Pursuit Subregion of the Frontal Eye Field Projects to the Caudate Nucleus in Monkeys

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INTRODUCTION

The role of the caudate nucleus in oculomotor control has been extensively studied with respect to saccadic eye movements. Neurons in the caudate show activity that is time locked to voluntary saccades (Hikosaka et al. 1989, 2000). Inactivation of the caudate by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a chemical agent that destroys dopaminergic neurons, causes disorders of both spontaneous and voluntary saccades (Kato et al. 1995; Kori et al. 1995). The saccade subregion of the frontal eye field sends direct projections to the caudate (Graybiel and Ragsdale 1979; Leichnetz and Gonzalo-Ruiz 1996; Stanton et al. 1988). Bilateral infarction involving the body of the caudate has been associated with saccade deficits in humans (Sharpe et al. 1987; White et al. 1983), as have studies of patients with metabolic disorders of the caudate nucleus (Corin et al. 1972; O’Sullivan and Kennard 1998; Anderson 1982; Kim et al. 1976; Kuo and Carpenter 1973; Nauta and Mehler 1966). Second, studies of patients with Parkinsonian disorders have described smooth pursuit abnormalities (Corin et al. 1972; O’Sullivan and Kennard 1998; Sharpe et al. 1987; White et al. 1983), as have studies of patients with metabolic disorders of the caudate nucleus (Ross et al. 1995). Third, a recent PET study has reported that activity in the caudate of human subjects was greater during visual pursuit tasks than during saccade tasks (O’Driscoll et al. 2000)

The goal of the present study is to obtain direct anatomical evidence that the caudate nucleus is involved in the control of pursuit eye movements. Low-threshold microstimulation was used to localize the FEFsac and FEFsem in Cebus apella monkeys. Either biotinylated dextran amines (BDA) or wheat germ aglutinin conjugated to horseradish peroxidase (WGA-HRP) was then injected into the identified cortical subregions to compare the distribution and density of anterogradely labeled terminal fields in the caudate nucleus. Dense terminal fields were observed in the caudate after both FEFsac and FEFsem injections. The density and area of the FEFsem terminal fields were comparable to the density and area of the FEFsac terminal fields. Preliminary reports of these results have been published in abstract form (Cui et al. 2000a,b).

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METHODS

Intracortical microstimulation was used to localize the FEFsem and FEFsac in seven hemispheres of four adult C. apella monkeys. The anterograde tracers BDA and WGA-HRP were injected into physiologically identified subregions of the frontal eye field (Table 1).

Surgical procedures

Surgical procedures have previously been described in detail (Tian and Lynch 1996a,b). All surgeries were performed under sterile conditions, following National Institutes of Health guidelines and a research protocol that was approved by the Institutional Animal Care and Use Committee. Each animal was pretreated with dexamethasone (0.5 mg/kg im) and atropine (0.04 mg/kg). Rocephin (50 mg/kg im) was given before and after surgery. Buprenex (10–30 μg/kg im) was given for postsurgical analgesia. Body temperature was maintained with a heating pad. Vital signs were monitored at regular intervals.

Electrical stimulation

Trains of negative, unipolar pulses were used to evoke eye movements. Pulse width was 0.5 ms, frequency was 300 Hz, and train duration ranged from 100 to 500 ms. Stimulus thresholds were determined for each site at which a movement was evoked. Stimulus amplitudes were limited to <150 μA. Each microelectrode penetration site in the FEF was photographed during the mapping procedure using either a film or digital camera attached to the operating microscope. Digital images of the brain were imported into CorelDraw to record the location of each electrode penetration on the image of the exposed cortex while the mapping was in progress.

Injections and histological processing

Following the electrophysiological mapping procedure, BDA was injected into the FEFsem or FEFsac in one hemisphere, and 12 days later WGA-HRP was injected into the FEFsem or FEFsac in the opposite hemisphere (see Table 1). Approximately 0.6 μl of tracer was injected at each site. After postsurgery survival periods of 14 days for BDA and 2 days for WGA-HRP, each of the monkeys (except C26) was deeply anesthetized with Nembutal and perfused transcardially with saline followed by a mixed fixative solution (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer). Monkey C26 was perfused similarly, but 4% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer). Monkey C26 was perfused similarly, but 4% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer). The brains were removed and placed in a 5% sucrose buffer. Sections were cut at 50 μm in the coronal plane on a freezing microtome. Every sixth section was cut at 100 μm in the coronal plane on a freezing microtome. Every sixth section was stained with cresyl violet for cytoarchitectural study. A series of sections adjacent to the cresyl violet sections was reacted for BDA (Chen and May 2000; Veenman et al. 1992). A second series of adjacent sections was reacted for WGA-HRP using the Molybdate-TMB protocol (Chen and May 2000; Olucha et al. 1985). Figure 1 shows typical injection sites.

