *Bar* denotes 1 mm. Cresyl violet-stained 50-μm section.

seen in seven neurons, which showed quantitatively minor changes ipsilaterally or which were modulated during a different phase of the movement.

Most changes in movement-related cell activity began after onset of EMG activity or key release. Changes starting after key release were seen in 24 neurons (10 activated, 14 depressed) and between 0 and 120 ms before key release in 10 neurons (5 activated, 5 depressed). Changes as early as 120 to 180 ms before key release, i.e., at a time corresponding to onset of EDC activity, were found in 16 neurons (10 activated, 6 depressed). Only four neurons (3 activated, 1 depressed) were modulated clearly before any EDC activity (i.e., >180 ms before key release).

In quantitative respects, movement-related changes in SNpr cell activity were generally moderate. Activations amounted maximally to a tripling of discharge rate, with a median of 70% increase above the level of spontaneous

	Neurons Activated		Neurons Depressed		Neurons With Only 1 Relationship	Neurons With Additional Relationships			
	N	%	N	%		Audit	Light	Mvmt	Mouth
Auditory signal	0	0	10	9	0				
Instructive light	0	0	10	9	1	4			
Forelimb movement	27	23	27	23	38	4	6		
Mouth movement	14	12	5	4	10	2	3	6	
Waiting period	5	4	9	8	7	4	0	5	0
Sum	46		61		56				

The total number of neurons activated ($n = 46$) or depressed ($n = 61$) in relation to a behavioral event is superior to the total number of neurons ($n = 74$) modulated in the task, because 18 neurons showed more than one behavioral relationship. The observed combinations of relationships in these neurons is given at right. The total number of quantitatively fully tested neurons was 117.

activity. The median reduction of activity observed was 50%, the maximum being 80%.

Somatosensory input from arm, shoulders, and upper trunk was tested with about half of the SNpr neurons. We moved both arms of the animal in a similar trajectory as that of active reaching toward the food box and stimulated glabrous and hairy skin without evoking pain. Surface or deep somatosensory input was never seen in a consistent and reproducible manner. Some neurons of SNpr responded to this stimulation for about four or five trials; thereafter the effect vanished and could not be regained. This was independent of eye movements. Although the nature of these responses is uncertain, it is unlikely that the majority of movement relationships of SNpr neurons is caused by somatosensory input. Similar passive movements of the arm evoked clear somatosensory responses in neurons of the ventral posterolateral nucleus of thalamus, which were recorded in a few more lateral tracks.

Changes related to mouth movements

Nineteen of 117 tested SNpr neurons changed their activity during mouth movements. Of these, 14 cells were activated and 5 cells depressed. Six of these neurons were, in addition, modulated during forelimb movements, while four responded also to the tone or a light or to both. Changes began when the hand entered the food box (4 neurons activated, 1 depressed), while the hand manipulated inside the box (3 neurons activated, 1 depressed), when the hand left the box or up to 250 ms afterwards (5 neurons activated, 1 depressed). Changes were continuous with arm movement-related modulations in four neurons (2 activated, 2 depressed). Activations amounted to 52–200% increases above spontaneous discharge rate, while decreases ranged from 25% to 50%.

The possibility of a role of trigeminal somatosensory input remains an open question. We could not detect perioral receptive fields in those neurons tested but did little intraoral testing. Mouth movement-related changes in SNpr neurons may thus be due to motor or somatosensory mechanisms or to both.

TABLE 2. *Detailed relationships of forelimb movement-related SNpr neurons*

	Activated	Depressed
Foreward reaching	13	8
Foreward reaching with hand manipulation	4	3
Total duration of arm movement	5	11
Hand manipulation only	5	5

Responses to an instructive light stimulus

Nine of the 117 SNpr neurons decreased their activity in response to the green light which invariably signaled the GO situation (Fig. 10). One additional neuron responded with a decrease to the red light, which signaled the NO-GO situation, but not to the green light (see Fig. 11). Nine visual neurons also covaried with other behavioral events, i.e., initial tone, forelimb movement, mouth movement, or waiting (see below). None of the visual cells were depressed between door opening and key release, which would have been suggestive of an oculomotor relationship.

