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Acute Activation and Inactivation of Macaque Frontal Eye Field With GABA-Related Drugs

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SUMMARY AND CONCLUSIONS

1. This project tests the behavioral effects of reversible activation and inactivation of sites within the frontal eye field of rhesus monkeys with microinjections of the γ-aminobutyric acid (GABA) related drugs bicuculline and muscimol.

2. Muscimol injections impaired the monkeys’ ability to make both visually and memory-guided saccades on targets at the center of the area represented by the injection site. The latencies of saccades to targets in regions flanking the injection were increased. For memory-guided saccades, saccades in the direction opposite to that represented by the injection site, were made with shorter latency than controls and often occurred before the movement cue.

3. Bicuculline injections produced irreversible saccades equivalent to the saccade vector represented by the injection site, often in a staircase of several closely spaced movements.

4. Both substances decreased the accuracy of fixation of a central light. The distribution of points of fixation on different trials was diffuse, and the angle of gaze tended to deviate towards the side of the injection.

5. The results of these acute injections are similar to those observed in the superior colliculus and are much more substantial than the effects observed in the long term after surgical removal of the frontal eye field. The results of this study promote a central role for the frontal eye field in the generation of all voluntary saccades and in the control of fixation.

INTRODUCTION

A wealth of anatomic and physiological data strongly suggest that the macaque frontal eye field (FEF) is intimately involved in the control and generation of voluntary saccadic eye movements (for reviews see Bruce 1990; Goldberg and Segraves 1989). In marked contrast to these findings are the data from surgical lesion experiments, which show few long-term effects of FEF removal upon saccadic eye movement performance (Lynch 1992; Schiller et al. 1980, 1987). These lesions produced an initial contralateral neglect that recovered completely within 2–4 wk. The most substantial long-term deficit was observed by Deng and colleagues (1986) who noted that unilateral FEF lesions strongly affected a monkey’s ability to make memory-guided saccades where targets were flashed briefly and extinguished 100 ms before the cue to make a saccade. In contrast, marked long-term deficits in saccadic eye movement behavior are produced when FEF lesions are combined with lesions of the superior colliculus (Keating and Gooley 1988; Schiller et al. 1980) or the posterior parietal cortex (Lynch 1992).

As an alternative to surgical ablation, we made small injections of γ-aminobutyric acid (GABA) related substances in the FEF of rhesus monkeys and tested their performance on oculomotor tasks. We used muscimol, a GABA agonist that has an inhibitory effect in the cerebral cortex to test the effect of inactivation of the FEF, and bicuculline, a GABA antagonist, that produces a disinhibition of cortical cells to test activation of the FEF (Andrews and Johnston 1979). These experiments allowed us to observe reversible, acute changes in oculomotor behavior that were substantially more dramatic than those observed with chronic lesions, which are not reversible and require a recovery time for the animal in which compensatory mechanisms are likely to mask many of the initial effects of the lesion.

METHODS

Two rhesus monkeys (MK1 and MK2) were trained on standard oculomotor tasks and prepared for chronic recording of eye movements and neuronal activity. Complete procedures for surgery, behavioral training, and electrophysiology have been described in detail elsewhere (Segraves 1992). All procedures to ensure the care and well being of these animals followed the guidelines of Northwestern University’s Animal Care and Use Committee.

Electrodes and injection cannulae were introduced into the FEF through guide tubes held in place by a plastic grid with 1-mm spacing between grid holes. The grid was fitted inside a surgically implanted stainless steel recording cylinder. Injection sites within the FEF were chosen based upon the identification of cells characteristic of that region and verification of threshold for electrically elicited saccades of <50 μA. The injection cannula was made from 30 gauge stainless steel tubing with insulated 0.05-mm diam stainless steel wire protruding from the tip for neuronal recordings. Injections of up to 1 μl, in 0.2-μl steps, were made by pressure that was controlled by a pneumatic picoclamp (WPI); the amount injected was measured by watching the movement of the fluid meniscus within a glass capillary tube that was attached to the end of the steel tubing. Concentrations were 5 μg/μl of muscimol and 1 μg/μl of bicuculline.

