Visual and Oculomotor Functions of Monkey Substantia Nigra Pars Reticulata. IV. Relation of Substantia Nigra to Superior Colliculus

OKIHIIDE HIKOSAKA AND ROBERT H. WURTZ
Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20205

SUMMARY AND CONCLUSIONS

1. The preceding studies (18–20) have shown that cells in the monkey substantia nigra pars reticulata have responses that are temporally correlated with visual or auditory stimuli and saccadic eye movements. In this study we determined whether these substantia nigra cells project to the superior colliculus.

2. We first recorded from a substantia nigra cell and identified its sensory and oculomotor properties using the behavioral paradigms developed previously (18–20). Then we stimulated the ipsilateral superior colliculus while moving the stimulating electrode through the layers of the colliculus to determine whether and from what depth the substantia nigra cell was activated antidromically. Finally, we identified the visual and oculomotor properties of the colliculus cells located near the points from which the substantia nigra cell was activated antidromically with the lowest thresholds.

3. Of 180 substantia nigra cells studied, 51 were activated antidromically from the ipsilateral superior colliculus. Nearly half of the substantia nigra cells that showed some visual or saccade-related responses were activated antidromically, whereas cells that did not show such responses were rarely activated.

4. For each of the substantia nigra cells that showed visual or oculomotor-related responses, we determined the center of the visual receptive or movement field of the cell and compared it with the field center of the superior colliculus cells found at the lowest threshold point. The closer the two field centers, the more probable the antidromic activation of the substantia nigra cell, which suggests that each substantia nigra cell projects to the superior colliculus so as to match its own field with that of the colliculus cells in its projection area.

5. The threshold stimulation current for antidromic activation of single substantia nigra cells varied, depending on the depth of the stimulating electrode within the superior colliculus, with several low-threshold points usually seen on a penetration through the colliculus. Regardless of the type of visual or oculomotor response of the substantia nigra cell, the low-threshold point was most commonly found among colliculus cells that showed a burst of spikes before saccades and, therefore, were probably located in the intermediate layers. A low-threshold point was also common in the deep layers but was relatively rare in the superficial layers. The latency of antidromic response ranged from 0.7 to 2.3 ms. The antidromic latency for single substantia nigra cells tended to decrease with the depth of stimulation in the superior colliculus.

6. We compared the visual and oculomotor activity of antidromically activated substantia nigra cells with that of superior colliculus cells near the point of lowest threshold for stimulation. An inverse relationship was found between saccade-related activities of such a neuron pair; a decrease in discharge rate of the substantia nigra cell occurred with an increase in discharge rate (frequently a burst of spikes) of the superior colliculus cell. However, such a relationship depended on how the saccade was initiated because saccade-related responses of substantia nigra cells. 
tia nigra cells were selective for the mode of saccade initiation (visually contingent or memory contingent; Refs. 18 and 20), whereas superior colliculus cells were usually nonselective. Correlation of visual responses of substantia nigra cells with activity of superior colliculus cells was less obvious.

7. The results of these antidromic stimulation experiments suggest a synaptic connection between substantia nigra cells and the superior colliculus, particularly those cells in the intermediate layers of the colliculus that give a burst of spikes before the onset of saccades. The relationship between cell discharge in the two structures is consistent with the idea that the substantia nigra cells discharge rapidly and inhibit superior colliculus cells tonically. A release of the tonic inhibition resulting from a decrease in substantia nigra cell activity would contribute to the generation of the burst of activity in the colliculus cells and, consequently, would contribute to the initiation of saccadic eye movements.

INTRODUCTION

The preceding reports in this series (18-20) have described the activity of substantia nigra cells temporally correlated with visual stimuli and saccadic eye movements. However, such temporal correlation itself does not indicate that the substantia nigra is involved in the initiation of saccadic eye movements. For example, saccade-correlated activity of substantia nigra cells might just be the signal to other brain areas that the monkey is making a particular saccade. To conclude that these substantia nigra cells contribute to the initiation of saccades, it is necessary to relate them to the neural circuits underlying saccade initiation.

Anatomical (1, 5-7, 14, 16, 21, 22, 24, 28, 31) as well as electrophysiological (3, 4, 12) studies have indicated that a substantial proportion of cells in the substantia nigra pars reticulata of the rodent, cat, and monkey project to the superior colliculus, and the superior colliculus is known to be involved in the initiation of saccades. In the monkey the nature of this involvement varies, depending on the layer of the superior colliculus in which the cell is located: cells in the superficial layers respond to visual stimulation, while cells in the intermediate layers have a burst of discharges before saccadic eye movements as well as have a response to visual stimulation (see Ref. 33 for summary). Although some of the anatomical studies have shown that the nigrocollicular projection is predominantly to the intermediate and deep layers (5, 16, 22, 24), which suggests a role for the substantia nigra in the initiation of saccades rather than in visual processing, we do not know whether the substantia nigra cells that we have found to have visual- or oculomotor-related activity are the ones projecting to the superior colliculus. Furthermore, we do not know how the different types of activity in substantia nigra cells contribute to the generation of the bursts of spikes in the superior colliculus cells that precede saccades.