Of the nine neurons responding to the green light, eight were also tested in the NO-GO situation (Fig. 10). Two of these responded to the red light, while the others remained unaffected or showed a mild and slow increase in activity. Ipsilateral performance was tested with six neurons responding to the green light. Four of them also responded to the ipsilateral green light, while two remained unaffected.

Latencies of responses to the contralateral green light ranged from 80 to 200 ms, with a median of 120 ms. Responses amounted to decreases in activity by 25–70% with a median of 47%.

Auditory responses

Ten of the 117 SNpr neurons decreased their activity in response to the tone that initiated each trial (Fig. 11). All of them also covaried with other behavioral events of the paradigm, three of them showing visual responses. Five auditory neurons were also tested for ipsilateral performance. Three of them showed a similar auditory response as for contralateral performance, two did not respond ipsilaterally.

Latencies of auditory responses ranged from 50 to 150 ms, with a median of 90 ms. Responses amounted to decreases of activity ranging from 35 to 80%, the median being 60%.

Changes related to waiting

Fourteen of the 117 SNpr neurons showed consistent and statistically significant changes in activity during performance in the paradigm that were not simply related to sensory stimuli or forelimb and mouth movements. Five neurons increased and nine decreased their activity during the period between an initial sensory

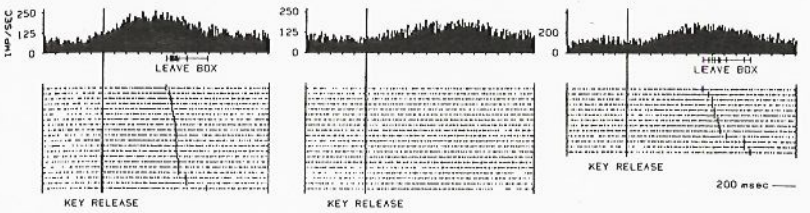

FIG. 7. The activity of an SNpr neuron which displayed an increased discharge rate during forelimb movement. Data are referenced to onset of movement ("key release"). In the *left* and *right* part of the figure, trials are rank-ordered according to the interval between movement onset and leaving the food box. The latter event is indicated by longer lines. Bin width is 10 ms.

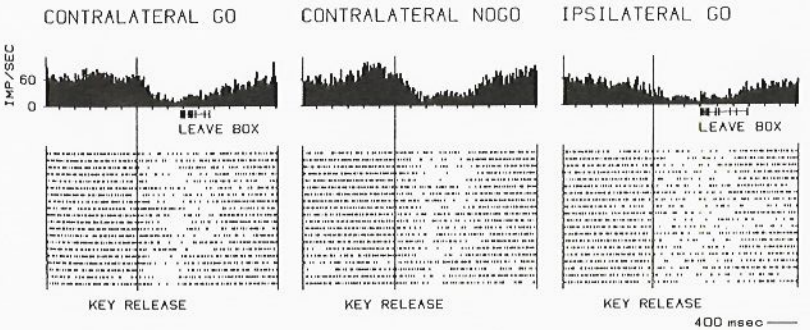

FIG. 8. An SNpr neuron whose activity decreased during forelimb movement. Data are referenced to onset of movement ("key release"). Longer lines below left and right histograms indicate the moment when the hand left the food box. Bin width is 20 ms.

