Punctate chemical lesions of striate cortex in the macaque monkey: effect on visually guided saccades

W.T. Newsome\textsuperscript{1}, R.H. Wurtz, M.R. Dürsteler\textsuperscript{3}, and A. Mikami\textsuperscript{2}

Laboratory of Sensorimotor Research, National Eye Institute, Bldg. 10, Rm. 6C-420, Bethesda, MD 20205, USA

\textbf{Summary.} Chemical agents which reversibly or irreversibly disrupt neural processing offer several advantages over traditional techniques for behavioral studies of the central nervous system. In order to evaluate the utility of chemical agents for a behavioral analysis of visual cortical function in primates, we have tested the effects of muscimol and ibotenic acid on the function of striate cortex in awake, behaving monkeys. We studied the monkey’s ability to generate saccadic eye movements to visual targets at various locations in the visual field following an injection of one or the other chemical solution into a topographically identified location in striate cortex. Our results show that deficits in the generation of visually guided saccades following such injections are similar to those that result from surgical ablation of striate cortex, although recovery is more rapid following the injections. The experiments indicate that, with certain restrictions, chemical inactivation is a useful technique for behavioral analysis of visual cortical function.

\textbf{Resume.} Chez le Cachat éveillé, l'activité électromyographique induite dans les muscles du cou par le réflexe vestibulo-collique a été enregistrée pendant des rotations sinusoidales en bloc du tronc et de la tête autour d'un axe longitudinal, de 0,2 à 1,2 Hz, à la lumière ou à l'obscurité. Les diagrammes de phase et de gain obtenus sont semblables à ceux décrits chez des animaux d'évéités ou anesthésiés. Chez l'animal éveillé, toutefois, la contraction des muscles est modulée par la position de l'œil, comme lors de rotations autour de l'axe stéréotaxique vertical. La contraction des muscles du cou serait donc sous le double contrôle de la position de l'œil et de l'angle d'inclinaison de la tête, ce qui permettrait la suppression des mouvements compensatoires d'origine vestibulaire pendant des réactions d'orientation.

\textbf{Key words:} Ibotenic acid – Muscimol – Striate cortex – Visually guided saccades

\textbf{Introduction}

Visual cortex in the macaque monkey is composed of the primary receiving area, striate cortex, and at least nine extrastriate visual areas (for reviews see Van Essen 1979; Zeki 1978). Together these areas occupy approximately 50% of the total expanse of neocortex in the macaque (Van Essen and Maunsell 1980). Anatomical experiments have resulted in clearer ideas concerning the general organization of extrastriate areas, and physiological experiments have provided clues concerning the nature of neural processing in several of them. However, direct behavioral evidence concerning the functional role of each area in visual perception and visually guided behavior is only beginning to be obtained (Cowey and Porter 1979).

Techniques for chemical inactivation of identified visual areas offer a promising approach to this problem (Newsome et al. 1983; 1985). Injection of the chemical agent may be accomplished quickly and without surgical trauma so that a monkey’s performance on specific visuomotor tasks can be evaluated immediately before and after the chemical inactivation. In addition, chemical injections permit inactivation of visual areas that cannot be selectively ablated with surgical techniques due to their location within deep sulci. Several problems, however, are associated with chemical techniques. First, it is unclear

\textsuperscript{1} Present address: Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794, USA
\textsuperscript{2} Present address: Department of Neurophysiology, Primate Research Institute, Inuyama 484, Japan
\textsuperscript{3} Supported by Schweizerische Stiftung für medizinische-biologische Stipendien

\textit{Offprint requests to:} R.H. Wurtz (address see above)
whether the quality of chemically induced lesions in monkey cortex is sufficiently comparable to a surgical ablation to warrant their use: some cells are known to survive kainic acid or ibotenic acid treatment (Köhler 1983), and neurotransmitter agonists and antagonists may not disrupt all neural processing in the affected region. Secondly, to create localized lesions, injections (and therefore lesions) must be kept small. If the anatomical target were a large cortical visual area, for example, it is uncertain whether a relatively small lesion would result in a detectable behavioral deficit.