In an attempt to answer these questions we applied electrical stimulation to the superior colliculus in order to activate the cells antidromically in the substantia nigra. In order to determine the relationship of both substantia nigra and superior colliculus cells to behavior as well as the relationship between the cells in these two areas, we did experiments in three steps: first, we identified the visual and oculomotor properties of a particular substantia nigra cell; then, we stimulated the ipsilateral superior colliculus, while moving the stimulating electrode through the layers of colliculus, to determine whether activity from what depth the substantia nigra cell was activated antidromically; finally, we identified the visual and oculomotor properties of the colliculus cells located near the points from which the substantia nigra cell was activated antidromically with the lowest thresholds.

We have found that nearly half of the substantia nigra cells with visual- or saccade-related activity project to the superior colliculus. The lowest threshold for this antidromic stimulation is predominantly among the saccade-related cells in the intermediate and deep layers. The results of these antidromic-stimulation experiments suggest a synaptic connection between the axons of the substantia nigra cells and the superior colliculus cells at these low-threshold points. The relationship between the cell discharge in the two structures is consistent with the idea that the substantia nigra acts on the superior colliculus at the time of saccadic initiation by a release of tonic inhibition.
A brief report has appeared previously (35).

METHODS

The general procedures concerning surgical preparation, recording of cell activity, data collection, and behavioral paradigms have been described in previous papers (18–20). All surgery was performed under aseptic conditions using sodium pentobarbital anesthesia. In the first step in these experiments, we recorded extracellular action potentials of a single cell in the substantia nigra of the alert monkey ipsilateral to the superior colliculus to be stimulated (Fig. 1.4). We used a glass-coated platinum electrode, and determined the relation of the cell to visual or auditory stimulation or the initiation of saccades using the different behavioral paradigms and response categories already described (18–20).

In the second step, we stimulated the superior colliculus with single electrical pulses to test for antidromic activation of the substantia nigra cell under study and the depth within the colliculus at which that activation occurred. We had previously implanted a stainless steel guide tube (19–23 gauge) so that it was directed toward the superior colliculus with its tip located about 5 mm above the collicular surface. This guide tube could be removed and directed toward different areas of the superior colliculus so that with successive positions of the guide tube, the whole representation of the visual field in the colliculus could be explored. At the start of an experiment we introduced a stimulating electrode through this guide tube into the superior colliculus. This electrode was a tungsten-wire microelectrode (F. Haer, Brunswick, ME) with an exposed area on the shaft of about 50 μm and a resistance measured at 10 kHz of 0.3 MΩ, and this microelectrode could be used for either stimulation or recording (Fig. 1.4).

While we continued to record from the substantia nigra cell, we moved the tungsten electrode up or down through the superior colliculus in 100- to 200-μm steps while applying current pulses through the electrode. If single spikes were evoked in the substantia nigra cell when the stimulating electrode was at a given depth below the surface of the colliculus, we determined the latency and the threshold for the evoked spike. In addition, we frequently checked to see if collision occurred between a spontaneously occurring spike in the substantia nigra cell and one produced by stimulation in order to make sure that this was an antidromic response resulting from the stimulation. D shows that the antidromic spike was reinstated by lengthening the interval between the spontaneous spike and the stimulus. Multiple sweeps are shown on all traces.
Antidromic response. We used a biphasic pulse with a negative and then positive component. Current and wave shape were monitored by displaying the voltage drop across a series resistor on an oscilloscope. Threshold current was the intensity of the negative pulse whose duration was 0.1 ms; the configuration or the intensity of the positive pulse had little effect on the threshold or latency of antidromic spikes. Stimulation was given at intervals of about 1 s. We did not attempt to stimulate the contralateral superior colliculus to activate substantia nigra cells antidromically.

In the third step of the experiment, we were able to identify the functional level within the colliculus where antidromic stimulation is effective by recording single and multiple cell activity within the colliculus through the microelectrode that was used for stimulation previously. By determining the types of visual- and saccade-related responses recorded along the penetration and matching these to previously established cell-layer relationships within the superior colliculus, we were able to establish the layer within the colliculus at which the electrode tip was probably located. Furthermore, we frequently switched the tungsten electrode back and forth between stimulation and recording modes to make sure that the locations of the superior colliculus cells were determined correctly in terms of the effectiveness of antidromic activation. The electrode was fine enough to record single, isolated spikes of cells in the intermediate and deep layers of the superior colliculus, although cells in the superficial layers were frequently difficult to isolate.

RESULTS

Antidromic activation of substantia nigra cells

We studied a total of 180 cells in the substantia nigra pars reticulata of two monkeys, and 51 of these cells were activated antidromically from the ipsilateral superior colliculus. Figure 1 shows an example of such antidromic activation. Following single-pulse stimulation in the superior colliculus (Fig. 1B), single spikes with a fixed latency (2.0 ms) were evoked in this substantia nigra cell. Threshold current at this particular depth was 18 μA. The evoked spike followed double-pulse stimulation at intervals as short as 0.8 ms. This interval varied for different cells, but never exceeded 2 ms.