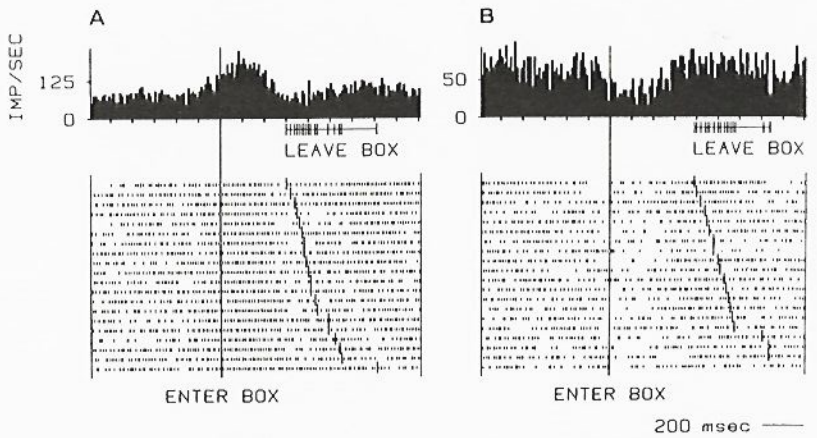

FIG. 9. The activity of 2 SNpr neurons that displayed changes of discharge rate during hand manipulation inside the food box (cell *A*: activated; cell *B*: depressed). Trials are rank-ordered according to the duration of hand manipulation inside the box. Leaving the box is indicated by *short lines* below histograms and in *dot displays*. Bin width is 10 ms.

stimulus and the final possibility to move for reward. Seven of these neurons showed additional changes in relation to sensory stimuli or movements.

In detail, changes began after the tone and before light onset in five neurons (4 increases, 1 decrease), and after the green light (GO situation) in seven neurons (4 decreases, 3 increases) (Figs. 12 and 13). Activation in one other neuron began after illumination of only the red light (signaling the NO-GO situation). Decreased activity in another neuron began after door opening in the NO-GO situation, after which the animal kept its hand on the

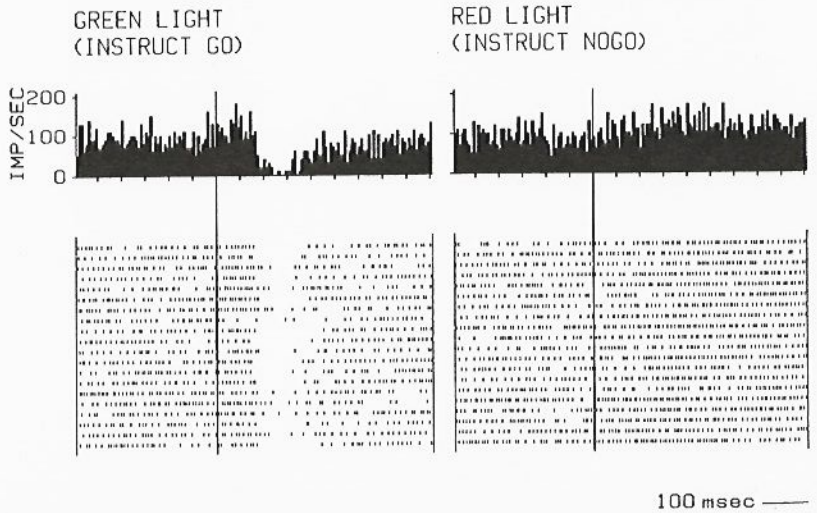

FIG. 10. Reduced activity of an SNpr neuron in response to a light stimulus in front of the animal. The green light signaled the GO situation, the red light the NO-GO situation. Bin width is 5 ms.