In an attempt to evaluate these problems, we have studied the effects of punctate chemical lesions of striate cortex in the macaque monkey. Striate cortex is large (≈ 1200 mm²) compared to the dimensions of a typical chemical lesion (4–12 mm²), and we were able to take advantage of the well studied topographic organization of striate cortex to assign behavioral effects to discrete loci of cortical damage. We evaluated visuomotor function by examining the monkeys' performance on visually guided saccadic eye movements since surgical ablation of striate cortex is known to disrupt performance on this task (Mohler and Wurtz 1977). We were therefore able to compare directly the effects of chemical agents to the effects of surgical ablation on the same brain structure using the same behavioral assay.

We employed a reversible and an irreversible chemical agent in this study: muscimol is a potent GABA agonist (Krnenšćic and Schwartz 1967; Andrews and Johnston 1979) which can cause a reversible and localized disruption of neural activity, presumably by activating intracortical inhibitory networks; ibotenic acid is a neurotoxin which selectively kills cell bodies in a localized area around the injection site (Schwartz and others 1975; Guldin and Markowitsch 1981). Following injections of either muscimol or ibotenic acid into striate cortex, monkeys were unable to make saccades to the portion of the visual field affected by each injection. Monkeys acted as though they did not see the target light when it fell within an affected portion of the visual field. The monkeys recovered from the deficits induced by both drugs, but the short-term effects on visually guided saccades were similar to the effects of surgical ablations.

Methods

Two monkeys (Macaca mulatta) were used. Each monkey underwent aseptic surgery under nembutal anesthesia in which a recording cylinder and head holding device were implanted on the skull. In addition, a scleral search coil was implanted around the eye for measuring eye movements. The recording cylinder covered a cranialotomy which permitted access to the lateral surface of striate cortex and the calcarine sulcus beneath. Details of the surgical procedures have been published elsewhere (Hikosaka and Wurtz 1983).

During training sessions monkeys were comfortably seated in a primate chair with their heads fixed in position. We trained the monkeys to fixate and to make saccadic eye movements to visual targets back-projected onto a tangent screen. Behavior control, stimulus presentation, and data collection were accomplished by means of a PDP 11/40 laboratory computer. Eye movements were measured by the magnetic search coil technique (Fuchs and Robinson 1966). The monkey's initial task was to keep his eyes within an electronically defined window centered on the fixation point for a variable period of time (1–4 s). The window was kept small (usually less than 1.5° in diameter) to minimize eye movements during fixation. After the monkey learned this task, the fixation point was extinguished at an unpredictable time and a saccade target appeared simultaneously at one of several possible locations on the tangent screen. The monkey was allowed 400 ms to move his eyes into a new window (usually 3° in diameter) centered on the target, and was required to maintain his eye position within that window for the remainder of the trial. The monkey was rewarded upon successful completion of a trial with a drop of water. Water intake was controlled from day to day so that monkeys were maintained in a healthy but motivated state. Monkeys were returned to their home cages after each session.

During experiments, monkeys performed blocks of trials in which the location of the saccade target was varied in pseudorandom order from trial to trial. A total of eight trials was usually presented for each target location. In each block of trials, about half of the target locations were selected so as to establish the boundaries of deficient regions of the visual field while the other half were control locations remote from deficient regions. Blocks of trials were obtained before an injection and at regular intervals following an injection until recovery was complete. We recorded the percentage of successfully completed saccades for each test location. We considered a saccade to be successful if the eye fell within 3° of the target within 400 ms of target onset. Visual inspection of the eye movements recorded at rates of 2000 Hz permitted us to tell whether missed trials were due to dynamic saccades or to a failure to attempt the saccade.

Injections were made with a one microliter syringe (Hamilton) which had been insulated with tape to permit multian electrodephysiological recording from the needle tip, thereby allowing positive identification of the target site before the injection was made. One monkey received a microliter injection of muscimol (1 µg µl, Sigma) in saline, and the second received a microliter injection of ibotenic acid (15 µg µl, Regis) in a basic saline solution. Each injection was made at a rate of 0.1 µl/min, and the syringe needle remained in place for 15 min following the injection before it was withdrawn. Both monkeys were also used for electrophysiological recording experiments unrelated to the present study.

Following completion of experimental protocols, both animals were deeply anesthetized with nembutal, and perfused through the heart with normal saline and 10% formalin. The brains were blocked and sectioned on a freezing microtome. Sections at regular intervals through the hemispheres were stained for cell bodies with cresyl violet and for myelinated fibers by the silver stain of Gallyas (1979).

