Effects on eye movements of a GABA agonist and antagonist injected into monkey superior colliculus

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(Accepted April 4th, 1983)

Key words: superior colliculus — substantia nigra — GABA — bicuculline — muscimol — saccades

Injection of muscimol, a GABA agonist, into the superior colliculus of awake monkeys produces saccades to visual targets in the movement field of colliculus cells near the injection site that have longer latency and shortened amplitude. Bicuculline, a GABA antagonist, leads to irrepressible saccades towards the movement field. These results are consistent with a tonic inhibition by the substantia nigra on the superior colliculus mediated by GABA which is reduced at the time of saccade initiation.

Cells in the lateral part of the substantia nigra pars reticulata (SN) of the monkey have been shown to respond to visual or auditory stimulation or in relation to the initiation of saccadic eye movements to visual targets or to remembered targets. The response of these SN cells is a pause in a high and steady discharge rate (frequently 80–100 spikes/s). The most prominent connection of these cells to brainstem oculomotor areas is through the superior colliculus (SC) since the SN in the monkey has been shown to project to the SC. The projection according to anatomical studies and antidromic stimulation is primarily to the intermediate layers of the SC. In the monkey, cells in these layers of the SC discharge before the onset of saccadic eye movements made to one area of the visual field, the movement field of the cell. The projection from the SN to the SC in the rat has been shown to be inhibitory and it seems likely that the transmitter is y-aminobutyric acid (GABA).

In light of this connection between the SN and SC, one hypothesis of the function of the SC related to the initiation of saccades advanced by Hikosaka and Wurtz was that SN cells exert tonic inhibition on SC cells and that the pause in SN cell discharge before saccadic eye movements allowed the burst of activity in the SC cells. If this hypothesis were correct, application of GABA agonists and antagonists in the SC should clearly affect the initiation of saccades. The present experiments show that this is indeed the case.

Two awake monkeys, trained to make saccades to visual targets, were implanted with a device for head restraint and recording of single cell activity in the SC (see ref. 9 for details). A stainless steel guide tube (19-gauge) was implanted which was directed to the SC and whose tip came within 5 mm of the surface of the SC. Through this guide tube we introduced a glass pipette filled with a drug solution which could be pressure injected into the brain. The pipette also contained a fine tungsten microelectrode that extended 100–200 μm beyond the 30–60 μm orifice of the glass pipette and allowed recording of single cell activity.

In each experiment we first recorded the monkey's saccades to visual targets and his spontaneous saccades made in light or dark. Saccades to visual targets throughout the central 30° of the visual field were tested by rewarding the monkey in a behavioral task that required a saccade from a central fixation point to the visual target (saccade task of Hikosaka and Wurtz). One block of experiments consisted of two trials for each point presented in a random sequence. The glass pipette then was introduced into the intermediate layers of the SC as identified by cell discharges preceding saccades. The movement field of
the SC cells was determined along with the area to which saccades were directed when stimulating currents were passed through the microelectrode. Following this, the injection was made over several steps, each being 0.2 or 0.4 μl; a successful injection was indicated by a temporary silencing of cellular activity, presumably due to mechanical displacement of the cells by the injected fluid. The pipette was usually removed about 20 min later. The monkey’s saccades were monitored over a period of several hours or until recovery was complete.

Fig. 1 shows the effect of muscimol, a GABA agonist. The pipette was in an area of the left SC where the cells discharged before saccades to the lower right (contralateral) visual field and where stimulation produced right-downward saccades about 15° long (Fig. 1A). 1B1 shows a sample saccade made to a visual target in the movement fields of cells near the pipette tip and Fig. 1C1 shows saccades to a symmetrical point in the ipsilateral visual field. The effect of the muscimol injection (0.8 μl of 1 μg/μl of saline) on saccades became apparent before the injection was completed. The injection of muscimol produced obvious changes in saccades to the target in the cells’ movement fields (Fig. 1B2, compared with B1). First, the latency increased by over 300 ms. Second, the saccades became hypometric; the hypometric saccades were usually followed by corrective saccades which also had a long latency (so long that the corrective saccade is not seen in Fig. 2B3). There was also a slowing of the saccade although this has not yet been analyzed quantitatively.

Fig. 1. Effect of muscimol in the SC on saccade initiation. In A, stimulation through the microelectrode within the pipette in the left SC produced saccades 10° in amplitude to the right (contralateral) and down (threshold = 9 μA). The vertical line indicates the onset of pulse train stimulation; its duration was 50 ms. Before injection of muscimol (B1 and C1) the monkey made saccades to two different visual targets. Eccentricity of the targets from the fixation point was 20° in both B1 and C1, but 45° down to the right in B1, and 45° down to the left in C1. Three min after injection of muscimol (0.8 μl) through the pipette (B2 and C2) the onset of saccades to the right-down target (B2) was delayed and the amplitude and velocity decreased while saccades to the left-down target (C2) were unaffected. In each section of the figure the top trace is the horizontal, the bottom is the vertical component of eye position; each trace consists of two superimposed eye movement records. The vertical line in B and C indicates the time at which the fixation point went off and the visual target came on. Dots at the end of the traces indicate the position of the visual target.
On the other hand, saccades to the ipsilateral field were relatively unaffected (Fig. 1C; compared with 1C1). Saccades to upper quadrants of both the ipsilateral and contralateral visual fields were similarly unaffected. However, there was some indication that the affected area enlarged slightly over time suggesting the diffusion of muscinol within the SC.