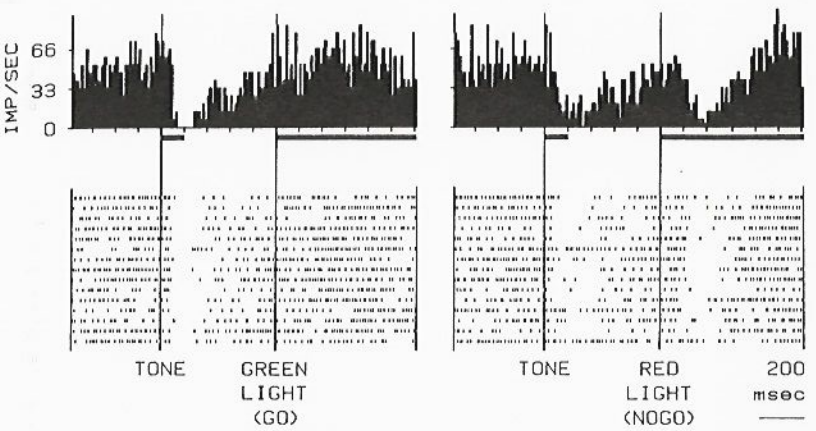

FIG. 11. Reduced activity of an SNpr neuron in response to an auditory stimulus of 66–67 dB. This neuron also responded to the red light signaling the NO-GO situation. *Heavy bars* below the histograms indicate the duration of the stimuli. Bin width is 10 ms.

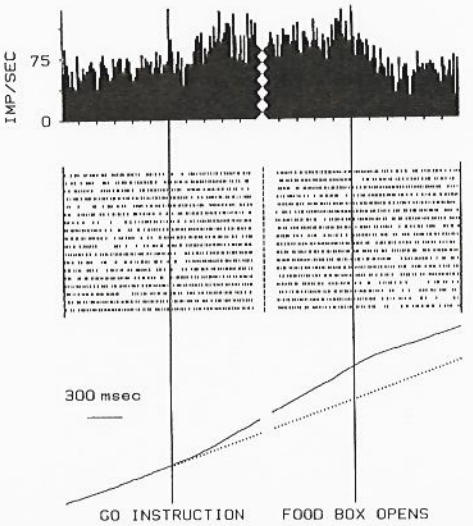

FIG. 12. Increased discharge rate of an SNpr neuron during the waiting period between the behavioral instruction and the moment at which reward became accessible. Time axes are split at *vertical interrupted lines* while maintaining the mean time interval between behavioral events. *Curves* below the *dot displays* are cumulative frequency distributions of cell activity shown above. Bin width is 15 ms.

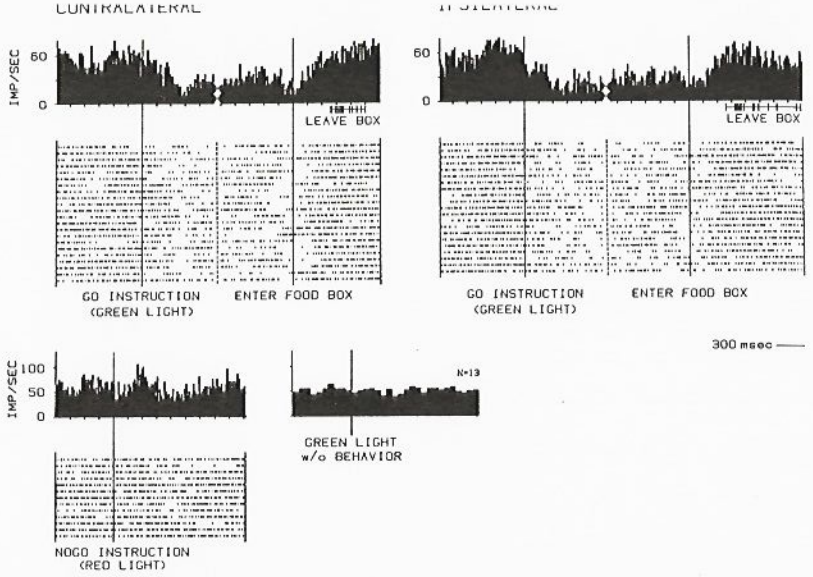

FIG. 13. Decreased discharge rate of an SNpr neuron between the behavioral instruction and the moment when the animal gained access to the reward ("enter food box"). This change was also seen with ipsilateral performance. No change in activity occurred during performance in contralateral NO-GO trials (*lower left*). There was no response to the green light outside of the behavioral context (*lower right*). Time axes are split at *vertical interrupted lines* while maintaining the mean time interval between behavioral events. Bin width is 15 ms.

key and waited for later reward. Changes continued until the door of the food box opened ($n = 2$; Fig. 12), the forelimb movement began ($n = 4$), the animal's hand reached the food box ($n = 6$; Fig. 13), or the hand left the box with a food morsel ($n = 2$). Changes were slower in onset than with other types of modulations.