Results

Muscimol injection

Figure 1 illustrates the results obtained from an injection of muscimol into striate cortex. We first
mapped the target region of striate cortex with a microelectrode, and a typical penetration is illustrated in the inset of Fig. 1A. We recorded a multiunit receptive field on the lateral, or opercular, surface of striate cortex, a second striate field in the "roof" of the calcarine sulcus and a third striate field in the dorsal bank of the calcarine sulcus. The receptive field recorded at each of the three locations is illustrated in the visual field diagram of Fig. 1A. Figure 1B shows the monkey's preinjection performance on the saccade task. The monkey performed at virtually 100% correct at every location tested, including locations which corresponded to the receptive fields illustrated in Fig. 1A. The targets were 0.05° in diameter and were one log unit in intensity above the background level.

A one microliter injection of muscimol was then made in the roof of the calcarine sulcus (recording site and receptive field 2 in Fig. 1A). Positive identification of the injection site was made by mapping the multiunit receptive field recorded through the laguer-insulated syringe needle: this closely matched receptive field 2 in Fig. 1A. The monkey began to omit saccades to some targets within 10 min after the injection was completed. The affected region of the visual field was centered on receptive field 2 and increased slowly over the next few hours. The "scotoma" induced by the muscimol reached its largest extent 5–6 h after the injection as is illustrated in Fig. 1C. The monkey successfully completed less than 10% of the trials within a region approximately 10° in diameter. In the most severely affected region, missed trials did not result from saccadic dysmetria; the monkey simply did not attempt to make the saccade within the allotted interval of time (400 ms), as if it never saw the target. The affected region spread further toward the periphery of the visual field than toward the center as would be expected from the well-known decrease of

Fig. 1A–D. Deficits in saccades that followed an injection of muscimol into striate cortex. A The visual field diagram shows the multiunit receptive fields (dashed circles) recorded at each of the three locations in striate cortex illustrated in the inset drawing. The inset drawing is the posterior half of a parasagittal section showing the electrode track from which the three recordings were obtained. The boundaries of striate cortex are indicated in the section drawing by line segments running perpendicularly between the pial surface and layer VI. B The monkey's performance on the saccade task was better than 90% correct at all locations before the muscimol injection as indicated in the key. C The behavioral deficit reached its largest extent 5–6 h after the injection at location 2 in A. The monkey successfully completed fewer than 10% of the trials for an area of the visual field approximately 10° in diameter. Control locations were unaffected. D The monkey was fully recovered from the deficit 24 h after the injection.
cortical magnification factor with eccentricity (Daniel and Whitteridge 1961). When the scotoma was remapped with a larger, brighter stimulus (0.2", 2.5 log units above background), its diameter was reduced to 3–4", but the deficit appeared to be just as strong within the reduced region.

The visual field location occupied by receptive field 1 was not affected, so backflow of the solution along the syringe needle was not a problem for this injection. The location occupied by receptive field 3 was near the boundary of the affected region, and the scotoma was not particularly enlarged in that direction. If diffusion of muscimol across the pia to the other bank of the calcarine sulcus occurred, it did not have a major effect. The monkey had fully recovered 24 h after the injection as shown in Fig. 1D. The track of the syringe needle was visible in histological sections, but we observed no effect of the muscimol on cortical morphology.

Ibotenic acid injection

We injected one microliter of ibotenic acid in a second hemisphere with a penetration similar to that illustrated in Fig. 1A. A preliminary penetration with a microelectrode intercepted striate cortex at three different locations, and a receptive field recorded at each of the three locations is illustrated on the visual field diagram in Fig. 2A. Receptive fields 1 and 2 were recorded from the representation of the horizontal meridian on the occipular surface and roof of the calcarine sulcus, respectively. Receptive field 3 was obtained from the ventral bank of the calcarine sulcus and was located in the upper quadrant of the visual hemifield (unlike receptive field 3 in Fig. 1A which was obtained from the dorsal bank of the calcarine sulcus). The ibotenic acid injection was made in the roof of the calcarine sulcus where receptive field 2 was recorded, and the injection site was again confirmed by recording from neurons through the insulated syringe needle.