The evoked spike collided with an orthodromic spike if the stimulus was applied immediately following the orthodromic spike. This was demonstrated by using a spontaneous occurring spike to trigger the stimulation (Fig. 1C and D). The minimal interval between the spontaneous spike and the stimulation by which a spike was still evoked by the stimulation was 2.6 ms. This corresponds approximately to the sum of the conduction time including the latent period for spike initiation (2.0 ms, approximated from the antidromic latency) and the presumed refractory period of the axon terminal (0.6 ms).

On the basis of these stimulation experiments, which showed a fixed-latency spike that followed stimulation at short intervals and that showed collision with orthodromic spikes, we concluded that the cells in the substantia nigra that were activated after stimulation of the superior colliculus were being antidromically driven. We did not see significant orthodromic responses in substantia nigra cells, as evidenced by either an increase or decrease of discharge rate, following the single-pulse stimuli of less than 50 μA that we used.

Specifity of nigrocollicular projection

The next question was whether the subset of substantia nigra cells that had activity related to visual stimulation or saccades project to the superior colliculus. For each of 125 substantia nigra cells obtained in one monkey (007), we studied the response types using the paradigms described in the preceding papers (18–20) and then determined whether the cell was activated antidromically from any point along a penetration through the superior colliculus. We limited the stimulus currents to 50 μA in all of the following experiments. The results shown in Table 1, which includes all cells from penetrations that extended through all layers of the colliculus, demonstrate that the probability of antidromic activation was higher when the substantia nigra cells had a visual or oculomotor response than when they did not. Of 65 cells that were related to visual or oculomotor behavior, nearly half (29 cells) were antidromically activated. In contrast, of 60 cells that were not related to visual or oculomotor behavior, only one was antidromically activated.

Table 2 shows the frequency of antidromic activation of cells with different types of sen-
**TABLE 1. Frequency of antidromic activation of substantia nigra cells with visual and oculomotor responses**

<table>
<thead>
<tr>
<th>Antidromic Response</th>
<th>Visual or Oculomotor Activity</th>
<th>Total</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>29</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>

+ or - indicates, respectively, the presence or absence of a visual or oculomotor response in substantia nigra cells or the presence or absence of an antidromic response (with threshold less than 50 μA) in substantia nigra cells; monkey 607.

sory or oculomotor response (see Refs. 18–20). For example, of 57 cells that showed a simple visual response, 34 cells were activated antidromically from the superior colliculus. The total number of responses is considerably larger than the number of cells studied (99 cells) because some cells showed more than one type of response. Every response type we have identified in the substantia nigra was represented by a cell that could be antidromically activated from the superior colliculus, suggesting that any visual or oculomotor activity in the substantia nigra is conveyed to the superior colliculus. There might be some difference in the pattern of projections for the different response types, but it is not possible to draw any conclusion from our experiments because the number of cases sampled was small for some response types and because in some cells we could not determine the presence of all response types before the cell was lost. One substantia nigra cell, which had an auditory response and was activated antidromically as well, also had a visual response. It should be noted that in both Tables 1 and 2, the number of cells in the substantia nigra with visual and oculomotor relationships that project to the colliculus is probably underestimated, since for any given substantia nigra cell the stimulating electrode in the colliculus could not be moved to parts of the colliculus related to other areas of the visual field.

Since the visual or saccade-related responses of both substantia nigra cells and superior colliculus cells have spatial selectivity (18–20), one might expect that substantia nigra cells would project to the parts of the superior colliculus whose visual receptive fields or movement fields correspond to those of the substantia nigra cells. To determine this field correspondence, we first found the receptive-field or movement-field center for the cells in the superior colliculus at the point of stimulation. As shown in previous studies (29, 34), superior colliculus cells along a penetrations through the collicular layers have visual receptive fields in the superficial layers that are in register with movement fields of saccade-related cells in the intermediate or deep layers. Even though the receptive fields and movement fields of substantia nigra cells were large, they have a gradient of response intensity within them (18–20), and we next determined the field center of the substantia nigra cell under study. Finally, we determined whether the field center of the substantia nigra cell fell within an arbitrary distance from the superior colliculus field center (as shown by a shaded area in Fig. 2). Most substantia nigra cells (16 of 18) whose field centers were within an arbitrary distance of those of superior colliculus cells at the site of stimulation were activated antidromically. On the other hand, less than half (15 of 35) of the cells with field centers that were remote from one another showed such antidromic activation.