Four neurons showing changes after light onset were also tested in the NO-GO situation. Three of them showed changes only after the green light (GO situation), and only when the light was illuminated in the behavioral context (Fig. 13). All five neurons tested during performance on both sides showed bilaterally symmetrical changes (Fig. 13).

DISCUSSION

The present findings indicate that the activity of many SNpr neurons in the primate shows temporal relationships to forelimb reaching movements. This adds a new aspect to their behavioral function, which is so far known to be concerned with auditory and visual responses and oculomotor mechanisms (12, 16), and with mouth movements (7, 22). Somatosensory input had been reported in cats (37), but this was not confirmed in primates by others (7) and by us. We could, however, demonstrate neuronal mechanisms that appear to be related to attentive waiting during the arm movement task. Similar complex relationships have been seen in SNpr neurons in relation to oculomotor mechanisms (13).

The GO/NO-GO paradigm in the present experiment was chosen in order to investigate a wider range of behavioral relationships of basal ganglia neurons than that permitted by a direct motor-control task. The paradigm involved alerting and instructing signals requiring different kinds of preparation for behavioral responses to trigger stimuli, response suppression, and movements under two different conditions. We also intended to compare SNpr neuronal activities with those of pars compacta dopaminergic neurons in the same behavioral situation (36).

The forelimb movements in the presently used paradigm involved proximal and distal muscles of the arm as well as shoulder muscles, but little activity of trunk muscles, in agreement with an earlier study (36). The results indicate that only a very small number of SNpr cells are modulated preferentially during the time of activity in distal arm muscles, as reported previously (7). The fact that the activity of forelimb-related SNpr neurons generally covaries throughout the time of the arm trajectory suggests a relationship to arm movement occurring independently of the temporal pattern of activity of individual muscles. We have seen no similarities between SNpr cell discharges and the activity of any of the tested muscles. A higher percentage of movement-related SNpr neurons was found than in a more direct motor-control task used by others (7). The movements required in the present task were of larger amplitude and involved more muscles than the forearm flexion-extension movements in the experiments of DeLong et al. (7). This should largely account for the observed differences. The changes of SNpr activity in temporal relation to arm movements were quantitatively minor when compared with those with eye movements. Until now, movement relationships in monkey SNpr neurons have been studied in a precise motor-control task (7) and in our paradigm involving more general, large-amplitude arm movements. It is therefore unclear whether SNpr neurons might show more dramatic changes in relation to other kinds of limb movements.

The relations of SNpr neurons to forelimb movements agree well with the known afferent input from movement-related striatal regions. The monkey SNpr receives fibers from both caudate nucleus and putamen (28, 40–42), although the caudate input appears to predominate in the SNpr as a whole (27). The forelimb-related region of the putamen (6, 19, 20) projects heavily to the lateral half of the SNpr (27). This nigral territory overlaps with the SNpr area in which we recorded in the present experiments. The majority of forelimb-related SNpr neurons also covaried with ipsilateral movements, mostly in a fashion similar to contralateral movements. This is compatible with the bilateral nature of input to the striatum (2, 14, 26).

Eye movements consisting of one horizontal saccade between door opening and onset of reaching movement occurred regularly under the present paradigm. They were seen immediately before and with the onset of forelimb muscle activity. About 50 SNpr neurons decreased their activity during this period. Although we have not submitted these neurons to further tests, most of them should be considered as eye movement related. Hence, SNpr neurons that were related to the simultaneously occurring initiation of forelimb muscle activity may have been missed. Neurons like these were only judged to be movement related when changes of activity continued during the period of forelimb reaching, when no eye movements were seen.