Fig. 2A-D. Deficits in saccades that followed an injection of ibotenic acid into striate cortex. A The visual field diagram shows multineuron receptive fields (dashed circles) recorded at three different locations on a single microelectrode penetration through striate cortex. B Deficits in visually guided saccades were present at three visual field locations one day after an injection of ibotenic acid at the sites of receptive field 2. Each deficit was centered on one of the receptive fields illustrated in A. C The monkey had recovered from the deficit at receptive field 1 on the third day after the injection. The deficient regions at locations 2 and 3 were smaller. D The monkey's recovery was almost complete by the tenth day after the injection.
Fig. 3A–F. Photomicrographs at progressively higher magnifications illustrating the damage to striate cortex caused by an injection of ibotenic acid. A, C, and E Parasagittal section stained for cell bodies with cresyl violet. B, D, and F A nearby section stained for myelinated fibers with a silver stain (Gallyas 1979). Photomicrographs in C–F are centered on the lesion boundary so that normal cortex is to the left and damaged cortex is to the right. Calibration bars: A = 2.0 mm; C = 400 μ; E = 100 μ. The calibration bars in A, C, and E apply also to B, D, and F, respectively.
Before the injection, the monkey made saccades to all three receptive field locations with a success rate greater than 95% (0.3° target, 1 log unit above background). Following the injection, deficits appeared at all three receptive field locations within 30 min and expanded in size over the next several hours. The monkey did not attempt saccades on trial 1, but spared the lower layers for the areas with single-diagonal hatching (3 and 4). The asterisks indicate the contour lines (one on the operculum and one in the calcarine sulcus) taken from the section shown in Fig. 3A. Lesion 1 was located on the operculum while the other three were in the calcarine sulcus.

On the tenth day post-lesion (Fig. 2D), the monkey had substantially recovered from all three deficits. Since ibotenic acid kills neuronal cell bodies, we were able to histologically reconstruct the cortical lesions which were related to the deficits illustrated in Fig. 2. Figure 3 illustrates the damage to striate cortex caused by ibotenic acid. Figures 3A, C, and E are progressively higher power micrographs of a section stained for cell bodies with cresyl violet. Figures 3B, D, and F are similarly magnified micrographs of an adjacent section stained for myelinated fibers by the method of Gallyas (1979). Figure 3A confirms the inference made from the behavioral data that ibotenic acid damage was present at all three locations from which recordings were obtained: the opercular surface, the roof of the calcarine sulcus, and the ventral bank of the calcarine sulcus. In addition, a small amount of damage was present on the adjacent dorsal bank of the calcarine sulcus. We did not behaviorally test the part of the visual field related to the latter location since our microelectrode recording did not intercept the dorsal bank. The damage to the opercular surface and roof of the calcarine sulcus affected all cortical layers while damage to the other locations in the calcarine sulcus involved only the upper layers. Figure 3C shows that there is a considerable loss of neuronal cell bodies in the damaged area and that the normal laminar appearance of striate cortex is completely disrupted by the lesion. In the highest power micrograph, Fig. 3E, a small number of apparently viable neurons can be seen, but most of the cells in the damaged area are gliosis.

Axons in the white matter always stain normally following ibotenic acid lesions as can be seen in Figs. 3B and D. This observation is consistent with previous reports that ibotenic acid does not affect fibers of passage (Schwarz et al. 1979; Glimy 1983). Figures 3D and F show, however, that fibers which terminate in the damaged grey matter appear discontinuous and stain relatively poorly. We do not know whether ibotenic acid directly affects intracortical fibers or whether intracortical fibers appear damaged because of loss of their cell bodies or targets. Another possibility is that these fibers are normal but stain poorly because of generalized damage to the cortical tissue following the lesion.

Figure 4 shows a two-dimensional map of this striate cortex created by the “unfolding” method of Van Essen and Maunsell (1980). The full extent of the ibotenic acid lesions illustrated in Fig. 3 are shown in relation to the total area of striate cortex. The fine solid lines in Fig. 4 are the layer IV contours from which the map was constructed, the heavy solid line shows the boundary of the calcarine sulcus, and
the dashed lines indicate the fundi of the calcaine (CS) and external calcaine (ECS) sulci. As is typically the case, this striate cortex was elliptically shaped, and about half of the surface area was contained within the calcaine sulcus. The hatched regions indicate the four locations in striate cortex that were damaged by the ibotenic acid. The asterisks indicate the two contour lines that were obtained from the cresyl violet stained section in Fig. 3A (one from the opercular surface and the other from the calcaine sulcus).