The following oculomotor tasks were used to test the effects of the injections: a fixation task during which the monkey fixated on a central spot of light that appeared on a tangent screen, a visually guided saccade task during which the monkey made a saccade from the fixation point to a peripheral target when the fixation spot was turned off, and a memory-guided saccade task during which the monkey maintained fixation on the central spot while a target was briefly flashed in the periphery; after a delay of 100 ms, the fixation spot was turned off and the monkey was required to make a saccade toward the place where the target flashed.

Relative target position and saccade vectors in this study are expressed in polar coordinates, radius and angle. An angle of 0° describes a rightward horizontal direction, and a 90° angle describes an upward vertical direction.

Histological localization of injections to the FEF was made in one monkey. At the time of this submission, the second monkey was still being used in related experiments.

RAPID PUBLICATION
RESULTS

We studied the effects of reversibly activating or inactivating eight sites in MK1 and five sites in MK2. We made a total of eight injections of bicuculline and five injections of muscimol in these sites. These injections were made over a period of several weeks for MK1, and during 2-wk long periods separated by 3 mo without injections for MK2. Injections of muscimol and bicuculline were interspersed, usually with a day or more left between injections. Two additional injections of saline were made as controls.

Injections of muscimol created an oculomotor scotoma. After the injection the monkeys had difficulty making saccades corresponding to the amplitude and direction of saccades represented at the injection site, though still making saccades to targets outside of this representation. The activity of the cells recorded from the tip of the injection cannula was shut down, and saccades could not be elicited with electrical currents as high as 150 μA. This effect was progressive. During the first 30 min, saccades affected by the injection had a longer latency but could still be made; however, after 30 min to 1 h, the monkeys began to have more difficulty making saccades represented at the center of the injection site, and the increase in latency spread over a wider portion of the representation.

Figure 1 is an example of such a site. Electrical stimulation yielded saccades of about 5° amplitude directed rightward and slightly below the horizontal meridian. Under normal conditions, the monkey made visually guided saccades to targets at 5° eccentricity with no mistakes and mean latencies of 186-229 ms. Following the injection of muscimol, the monkey did not make saccades to targets located at angles of 0° and 315° with the exception of one rewarded saccade (arrow on left side) was made with very short latency. Liberal acceptance criteria for eye position were applied after the injection to induce the monkey to complete the trials. In all figures, single dots represent eye position sampled at a frequency of 1 kHz.
The monkey could not make saccades into the movement field represented by the injected site, while easily making saccades to targets outside the movement field. The latencies of saccades to targets adjacent to those most affected by the injection increased in comparison to the latencies of saccades to the same targets before the injections. The latencies for rewarded saccades in directions opposite the injection site field were reduced; however, examination of Fig. 2, middle, suggests that the monkey had difficulty making these saccades. In fact, many saccades to targets in the left hemifield were made prematurely, before the fixation light was turned off (Fig. 2, bottom).

Outside of the task conditions described above, the monkeys also seemed to have difficulty generating the saccades most affected by the injection site. For example, after the injection described above, trials where the monkey made a leftward eye movement required that the monkey make a rightward eye movement during the intertrial interval to return her gaze to the central fixation light at the beginning of the next saccade. This rightward movement was the one most affected by the injection, and the monkey would adopt
an alternate strategy for returning gaze to the center of the tangent screen. This might include a large downward saccade followed by an oblique saccade up and to the right placing the eyes on the fixation point. These effects were observed for the duration of the testing session, which lasted for ~3 h after the time of the injection. By the next day the monkeys made normal saccades in all directions.

Bicuculline injected at approximately the same site but on a different day produced an effect that was nearly opposite to that produced by muscimol (Fig. 3). During the time that the injection was being made, cellular activity recorded from the injection cannula began to increase producing bursts of cell activity lasting for the duration of each sequence of saccades. After bicuculline injections, the monkeys were hampered in their ability to correctly perform a visually guided saccade task. Frequently, the first saccade, made after the fixation point was extinguished, went towards the area represented by the injection site regardless of the position of the target. After this initial movement, a corrective saccade was made toward the location of the visible target. Fixation was less accurate and deviated toward the side of the injection, although these effects were not as substantial as those observed after muscimol injections. The monkeys were incapable of doing the memory-guided saccade task because they could not maintain fixation long enough for the task to proceed to the point where they were allowed to make a saccade to the remembered location of the target. The effects of the bicuculline injection began to subside after 1.5–2 h.