Several neurons showed changes during the period of attentive waiting before the arm movement towards the food-containing box. Some temporal similarities exist between these changes and one of the three types of memory-contingent saccade-related responses that were seen before in monkey SNpr neurons at a comparable frequency (13). Several neurons in the other categories of our sample showed behavioral relationships that also did not appear to be of primarily motor or sensory nature. Some forelimb-related neurons did not covary with movement in the NO-GO situation, or showed an inverse relationship (activation vs. depression). Some visual SNpr neurons were only influenced by one of the two behaviorally significant lights, although we cannot exclude spectral selectivity because of the invariable behavioral significance of each color. Several SNpr neurons both responded to a sensory signal of the task and covaried with forelimb or mouth movements. It appears possible that many of these complex relations to the task, in particular those during waiting, represent neuronal mechanisms that are involved in certain aspects of preparation for the correct response according to the indicative stimuli (GO vs. NO-GO). Alternative interpretations may include attentional, motivational, and short-term mnemonic processes, which are operational during a short, well-defined period before the animal may actively approach the rewarding situation. Although we presently possess no specific data to favor one or the other of these or similar hypotheses, the results indicate an activity of basal ganglia

neurons during the intermediate term planning of a movement before the immediate initiation in response to the triggering signal occurs. Some of these questions may be answered in future studies with specifically developed paradigms.

The behavioral relations of SNpr neurons appear, on first sight, to be very heterogeneous. This is not surprising in view of the currently held notion that proposes that the SNpr functions as a major output station of the basal ganglia. The different relationships of SNpr neurons should reflect the results of neuronal operations in the striatum dealing with multimodal sensory processing, cognitive mechanisms, and the initiation and conduction of limb movements (1, 4, 6, 18, 23, 25, 29). Neuronal activity progresses from SNpr to ventral thalamic areas, midbrain tegmentum and superior colliculus (2, 5, 10, 26). Ventral thalamic areas with nigral input project to supplementary motor area (31) and, more rostrally, to prefrontal cortex (17). In this way, the SNpr would form part of a neuronal loop from the frontal cortex through the striatum, SNpr, and thalamus back to frontal cortical areas. The existence of these frontal associational and motor loops (8) would be compatible with the observed relationships of SNpr neurons to motor and complex processes.

The behavioral relationships of SNpr neurons are distinctly different from those of dopaminergic neurons of the pars compacta of the SN. In the same behavioral situation in monkeys, dopaminergic neurons show an increase in activity during the whole movement phase without obvious relationships to detailed movement parameters and no responses to instructive sensory signals (36). In a more direct reaction-time paradigm, dopaminergic neurons responded strongly to stimuli that led to an immediate behavioral reaction (32). Thus, the considerable differences between the two substructures of SN in terms of cytology, connectivity, and neurotransmitters are also represented in their relationships to behavioral acts. Whereas SNpr cells appear to be part of specific information transfer and processing circuits of the basal ganglia, nigral dopaminergic cells may subserve a more basic mechanism dealing with behavioral responsiveness (33).