If there was no target on the screen either in light or dark, the monkey made many fewer 'spontaneous saccades' particularly in the direction of the cells' movement fields. The eye tended to stay within a small area; this area was on the ipsilateral side usually symmetrical to the cells' movement fields relative to the center of the orbit. About 30 min after injection the monkey never looked at objects on his right side, such as the investigator or food pellets, although he ate the pellets very eagerly. Slow horizontal deviation of the eye to the contralateral side alternating with fast phases to the ipsilateral side appeared within 35–100 min after injection.

Bicuculline dramatically increased the frequency of contralateral saccades of a particular direction and

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Fig. 2. Effects of bicuculline in the SC on spontaneous eye movements and eye position. A shows a saccade (10° to the left) evoked by stimulation through the pipette in the right SC. In A2, the top trace is horizontal, and the bottom trace is the vertical component of eye position. In A3, the same eye movement is shown on a two-dimensional plane with the location of the fixation point shown by a central dot; the square of dots is 40° on each side. B2 and C2 show spontaneous saccades after injection of bicuculline (0.4 µg) when no target was present on the screen; B3 and C3 are corresponding two-dimensional displays. In B, leftward saccades occurred repetitively bringing the eye from the center of the orbit to the left (contralateral) periphery. In C, square wave saccades (leftward and then rightward) occurred repeatedly, and the starting points of the square wave saccades C2 were located near the end point of the stimulus-evoked saccade (A3).
amplitude which corresponded to the movement fields of SC cells near the injection site (Fig. 2). In Fig. 2A, stimulation through the pipette in the right SC produced horizontal, leftward saccades that were about $10^\circ$ in amplitude. Fig. 2B, shows that immediately after injection of bicuculline (0.4 $\mu$l of 1 $\mu$g bicuculline methiodide of saline), leftward saccades occurred repetitively so that the eye was moved from the center of the orbit to the left periphery; the eye was usually brought back to the central area by a large rightward saccade. Each leftward saccade was similar in size and direction to saccades evoked by stimulation (compare Fig. 2B, with Fig. 2A). The repeated saccades sometimes took the form of square wave jerks (Fig. 2C) with the first leftward component similar to the stimulus-evoked saccades. The square wave jerks usually started from a point in the orbit which was near the end point of stimulus-evoked saccades that started from the center of gaze (compare Fig. 2C with Fig. 2A). Then a slow deviation of the eye to the ipsilateral side developed which alternated with a contralateral fast phase similar to the stimulus-evoked saccades. Under any of these conditions the monkeys could not suppress contralateral saccades, could not fixate the center spot, and therefore could not perform the task. They showed no sign of discomfort.

About 40 min after the injection the monkey became able to acquire the visual target, but the targeting saccades were preceded or followed by square wave saccades similar to those in Fig. 2C. When the target was outside the movement fields of SC cells near the injection site, the monkey tended to make a saccade to the movement fields of these cells and then make another saccade to the target.

With the doses of muscimol and bicuculline we used no apparent changes in eye movements were observed on subsequent days and SC cell activity recorded using the same guide tube looked normal. Injection of saline (2 $\mu$l) in the SC produced no apparent change in eye movements.

The results of these experiments are consistent with the hypothesis that the SN acts to tonically inhibit the SC and that this inhibition is mediated by GABA. Bicuculline, a GABA antagonist, applied in the SC probably blocked the tonic inhibition on saccade-related SC cells: more saccades occurred whose direction and amplitude corresponded to the movement fields of SC cells where the injection was made. In contrast, muscimol, a GABA agonist, produced effects consistent with an increase in tonic inhibition: saccades toward the movement field areas were delayed, reduced in amplitude, and probably had lower velocity. Furthermore, such spatial specificity of drug actions suggests that a large proportion of the injected drugs stayed in a relatively small area of the SC.

Our experiments confirm and further emphasize the important role of the SC in saccade generation (see ref. 22, for summary). Disruption of saccades by muscimol appears more pronounced and yet spatially localized than that after surgical lesions of the SC (11, 28). Unlike these lesion studies we were able to see instantaneous effects of alteration of SC cell activity during which functional compensation by other brain areas might not yet have developed. This suggests that direct efferent pathways to the brainstem oculomotor system from such areas as the frontal eye fields (15) usually make a relatively small contribution but assume a more prominent function with time after removal of the SC.

The striking effects on eye position — toward the movement field with the GABA antagonist, away from it with the GABA agonist — is difficult to interpret. Such a shift might simply result from a preponderance of saccades in one direction or it might reflect an influence of SC activity on eye position.