Data analysis

BDA and WGA-HRP sections were studied using a Leitz Diaplan DMR microscope and a Metamorph image analysis system. The sections were viewed with light-field and dark-field optics. Digital images of cresyl violet sections were captured with an M2 image analysis system (Imaging Research). The regions of labeled axon terminals were then plotted on the digital images using CorelDraw software.

RESULTS

Fields of densely labeled axon terminals were observed in the body and the head/body junction of the caudate nucleus following tracer placements in both the FEFsem and the FEFsac. In general, the terminal fields labeled by injections into the smooth eye movement subregion of the FEF were equal in density and area to the terminal fields labeled by injections into the saccadic subregion. Figure 2 illustrates the levels of representative coronal sections through the caudate nucleus for monkey C21. The section numbers in this drawing correspond to section numbers of the C21 sections shown in Figs. 3 and 4. Figure 3A shows typical distributions of terminals labeled by an FEFsem injection (blue dots) and Fig. 3D shows the distribution of terminals labeled by an FEFsac injection (red dots). A typical zone of terminals labeled by a BDA injection into the FEFsem is illustrated in Fig. 3B. The location of this zone is

TABLE 1. Summary table of tracer placements

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Tracer</th>
<th>Subregion of FEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C21</td>
<td>Left 10% BDA</td>
<td>Saccade</td>
</tr>
<tr>
<td></td>
<td>Right 2% WGA-HRP</td>
<td>Pursuit</td>
</tr>
<tr>
<td>C22</td>
<td>Left 10% BDA</td>
<td>Pursuit</td>
</tr>
<tr>
<td></td>
<td>Right 10% WGA-HRP</td>
<td>Pursuit</td>
</tr>
<tr>
<td>C23</td>
<td>Left 10% BDA</td>
<td>Pursuit</td>
</tr>
<tr>
<td></td>
<td>Right 10% WGA-HRP</td>
<td>Saccade</td>
</tr>
<tr>
<td>C26</td>
<td>Left 10% BDA</td>
<td>Pursuit</td>
</tr>
</tbody>
</table>

FEF, frontal eye field.
indicated by the white rectangle in Fig. 3A. The black rectangle in Fig. 3B indicates the location of the high-power photomicrograph in Fig. 3C, in which labeled axon terminals, terminal boutons, and boutons en passage are visible. Typical terminal labeling after a BDA injection into the FEFsac is shown in Fig. 3E. Figure 3F is a high-power photomicrograph of terminal labeling following an FEFsac injection. The density of the FEFsac terminal labeling is comparable to the density of the FEFsac labeling.

Zones of terminal labeling were also observed in the putamen (indicated by red and blue dots in Fig. 3, A and D). The putamen labeling was less dense and less extensive than the caudate labeling and will be described in detail in a later paper. The results using WGA-HRP as a tracer in this study were qualitatively the same as the results obtained using BDA. Only BDA terminal fields are illustrated here because of the superior detail provided by BDA.

The edges of the zones of BDA terminal labeling were remarkably sharp. The terminal fields could therefore be traced with a high degree of accuracy. The regions of dense labeling were traced in 22 equally spaced sections through the caudate in four hemispheres. Seven representative sections from C21 (FEFsac injection and FEFsac injections), C22 (FEFsac injection), and C23 (FEFsac injection) are shown in Fig. 4. The FEFsac sections from monkeys C22 and C23 were selected to be at approximately the same levels of the caudate as the sections from monkey C21. The sections with FEFsac terminals were reversed as necessary (see Table 1) to allow each FEFsac section to be superimposed on the corresponding FEFsac section from C21. All terminal field outlines are taken from BDA cases, except for the FEFsac fields from C21, which are from a WGA-HRP injection. It is obvious from inspection that the areas of the FEFsac terminal fields are comparable in area to the areas of the FEFsac terminal fields, indicating that signals from the visual pursuit subregion of the FEF have the opportunity to exert a strong influence on the caudate nucleus. Furthermore, although there is some overlap between the FEFsac and FEFsac terminal fields, particularly in some more posterior sections, there is a strong tendency for the FEFsac fields to be located more laterally than the FEFsac fields.