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REFERENCES

1. ALDRIDGE, J. W., ANDERSON, R. J., AND MURPHY, J. T. The role of the basal ganglia in controlling a movement initiated by a visually presented cue. *Brain Res.* 192: 3–16, 1980.
2. BECKSTEAD, R. M. AND FRANKFURTER, A. The distribution and some morphological features of substantia nigra neurons that project to the thalamus, superior colliculus and pedunculopontine nucleus in the monkey. *Neuroscience* 7: 2377–2388, 1982.
3. BOND, H. W. AND HO, P. Solid miniature silver-silver chloride electrodes for chronic implantation. *Electroencephalogr. Clin. Neurophysiol.* 28: 206–208, 1970.
4. BUSER, P., POUDEROUX, G., AND MEREAUX, J. Single unit recording in the caudate nucleus during sessions with elaborate movements in the awake monkey. *Brain Res.* 71: 337–344, 1974.
5. CARPENTER, M. B., NAKANO, K., AND KIM, R. Nigrothalamic projections in the monkey demonstrated by autoradiographic technics. *J. Comp. Neurol.* 165: 401–416, 1976.
6. Crutcher, M. D. AND DELONG, M. R. Single cell studies of the primate putamen. I. Functional organization. *Exp. Brain Res.* 53: 233–243, 1984.
7. DELONG, M. R., CRUTCHER, M. D., AND GEORGOPOULOS, A. P. Relations between movement and single cell discharge in the substantia nigra of the behaving monkey. *J. Neurosci.* 3: 1599–1606, 1983.
8. DELONG, M. R., GEORGOPOULOS, A. P., AND CRUTCHER, M. D. Cortico-basal ganglia relations and coding of motor performance. *Exp. Brain Res. Suppl.* 7: 30–40, 1983.
9. DIVAC, I. AND ÖBERG, R. G. E. (Editors). *The Neostriatum.* Oxford, UK: Pergamon, 1979.
10. GRAYBIEL, A. M. AND RAGSDALE, C. W. Fiber connections of the basal ganglia. In: *Development and Chemical Specificity of Neurons.* Amsterdam: Elsevier/North-Holland, 1979, p. 239–283.
11. HELLWEG, F.-C., SCHULTZ, W., AND CREUTZFELDT, O. D. Extracellular and intracellular recordings from cat's cortical whisker projection area: thalamocortical response transformation. *J. Neurophysiol.* 40: 463–479, 1977.
12. HIKOSAKA, O. AND WURTZ, R. H. Visual and oculomotor functions of monkey substantia nigra pars reticulata. Relation of visual and auditory responses to saccades. *J. Neurophysiol.* 49: 1230–1253, 1983.
13. HIKOSAKA, O. AND WURTZ, R. H. Visual and oculomotor functions of monkey substantia nigra pars reticulata. III. Memory-contingent visual and saccade responses. *J. Neurophysiol.* 49: 1268–1284, 1983.
14. JAYARAMAN, A., BATTON, R. R., III, AND CARPENTER, M. B. Nigrotectal projections in the monkey: an autoradiographic study. *Brain Res.* 135: 147–152, 1977.
15. JONES, E. G., COULTER, J. D., BURTON, H., AND PORTER, R. Cells of origin and terminal distribution of corticostriatal fibers arising in the sensory-motor cortex of monkeys. *J. Comp. Neurol.* 173: 53–80, 1977.
16. JOSEPH, J. P. AND BOUSSAOUD, D. Role of cat substantia nigra pars reticulata in eye and head movements. I. Neural activity. *Exp. Brain Res.* 57: 286–296, 1985.
17. KIEVIT, J. AND KUYPERS, H. G. J. M. Organization of the thalamo-cortical connections of the frontal lobe in the rhesus monkey. *Exp. Brain Res.* 29: 299–322, 1977.
18. KIMURA, M., RAJKOWSKI, J., AND EVARTS, E. V. Tonically discharging putamen neurons exhibit set-dependent responses. *Proc. Natl. Acad. Sci. USA* 81: 4998–5001, 1984.
19. KÜNZLE, H. Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in *Macaca fascicularis. Brain Res.* 88: 195–210, 1975.
20. LILES, S. L. Activity of neurons in the putamen associated with wrist movements in the monkey. *Brain Res.* 263: 156–161, 1983.
21. MERRILL, E. G. AND AINSWORTH, A. Glass-coated platinum-plated tungsten microelectrodes. *Med. Biol. Eng.* 10: 662–672, 1972.