These antidromic stimulation experiments show a specificity of projection from the substantia nigra to the superior colliculus: cells with visual or oculomotor activity are more

**TABLE 2. Antidromic activation and response types**

<table>
<thead>
<tr>
<th>Response Type</th>
<th>Fraction of Cells Antidromically Activated</th>
</tr>
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<tbody>
<tr>
<td>Visual</td>
<td>34/57</td>
</tr>
<tr>
<td>Saccade-modulated visual</td>
<td>3/5</td>
</tr>
<tr>
<td>Memory-contingent visual</td>
<td>3/5</td>
</tr>
<tr>
<td>Nonfixation-contingent visual on</td>
<td>6/21</td>
</tr>
<tr>
<td>Fixation-contingent visual off</td>
<td>2/14</td>
</tr>
<tr>
<td>Visual-contingent saccade</td>
<td>14/23</td>
</tr>
<tr>
<td>Memory-contingent saccade</td>
<td>4/9</td>
</tr>
<tr>
<td>Memory-contingent sustained</td>
<td>1/3</td>
</tr>
<tr>
<td>Auditory</td>
<td>1/2</td>
</tr>
</tbody>
</table>

Response types are described in the preceding papers (18–20).
likely to project to the superior colliculus than those that do not have such activity. This projection to the superior colliculus is to cells that have visual receptive or movement fields in the same area of the visual field as do the substantia nigra cells.

**Collicular depth and threshold and latency for antidromic activation**

The threshold for antidromic activation of each substantia nigra cell was dependent on the depth of the stimulating electrode within the superior colliculus, and an example of this depth-threshold relationship is shown in Fig. 3. The ordinate shows the depth of the stimulating electrode and the abscissa shows the threshold current required to evoke an antidromic response. This substantia nigra cell showed two low-threshold regions whose minimum thresholds were less than 10 μA: between 2 and 3 mm below and between 4 and 5 mm below the surface of the superior colliculus.

To determine the intracollicular localization of the low-threshold regions, we studied discharge patterns of cells along the penetration using the stimulating electrode for recording, and the type of antidromic responses found at different depths are shown beside the ordinate in Fig. 3 (using the same paradigms for visually related and saccade-related activity as in the first paper in this series, Ref. 18). The superficial part of the penetration (0–1 mm) was dominated by visual cells with or without slight saccade-related activity (VIS or VIS + SAC). These cells were usually not isolated but were recorded in groups. The intermediate part of the penetration (1.5–3 mm) was dominated by cells that showed a saccade-related burst of activity with or without a slight visual response (SAC or SAC + VIS). The deep part (3–6 mm) consisted of a nonuniform population of cells, including cells with a saccade-related increase in activity superimposed on a tonic discharge rate (SAC). By virtue of a previous series of studies in this laboratory (15, 34), which showed that these different cell populations were recorded, respectively, in the anatomically defined superficial layers (primarily the superficial gray and superficial white layers), the intermediate layers (intermediate gray and white layers), and the deep layers (deep gray and white layers) of the superior colliculus, we were able to localize the position of the stimulating electrode functionally. Therefore, we can conclude that the two low-threshold regions for the substantia nigra cell in Fig. 3 were located in two different layers: one at the level of saccade burst cells was probably in the intermediate layers; another at the level of saccade cells with higher background activity was presumably in the deep layers.

We found several patterns of depth-threshold relationship within the colliculus, and an indication of these patterns is illustrated in Fig. 4. The most common pattern had two peaks, one among the saccade cells and one deeper, as in Fig. 4A (and Fig. 3). Note, however, that while the deep low-threshold peak in Fig. 3 is among saccade-related cells, the deep low-threshold peak in Fig. 4A is clearly below the cells with visual and saccade-related activity that can be identified as a part of the superior colliculus. We conclude that this low-threshold peak was below even the deepest layers of the superior colliculus. One possible interpretation of this deep low-threshold point is that it results from stimulation of the substantia nigra axons as they ascend into the superior colliculus, a route that is evident in the anatomical descriptions.
Fig. 3. Threshold for antidromic response (absissa) of a substantia nigra cell as a function of the depth of stimulation within the superior colliculus (ordinate). Notations along the ordinate indicate types of superior colliculus cells recorded with the electrode that was previously used for stimulation. VIS, visual response; VIS + SAC, visual response with slight saccade-related activity; SAC + VIS, saccade-related cell with slight visual response; VIS = SAC, visual and saccade-related response equal; SAC, saccade-related cell without visual response; AUD, auditory cell; No R: cell without identifiable visual, auditory, or saccade-related activity. Latencies of antidromic responses at different depths were shown on the right (ms). The asterisk indicates the point at which the superior colliculus cell in Fig. 6 was recorded. The substantia nigra cell showed primarily a memory-contingent saccade response.

of the nigrocollicular fibers in the monkey by Jayaraman et al. (22).

Figure 4B shows a penetration with two low-threshold peaks but with the first low-threshold point among the visually activated cells presumably lying in the superficial layers and the second among the saccade-related cells. Figure 4C shows two low-threshold peaks but both are among the saccade-related cells. In both Fig. 4B and C there is also some indication of a third low-threshold peak again, probably at a point below the superior colliculus. Figure 4D shows an example of the remaining type of penetration, one with only one peak. Some other penetrations showed only a single peak but one that was broader than in 4D, and that included more than the saccade-related cells.