DISCUSSION

The primary result of this study is the observation of a large direct projection from the pursuit subregion of the FEF to the caudate nucleus. A projection from the FEFsac to the caudate is well established (Graybiel and Ragsdale 1979; Leichnetz and Gonzalo-Ruiz 1996; Stanton et al. 1988), but the FEFsac-to-caudate pathway has not been previously described. Our finding that the FEFsac projection to the caudate is equivalent in both density and area to that of the FEFsac suggests that, in addition to its well-known role in controlling saccadic eye movements, the caudate may play an important role in the control of pursuit eye movements. Figure 4 illustrates the classical conception of the neural circuits that control pursuit (gray) and saccadic (red) eye movements (e.g., Leigh and Zee 1999), with our modifications to the pursuit pathway indicated in blue. The thick blue arrow indicates the results of the present study; the arrows from the VL/VA to the FEFsac and the arrow from FEFsac to the pontine nuclei represent the previous work from this laboratory (Tian and Lynch 1997; Yan et al. 1999). The arrows from the putamen/caudate to the globus pallidus (GP) and from the GP to the thalamus represent connections demonstrated in other laboratories (Carpenter et al. 1976; DeVito and Anderson 1982; Kim et al. 1976; Kuo and Carpenter 1973; Nauta and Mehler 1966). These results indicate that, in addition to the well-known direct pursuit pathway from the cortex to the oculomotor nuclei via the cerebellum and vestibular nuclei, there is the possibility of additional feedback control of pursuit eye movements via a circuit through the striatum. The recent demonstration of a tectothalamostriate circuit suggests the possibility of feedback influences on visuomotor control in the saccadic system (Harting et al. 2001), although in this case the feedback arises from the superior colliculus rather than the cortex. There appears to be only limited direct overlap of the FEFsac- and FEFsac-labeled terminal fields. Nevertheless, the presence of large areas of FEFsac-labeled terminals in a structure that has previously been associated primarily with saccadic eye movements suggests that the caudate may be a site at which pursuit eye movements and saccadic eye movements are coordinated with each other, as has recently been suggested for several other structures, including the superior colliculus, cerebellar vermis, rostral interstitial nucleus of the medial longitudinal fasciculus, central mesencephalic reticu-

Several clinical reports have described pursuit eye movement deficits in patients with Parkinson’s disease (Corin et al. 1972; O’Sullivan and Kennard 1998; Sharpe et al. 1987; White et al. 1983), an illness that involves the striatum, but there has been some question as to whether the deficits are related directly to the Parkinson’s disease or are primarily related to the normal aging process (O’Sullivan and Kennard 1998). The strongest prior evidence for a pursuit eye movement function for the caudate comes from a PET study in humans by O’Driscoll et al. (2000). Subjects were asked to complete visual pursuit and visual saccade tasks. Greater activation was found in the caudate nucleus during the pursuit task than during the saccade task. Our results provide an anatomical foundation for these clinical and human imaging observations and thus contribute additional support to the proposal that the caudate nucleus plays a role in the control of visual pursuit.

The FEFsac and FEFsem appear to project to different, nonoverlapping or only slightly overlapping regions within the caudate. This finding differs from the observation of Parasarathy et al. (1992), who proposed that cortical regions that are functionally similar and are connected transcor-tically project to overlapping zones within the striatum. Our differing results may be due to the functional difference between the saccade-related and pursuit-related regions that were injected. In contrast, Parthasarathy et al. (1992) injected saccade-related regions in the FEF and supplemental eye field (SEF) to produce overlapping terminal fields in the caudate. Although the degree of overlap seen in our cases appears to be small, the question of the extent of terminal field overlap should be addressed more definitively in the future by making placements of distinctive anterograde trac-

FIG. 4. Comparison of the areas of dense terminal labeling that originate in the FEFsem (blue) and the FEFsac (red) demonstrates that the terminal fields of the FEFsem projections to the caudate occupies at least as large an area within the caudate as the FEFsac terminal fields. Levels of the C21 sections are indicated in Fig. 2; a series of comparably spaced sections from each of 3 FEFsem hemispheres is superimposed on the C21 sections.

FIG. 5. Summary diagram of pursuit and saccade pathways from the cerebral cortex to the brain stem oculomotor system. Thick arrow from the FEFsem to the putamen/caudate represents the results of the present study. See text for additional details. PEF, parietal eye field; MT, middle temporal area; MST, medial superior temporal area; VLcr, rostral portion of the ventral lateral nucleus, pars caudalis; VApC, ventral anterior nucleus, pars parvocellularis; MD, medial dorsal nucleus; GP, globus pallidus; SNr, substantia nigra, pars reticulata; SC, superior colliculus; PPRF, paramedian pontine reticular formation and other premotor regions in the reticular formation.
ers in the same hemisphere. We have thus far had only very limited success in our attempts to find two compatible anterograde tracers to use in this capacity.

Our present observations of nearby but largely nonoverlapping terminal fields in the caudate is similar to the observation of Tian and Lynch (1996b), who described the afferent corticocortical connections of the FEFsem and FEFsc. They found that, within each of five eye movement–related areas (parietal eye field, SEF, medial superior temporal area, prefrontal eye field, and 7m), the distribution of labeled neurons that projected to the FEFsem was adjacent to, but distinct from, the distribution of labeled neurons that projected to the FEFsc. Our results thus provide additional support for the proposal of Alexander and Strick that the cortico–striatal–thalamo–cortical loop circuits are spatially segregated to a high degree (Alexander et al. 1986; Hoover and Strick 1993, 1999).

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