The pattern of receptive field locations, lesions, and behavioral deficits is consistent with the known visual topography of macaque striate cortex (Daniel and Whitteridge 1961; Van Essen et al. 1984). Receptive fields 1 and 2 were recorded on the horizontal meridian, and lesions 1 and 2 were located correspondingly in the middle of striate cortex. Receptive field 1 was closest to the center of gaze as was expected since lesion 1 was on the opercular surface of striate cortex while the other lesions were in the calcaine sulcus. Receptive field 3 was recorded in the upper visual quadrant near the superior vertical meridian, and lesion 3 was appropriately located near the preprostriate border (vertical meridian representation) in the ventral half of striate cortex (upper quadrant representation). There was a striking difference in the size of the visual field deficits at locations 1 and 2 despite the rough similarity in the size of lesions 1 and 2. This observation is in accord with previous studies since cortical magnification factor in striate cortex has been shown to be proportional to the inverse of retinal eccentricity. The deficient region at location 3 was smaller than would be expected from the size of lesion 3, presumably because lesion 3 spared the lower layers of cortex.

Discussion

The results of these experiments indicate that the impairment of visually guided saccades immediately following small chemical lesions of striate cortex is similar to the impairment which follows surgical ablation of striate cortex as shown by Mohler and Wurtz (1977). The visual stimuli used as saccade targets in these experiments were similar in size and contrast to those used by Mohler and Wurtz, and the behavioral paradigms were similar. Mohler and Wurtz noted that their monkeys could not see the visual targets after the lesions since the monkeys failed a detection task as well as the saccade task. Although we can not address this point with certainty since we did not include a visual detection task in our experiments, the monkeys gave every indication of failure to see the target. The monkeys were not simply making errors in the size and amplitude of saccades to these targets; they completely failed to attempt them.

The major difference between our results and those of Mohler and Wurtz was in the time course of recovery from the deficits. Their monkeys recovered with practice over a three week period of time. Ours recovered within hours from the effects of muscimol and within several days from the effects of ibotenic acid. The quick recovery from the muscimol was excepted since its action is presumably mediated by reversible interactions with GABA receptors. Ibotenic acid, however, results in irreversible damage to neurons, and the difference in the time course of recovery between our experiment and those of Mohler and Wurtz needs explaining. We think the most likely explanation is that the small size of our lesions facilitated the recovery process. Mohler and Wurtz were able to show that with their relatively large striate lesions, behavioral recovery was mediated by the superior colliculus. For the small lesions caused by our ibotenic acid injection, the more rapid recovery may reflect limited changes occurring within striate cortex itself. Such changes might include local expansion of receptive field of neurons around the damaged area of striate cortex. Experiments in which ibotenic acid injections of striate cortex are followed by cellullar lesions should clarify this point. A second factor which may influence the time course of recovery is that monkeys which undergo surgical ablation are generally not available for behavioral testing for several days after surgery. Since our monkey was tested in the hours immediately following the ibotenic acid injection and on each ensuing day, effects of practice may have resulted in an accelerated rate of recovery.

While muscimol and ibotenic acid resulted in similar short-term effects in the present study, advantages and drawbacks to each technique will determine their utility in any given experiment. Muscimol may be useful when repeated injections are desired and when histological localization of the lesion is not required. The effects of muscimol may, however, vary among cortical areas due to differences in the density of GABAergic pathways (A.E. Hendrickson, personal communication). Ibotenic acid is more appropriate for studies in which histological verification of the lesion is necessary, but the irreversible damage inflicted on the cortex limits the number of experiments that may be performed with one animal. Due to the rapid recovery, both techniques will be most useful for experiments in which informative data can be gathered in hours rather than weeks.
Since the small size of the lesions may preclude complete destruction of a visual area, both techniques are most useful for a visual area with regular topography and for behavioral paradigms which force the monkey to use the affected portion of the visual field. With these restrictions, these experiments and others in our laboratory suggest that reversible and irreversible chemical lesions can be highly useful for analysis of visual cortical function.

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References


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