Figure 4 shows the variation in types of low-threshold profiles within the superior colliculus, and while there were multiple peaks for most penetrations, the most com-
FIG. 4. Threshold-depth relationship for four substantia nigra cells. Same conventions as in Fig. 3. In A two peaks are seen, one among saccade-related cells and one probably below the colliculus, while in B one peak is among visual cells and the other among saccade-related cells. In C both of two prominent peaks are among saccade-related cells, while in D only one peak is evident, which is also in the midst of saccade-related cells. Asterisks in A and B indicate the location of the superior colliculus cells shown in Figs. 7 and 8, respectively. The substantia nigra cell in A had a visually contingent saccade response, a visual response, and a saccade-modulated visual response; in B, a visual response and a visually contingent saccade response; in C, a memory-contingent saccade response; a memory-contingent sustained response, and a no-stimulation-contingent visual on response; in D, a visual response.

The common observation was that one low-threshold peak fell among the saccade-related cells in the intermediate layers. For 36 substantia nigra cells in which the depth-threshold relationship was tested in all collicular layers, 27 cells had a low-threshold peak in the presumed intermediate layers, 21 cells in the presumed deep layers or the underlying reticular formation, and 8 cells in the presumed superficial layers.

The latency of the antidromic response in single substantia nigra cells generally decreased as the point of stimulation moved deeper in the superior colliculus. In Figs. 3 and 4, the latency of the antidromic response measured using a current about 1.5 times the threshold is shown for successive depths on the right of each depth-threshold graph. Those points of depth forming a single low-threshold region usually had uniform or gradually changing latencies, while those forming different regions tended to have discrete latencies (Fig. 4B). Even within a single low-threshold peak, however, some substantia nigra cells showed two or more discrete latencies, depending on the intensity of stimulus currents, which suggests the activation of several different axonal branches.
Figure 5 shows the change in antidromic latency with depth in the superior colliculus for cells studied in one monkey. Latencies at different depths for each substantia nigra cell are connected by straight lines. Since the thickness of the layers of the superior colliculus varied for different penetrations and since we have found that the most common projection was to the saccade-related cells of the intermediate layers, we plotted the depth for all penetrations relative to the midpoint of these saccade-related cells; the estimated surface of the superior colliculus is shown on the ordinate. The latency in all penetrations ranged from 0.7 to 2.3 ms. For almost every cell the antidromic latency decreased as the point of stimulation moved deeper within the colliculus. Such a decrease in latency was particularly obvious for points above the saccade cell layer.

These results on antidromic latency are also consistent with the anatomical observations that the axons of the substantia nigra cells approach the superior colliculus upward from the underlying reticular formation. Our observations also suggest that these axons might branch in the deep and intermediate layers, since this would be consistent with a longer latency response in more superficial layers due to a slowing of their conduction velocities. We have not attempted to calculate conduction velocities, since the actual geometry of the pathway traversed by the substantia nigra axons is unknown.

**Comparison of discharge of substantia nigra and superior colliculus cells**

The prominence of the low-threshold points among the saccade cells in the superior colliculus makes it likely that the substantia
nigra cells have synaptic connections with at least some of the cells in the intermediate- and deep layers of the superior colliculus. If the connections are sufficiently strong, some relationship between the discharge patterns of these cells might be expected. Figure 6 shows that this is the case. The intracollicular depth-threshold relationship for antidromic activation of this substantia nigra cell is shown in Fig. 3. We chose the superior col-

FIG. 6. Correspondence of a decrease in the substantia nigra cell activity with an increase in the superior colliculus cell activity in relation to memory-contingent saccades. The same substantia nigra cell (upper trace) whose antidromic threshold curve is shown in Fig. 3, and a superior colliculus cell (lower trace) whose depth is indicated by the asterisk in Fig. 3. A shows activities related to a visually contingent saccade (saccade with overlap task). Note that the saccade-related spike burst of the superior colliculus cell was preceded by a weak visual response to the onset of the target. B shows activities related to a memory-contingent saccade (delayed saccade task—the flashed position of the target T had occurred before the beginning of the part of the trial shown). While the superior colliculus cell discharged before visually contingent, memory-contingent (and spontaneous) saccades, this substantia nigra cell paused primarily before memory-contingent saccades. H and V indicate horizontal and vertical eye positions. Each action potential is indicated by a dot. Consecutive trials are shown as a dot raster display for each condition, and a time histogram and a cumulative spike time histogram are constructed from the raster. Calibration line on the left to the histogram indicates 100 spikes/s per trial. Time scale (200 msec) indicates the period between large dots. Monkey number and cell number are on the lower left. A small bar on each raster line indicates the onset of the target point (T) for A and the offset of the fixation point (F) for B.
luculus cell closest to the low-threshold peak in the intermediate layers (as shown by the asterisk in Fig. 3) and compared its discharge pattern (lower record in Fig. 6) with the discharge pattern of the substantia nigra cell (upper record in Fig. 6). The substantia nigra cell decreased its discharge rate in relation to a saccade to a remembered target (Fig. 6B upper, delayed saccade task) but showed only a slight decrease in activity when the monkey made the same saccade to a visual target that was present (Fig. 6A upper record, saccade with overlap task). No change in discharge rate was seen in relation to spontaneous saccades made in darkness (not shown). This substantia nigra cell, therefore, had primarily a memory-contingent saccade response (see Ref. 20). On the other hand, the superior colliculus cell showed an almost identical burst of spikes before either a visually contingent saccade (Fig. 6A, lower), a memory-contingent saccade (Fig. 6B, lower) or a spontaneous saccade (not shown). Some discharges preceding the saccade-related burst in Fig. 6A were time-locked to the onset of the target (indicated by the small vertical bars on the raster lines) and were, thus, visual responses that can be separated from the sac-