22. MORA, F., MOGENSON, G. J., AND ROLLS, E. T. Activity of neurons in the region of the substantia nigra during feeding in the monkey. *Brain Res.* 133: 267–276, 1977.
23. NIKI, H., SAKAI, M., AND KUBOTA, K. Delayed alternation performance and unit activity of the caudate head and medial orbitofrontal gyrus in the monkey. *Brain Res.* 38: 343–353, 1972.
24. NISHINO, H., ONO, T., FUKUDA, M., AND SASAKI, K. Monkey substantia nigra (pars reticulata) neuron discharges during operant feeding. *Brain Res.* 334: 190–193, 1985.
25. NISHINO, H., ONO, T., FUKUDA, M., SASAKI, K., AND MURAMOTO, K.-I. Single unit activity in monkey caudate nucleus during operant bar pressing feeding behavior. *Neurosci. Lett.* 21: 105–110, 1981.
26. PARENT, A., MACKEY, A., SMITH, A., AND BOUCHER, R. The output organization of the substantia nigra in primate as revealed by a retrograde double labeling method. *Brain Res. Bull.* 10: 529–537, 1983.
27. PARENT, A., BOUCHARD, C., AND SMITH, Y. The striatopallidal and striatonigral projections: two distinct fiber systems in primate. *Brain Res.* 303: 385–390, 1984.
28. PERCHERON, G., YELNIK, J., AND FRANÇOIS, C. A Golgi analysis of the primate globus pallidus. III. Spatial organization of the striatopallidal complex. *J. Comp. Neurol.* 227: 214–227, 1984.
29. ROLLS, E. T., THORPE, S. J., AND MADDISON, S. P. Responses of striatal neurons in the behaving monkey. I. Head of the caudate nucleus. *Behav. Brain Res.* 7: 179–210, 1983.
30. ROUZAIRE-DUBOIS, B., SCARNATI, E., HAMMOND, C., CROSSMAN, A. R., AND SHIBAZAKI, T. Microiontophoretic studies on the nature of the neurotransmitter in the subthalamo-entopeduncular pathway of the rat. *Brain Res.* 271: 11–20, 1983.
31. SCHELL, G. R. AND STRICK, P. L. The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. *J. Neurosci.* 4: 539–560, 1984.
32. SCHULTZ, W. Primate dopamine cell activity in relation to behavioral acts. *Clin. Neuropharmacol.* 7, Suppl. 1: 48–49, 1984.
33. SCHULTZ, W. Recent physiological and pathophysiological aspects of parkinsonian movement disorders. *Life Sci.* 34: 2213–2223, 1984.
34. SCHULTZ, W., RUFFIEUX, A., AND AEBISCHER, P. Substantia nigra pars reticulata neurones in monkeys are related to arm or mouth movements. *Neurosci. Lett. Suppl.* 7: S317, 1981.
35. SCHULTZ, W., AEBISCHER, P., AND RUFFIEUX, A. The encoding of motor acts by the substantia nigra. *Exp. Brain Res. Suppl.* 7: 171–180, 1983.
36. SCHULTZ, W., RUFFIEUX, A., AND AEBISCHER, P. The activity of pars compacta neurons of the monkey substantia nigra in relation to motor activation. *Exp. Brain Res.* 51: 377–387, 1983.
37. SCHWARZ, M., SONTAG, K.-H., AND WAND, P. Sensory-motor processing in substantia nigra pars reticulata in conscious cats. *J. Physiol. Lond.* 347: 129–147, 1984.
38. SHANTA, T. R., MANOCHA, S. L., AND BOURNE, G. H. A stereotaxic atlas of the Java monkey brain (*Macaca irus*) Basel: Karger, 1968.
39. SOLTYSIK, A., HULL, C. D., BUCHWALD, N. A., AND FEKETE, T. Single unit activity in basal ganglia of monkeys during performance of a delayed response task. *Electroencephalogr. Clin. Neurophysiol.* 39: 65–78, 1975.
40. SZABO, J. Topical distribution of striatal efferents in the monkey. *Exp. Neurol.* 5: 21–36, 1961.
41. SZABO, J. The efferent projections of the putamen in the monkey. *Exp. Neurol.* 19: 463–476, 1967.
42. SZABO, J. Projections from the body of the caudate nucleus in the rhesus monkey. *Exp. Neurol.* 27: 1–15, 1970.
43. YOSHIDA, M. AND PRECHT, W. Monosynaptic inhibition of neurons of the substantia nigra by caudatonigral fibers. *Brain Res.* 32: 225–228, 1971.