Control injections of saline at the same locations where muscimol and bicuculline injections were made had no effect on the monkeys’ oculomotor performance.

DISCUSSION

We have demonstrated that injections of GABA-related substances into small regions of the FEF produce profound changes in the oculomotor performance of rhesus monkeys. The effect of bicuculline is similar to that observed with microstimulation using long trains of electrical pulses (Robinson and Fuchs 1969). The effect of muscimol may be compared to that of surgical removal of the FEF although the effects of muscimol are much more substantial. Surgical lesions produce an initial contralateral neglect with a decrease in the number of saccades to objects in the contralateral hemifield. After several weeks, however, the monkeys recover from the acute effects of the lesion and make visually guided saccades with normal latencies (Schiller et al. 1980, 1987). FEF lesions do appear to have a permanent effect on eye movements that require higher level cognitive processing, including saccades to remembered target locations (Deng et al. 1986) and the ordered sequences of saccades generated during visual scanning (Collin et al. 1982). Cooling of the entire FEF also produces a contralateral neglect, with a return of the ability to make visually guided saccades, albeit with increased latencies, when the cooling temperature is raised slightly (Keating and Gooley 1988). In the present study, small muscimol injections produced deficits in both visually and memory-guided saccades. These deficits included an elimination of saccades directed toward the center of the area represented by the injection site, increases in latency in flanking regions, and decreases in latency for memory-guided saccades in the opposite direction, including many premature saccades in the memory task. The accuracy of fixation decreased, and the angle of fixation was shifted toward the side of the injection. The severity of the effects we observed can be attributed, at least in part, to the elimination of the possibility for reorganization and recovery of function that is likely to occur after a surgical ablation.

An interesting component of our findings is the decrease in latencies accompanied by a high number of saccades an-
participating the movement cue in the memory task for stimuli located outside of the area represented by the muscimol injection site. A recent report has described a fixation disengagement signal in the FEF that is carried by about one-half of the cells active before saccades (Dias and Bruce 1994). Our results suggest that a localized inactivation of this signal causes an imbalance in this population of FEF cells resulting in changes in latency and a decrease in the ability to suppress saccades during fixation. This is reminiscent of the decrease in the ability to suppress inappropriate saccades in the anti-saccade task observed in humans with damage to prefrontal cortex (Guitton et al. 1985).

In the presence of bicuculline the balance appears to go the other way. The bursts of neuronal activity recorded from the injection cannula are likely to assist in the disengagement of fixation. Further analysis of these bursts will indicate whether they provide a constant signal specifying a saccade of the represented vector or contain dynamic information relevant, perhaps, to the time of initiation of each saccade within a sequence.

Our results are similar in many ways to the oculomotor deficits reported after injections of muscimol and bicuculline in the superior colliculus (SC) (Hikosaka and Wurtz 1985). This similarity is not unexpected considering the strong FEF input to the SC and suggests that these two regions share many functions in parallel. This is not meant to imply that FEF and SC contributions to oculomotor control are identical but rather that this preliminary analysis is not refined enough to reveal the essential differences between these two structures.

In combination with the results of earlier work, the present findings support the idea that the FEF is a crucial component of a complex network of cortical and subcortical structures responsible for the generation of saccadic eye movements. Although the FEF may be absolutely essential for the generation of some high-order types of saccades, our results emphasize its importance in the maintenance and disengagement of fixation and the generation of all voluntary saccades.

We are grateful to P. Ko and the staff of Northwestern’s Center for Experimental Animal Resources for animal care, Northwestern’s instrument shop for machining, and the chemistry electronics shop for electronic hardware. This work was supported by National Eye Institute Grant EY-08212.

Received 31 July 1995, accepted in final form 7 September 1995.

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