**Fig. 7.** Correspondence of a decrease in the substantia nigra cell activity with an increase in the superior colliculus activity in relation to visually contingent saccades. The same substantia nigra cell (upper trace) whose antidromic threshold curve is shown in Fig. 4A and a superior colliculus cell (lower trace) whose depth is indicated by the asterisk in Fig. 4A. A shows activities related to a visually contingent saccade (saccade task), while B shows activities related to a memory-contingent saccade (delayed saccade task). The superior colliculus cell discharged in both of these conditions (as well as before spontaneous saccades), but this substantia nigra cell decreased its discharge rate only before the visually contingent saccade.
cad- related activity. Comparison of the pattern of discharge of these cells shows that substantia nigra cell activity decreases as superior colliculus cell activity increases when the monkey makes a memory-contingent saccade (Fig. 6D). The onset of the change in activity in both cells was approximately the same, but the decreased activity of the substantia nigra cell was more prolonged than that of the superior colliculus cell. A decrease in the discharge rate in the substantia nigra was accompanied by an increased rate in the superior colliculus, and this relationship between the two cell activities would be expected if the substantia nigra cell had an inhibitory connection onto the superior colliculus cell. More carefully controlled experiments using, for example, cross-correlation of spikes would be necessary to demonstrate a functional inhibitory connection between this particular pair of cells. In contrast, such a clear relationship between the activity of these two cells did not occur with visually contingent saccades (Fig. 6A).

The substantia nigra cell shown in Fig. 7 was also activated antidromically from the superior colliculus, and its depth-threshold relationship is shown in Fig. 4A. This substantia nigra cell, however, showed a visually contingent saccade response (Fig. 7A, upper record) rather than a memory-contingent saccade response (Fig. 7B, upper record) or a response related to spontaneous saccades (not shown). The superior colliculus cell, which was recorded near the low-threshold peak in the intermediate layer (shown by an asterisk in Fig. 4A), again showed a burst of spikes before any appropriate saccade to a visual target (Fig. 7A, lower record), to a remembered target (Fig. 7B, lower record), or to a spontaneous saccade (not shown). The burst of activity in the superior colliculus cell fell within the duration of the decrease in discharge rate of the substantia nigra cell. The inverse relationship between the visually contingent saccade activity of the substantia nigra cell and that of the colliculus cell is again consistent with the idea that the substantia nigra cell had an inhibitory connection onto the colliculus cell. The lack of such a response for memory-contingent saccades or spontaneous saccades, however, indicates that the colliculus cell must receive other inputs to produce the saccade-related spike burst in these latter cases.

A comparison of the discharge of a visual cell in the substantia nigra with a visual cell in the superior colliculus is shown in Fig. 8. The substantia nigra cell with a visual response (Fig. 8A) was antidromically activated with a low-threshold point in the superficial layer (the upper low-threshold point shown in Fig. 4B). The superior colliculus cell with a visual response (Fig. 8B) was close to the low-threshold point (as shown by the asterisk).
in Fig. 4D). The striking point in comparing these visual responses is that the decrease in discharge rate in the substantia nigra cell occurs after the burst of activity in the superior colliculus cell. This is to be expected since the latency for a visual response is always longer in the substantia nigra than it is in the superior colliculus (15, 18). The substantia nigra cell can therefore influence only the late discharge of the superior colliculus cell.

DISCUSSION

These experiments reveal two points about the relationship of the substantia nigra pars reticulata and the superior colliculus. First, a subset of monkey substantia nigra cells whose activity changes in relation to visual stimulation and saccadic eye movements projects to the superior colliculus. The visual receptive fields and movement fields of these substantia nigra cells tend to correspond with those of the superior colliculus cells lying near the axons of the substantia nigra cells. Second, these cells in the substantia nigra and superior colliculus change their discharge rate in inverse ways in relation to visual stimulation or saccadic eye movements: a decrease in the substantia nigra cells, an increase in the superior colliculus cells. The timing of the change in discharge rate indicates that the substantia nigra could contribute to the initiation of saccade-related activity in superior colliculus cells but not to the visual on-response of colliculus cells. We shall discuss each of these two major points.

Characteristics of nigrocollicular projection

The characteristics of the antidromic activation of substantia nigra cells by collicular stimulation in this study are consistent with data in other electrophysiological and anatomical studies. The antidromic latency in our experiments on the monkey ranged from 0.7 to 2.3 ms (largely between 0.8 and 1.5 ms), which is similar to the latency shown for substantia nigra pars reticulata cells after stimulation of the superior colliculus of the cat (0.5-2.2 ms; Ref. 3). Most substantia nigra cells were activated antidromically from the intermediate or deep layers of the superior colliculus while only a few cells were activated from the superficial layers, and this is consistent with the anatomical demonstration of labeled terminals primarily in the intermediate and deep layers following the injection of radioactive amino acids into the substantia nigra of the cat (16), the monkey (22), the rat (5), and the gray squirrel (24). The consistent decrease in antidromic latency with depth in the superior colliculus suggests that the axons from the substantia nigra reach the superior colliculus from the underlying reticular formation and continue ascending within the superior colliculus, as is clearly evident from the course of the axons shown in the figures of Jayaraman et al. (22). Although the locations of antidromically activated substantia nigra cells were not verified histologically in our experiments, we can safely assume that they were located in the lateral part of the pars reticulata, since cells with visual and oculomotor responses were shown to lie primarily in this lateral area in the first paper in this series (18). This lateral location of the antidromically activated cells is consistent with the localization of labeled cells in the monkey substantia nigra following horseradish peroxidase (HRP) injection into the superior colliculus (1, 6). Anatomical and physiological experiments indicate that these nigrocollicular cells have axon branches that also project to the thalamus and the reticular formation (3, 4, 7, 12, 27, 31).

Nearly half of the cells (45%) that showed some visual or oculomotor response were activated antidromically from the superior colliculus, whereas cells that did not show such responses were rarely activated antidromically. Furthermore, these cells that were not activated antidromically but did show visual or oculomotor responses usually had visual receptive or movement fields that did not correspond to the fields of superior colliculus cells along the stimulation penetration, suggesting that these cells might also have been activated antidromically from other parts of the superior colliculus that had corresponding fields. Taken together, these results suggest the presence of a subset of cells within the substantia nigra pars reticulata that carries visual or oculomotor signals to the superior colliculus.

A substantia nigra cell tended to project to that part of the superior colliculus whose
cells had visual receptive fields or movement fields in register with that of the substantia nigra cell. However, in some cases antidromic activation was obtained even when the field centers of the substantia nigra cell and the superior colliculus cells were fairly far apart. This might be explained by a peripheral overlap of their visual receptive or movement fields. For example, the field of a substantia nigra cell whose center is on the horizontal meridian and a collicular field whose center is on the vertical meridian could easily overlap each other, particularly since the field of a substantia nigra cell is sometimes nearly a hemi-field. We did not attempt to test this possibility because we usually did not determine the exact edge of the field of substantia nigra cells (18). We also did not determine the area of the superior colliculus influenced by a given substantia nigra cell, particularly by such a cell with a large visual or movement field.

**Contribution of substantia nigra cells to initiation of saccades**

Several lines of evidence have suggested that in the rat the nigrocollicular connection is inhibitory. Although York and Faber (36) observed excitatory responses in superior colliculus cells following substantia nigra stimulation, a subsequent carefully controlled study by Chevalier et al. (10) showed that such excitation resulted from activation of axons in the cerebral peduncle or the optic tract and that after transection of these fibers only inhibitory responses (with short latencies, 1–5 ms) were obtained in cells in the intermediate and deep layers of the colliculus. Biochemical studies (13, 32) have suggested that the nigrocollicular connection is mediated by γ-aminobutyric acid (GABA) as a transmitter; after lesion of the substantia nigra the activity of glutamic acid decarboxylase (GAD), a marker for GABA-containing neurons, was reduced in the superior colliculus. Finally, the inhibition of superior colliculus cells following substantia nigra stimulation was blocked by the iontophoretic application of bicuculline, a GABA antagonist (11).

The results of our experiments indicate that the nigrocollicular synaptic connection is also present in the monkey, that the connection is primarily to the intermediate layer, and that it is inhibitory. First, low-threshold points for antidromic activation of substantia nigra cells were obtained at specific depths within the superior colliculus and these low-threshold points can be taken to indicate the location of stimulated axons in the superior colliculus. Because the penetrations were vertical through essentially horizontal layers, the minimal threshold must be reached when the electrode tip was at the same depth as the stimulated axon. Although the horizontal separation between the electrode tip and the axon in this situation was unknown, they were highly likely to lie in the same collicular layer. This localization of low-threshold points also clearly excludes the possibility that we stimulated passing axon fibers that did not enter the superior colliculus. Second, multiple low-threshold points were frequently found even on a single penetration, and this suggests the presence of a substantial number of axon branches within the superior colliculus. The axons we stimulated were, therefore, probably not main stem axons but fine axon branches close to terminal arborization. Large increases in antidromic latency above the intermediate layers, as well as the unusual distribution of threshold with distance from a low-threshold peak, also suggests that we stimulated axons of small diameter; stimulation of stem axons of other areas has been shown to lead to gradual changes in threshold (17, 30). Third, comparison of the activity of a substantia nigra cell and a superior colliculus cell close to the low-threshold point (and most likely to be innervated by the substantia nigra cell) showed that a decrease in discharge rate of the substantia nigra cell was coupled with an increase in discharge rate of the superior colliculus cell in relation to saccades and visual stimulation. This inverse relationship suggests that the presumed synaptic connection is inhibitory, although more careful experiments on spike cross-correlation would be necessary to demonstrate the inhibitory synaptic connection between this particular neuron pair.

If the above hypothesis is correct, substantia nigra pars reticulata cells, which discharge rapidly most of the time, continuously inhibit superior colliculus cells, particularly saccade-related cells in the intermediate layers. A decrease in discharge rate of these sub-
stantia nigra cells would reduce the tonic inhibition, increase the excitability of the colliculus cells, and probably facilitate the initiation of saccades. For a particular type of substantia nigra cell, however, this saccade-facilitatory effect depends on how the saccade is initiated. Regardless of whether the saccade is made to a visual target, to a remembered target, or spontaneously without any particular target, most superior colliculus cells show a virtually identical increase in discharge rate as long as the direction and the amplitude of the saccade are unchanged. Substantia nigra cells, on the other hand, decrease their discharge rate in relation to a saccade made to a real target or a remembered target but never in relation to a spontaneous saccade. No one type of substantia nigra cell, by itself, can exclusively determine the behavior of a saccade-related superior colliculus cell; the activity of the superior colliculus cell must be the result of a convergence of different types of inputs including those from other brain areas.

The exception to such a nonselective nature of saccade-related collicular activity is the visually triggered movement cell in the colliculus described by Mohler and Wurtz (26), which discharges before saccades to a visual target but not in relation to spontaneous saccades. This activity is similar to the visually contingent saccade response in the substantia nigra, although whether visually triggered movement cells also discharge before a saccade to a remembered target has not been determined. Therefore, the selectivity of saccade-related responses of substantia nigra cells might be reflected in this type of superior colliculus cell and possibly in other types of colliculus cells, which are so far unknown.

How, then, can the substantia nigra contribute to the initiation of saccades? The two schematic diagrams in Fig. 9 show two conceptually different roles for the action of the substantia nigra on the superior colliculus, one in which the substantia nigra modulates the saccade-related response in the superior colliculus, the other in which it mediates this response. For the sake of simplicity, we will consider only two types of substantia nigra cells, each of which has only one of the two types of saccade-related responses, visually contingent saccade response (SAC/VIS) or memory-contingent saccade response (SAC/MEM). We will also assume that a given colliculus cell receives inputs from both types of these substantia nigra cells.

The modulatory function in the upper diagram of Fig. 9 shows the input from other brain areas that have saccade-related activity (SAC/VIS, SAC/MEM, SPON; activity related to spontaneous saccades) that have excitatory connections with the superior colliculus cell and that produce an increased rate of discharge preceding saccadic eye movements. The tonic inhibition from the substantia nigra would normally reduce the discharge rate of the superior colliculus cell; any excitatory inputs would be attenuated by this inhibition. Before each saccade a subset of substantia nigra cells, depending on whether the saccade is made to a visual target or to a remembered target, would release the superior colliculus cells from the tonic inhibition. This disinhibition would provide an increase in excitability and would enhance the effect of the saccade-related inputs from other brain areas, which would then be able to produce the presaccadic activity of the superior colliculus cell. One source of such sac-
cade-related activity, particularly to visual targets, might be the frontal eye fields (9), which have been shown to project to the intermediate and deep layers of the superior colliculus (23). This source could not, however, provide the input related to spontaneous saccades, since frontal eye field cells rarely change their activity before such saccades (8, 25).

The lower diagram in Fig. 9 shows inputs from the substantia nigra to the superior colliculus acting as a mediator of the saccade-related activity. This schema assumes tonic excitatory inputs or an intrinsic mechanism that, if acting alone, would produce continuous activity. The tonic inhibition from the substantia nigra input acts to balance this excitatory drive. Decrease of the inhibitory drive from a subset of substantia nigra cells before a saccade would release the colliculus cell from inhibition and lead to an increased rate of discharge before a saccade.

In both of these schemata an additional neural mechanism underlying spontaneous saccades is necessary, since we have found no activity changes in substantia nigra cells related to spontaneous saccades. This could be accomplished either by an external input, as indicated in Fig. 9, or by some mechanism within the superior colliculus. The latter possibility is particularly attractive because neither of the inputs we have considered, the frontal eye fields and the substantia nigra, show activity in relation to spontaneous saccades, and ablation of the superior colliculus has as one of its most striking concomitants the reduction in the frequency of spontaneous saccades (2).

While we have been focusing on the relationship between saccade-related substantia nigra cells and saccade-related superior collicular cells, many of the substantia nigra cells with visual responses also project primarily to the intermediate layers of the superior colliculus. If we continue to assume that the connections are also inhibitory, this activity would also result in a removal of tonic inhibition and consequently an increase in activity of colliculus cells. However, the activity of substantia nigra cells cannot be the only determinant of the visual response of colliculus cells because the latency of the visual response is longer for substantia nigra cells than for superior colliculus cells. Substantia nigra cells could contribute only to later components of the visual response of colliculus cells. This is also the case for the possible connection between substantia nigra cells with a simple visual response and the visual cells in the superficial layer of the superior colliculus (see Fig. 8).

In any case, the consequence of the activity of these visual or saccade-related substantia nigra cells is probably most consistent with the hypothetical organization shown in the upper part of Fig. 9, in which substantia nigra cells modulate the excitability of colliculus cells by releasing them from tonic inhibition.

REFERENCES


