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Reversible inactivation of visual processing operations in middle suprasylvian cortex of the behaving cat

(extrastriate cortex/perception/motion/figure–ground separation/cooling)

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ABSTRACT **Extrastriate visual areas on the banks of the middle suprasylvian sulcus were inactivated by cooling to assess the behavioral contribution of this cortical region to the extraction of a stationary figure from a moving mask. Cooling blocked figure–ground separation when the mask was moving but had no influence when the mask was static. This difference provides strong evidence that the areas bounding the middle suprasylvian sulcus contribute to the neural separation of stationary from moving visual stimuli.**

Lesion studies have shown that the visual cortex of cats and monkeys is composed of several regions, each of which plays one or more specific roles in visual processing and cognition (1). This functional division of cortex is related to the multiple visual maps and to the unique patterns of connections each region possesses (2). In the present study we used cooling to reversibly inactivate the middle suprasylvian (MS) cortex in the behaving cat to investigate the contribution of this cortical region in solving a complex visual perceptual problem that involves movement. Our belief that motion processing is one function of MS cortex is based on the facts that (*i*) many neurons in this region are highly sensitive to simple motion (3–6) and respond well to differential motion (7); (*ii*) there is a systematic representation of movement direction (8); and (*iii*) permanent lesions interfere with movement velocity discrimination (9). By extension, the purported homologous (1) cortical area V5, or MT, contributes in a similar way to vision in primates (10–14).

However, most previous behavioral studies have examined motion processing in the absence of any other potentially interactive features. This situation is akin to the rather simple process of identifying the coherence, direction, or speed of flight of a flock of birds in a clear sky. However, under natural conditions there is typically relative motion between figures and either complex foregrounds or complex backgrounds. Our cats were trained to solve a discrimination in which stimuli composed of two geometrical outline patterns are partially obscured by either a moving grid pattern or a moving, nonsystematically arrayed pattern comprised of variable spatial frequencies. In this task the figure remains stationary and the mask moves coherently in the standard directions of up and down. This task is akin to identifying an object either behind a moving mesh (e.g., chain-link fence) or in a wind-driven black-snow storm. Our results show that (*i*) motion is a visual cue useful to cats for separating a static figure from a moving foreground mask and that the MS cortex plays a critical role in this separation, and (*ii*) the perceptual deficits in vision induced by the cooling of this region are completely reversible.

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EXPERIMENTAL METHODS

Three cats (S, Sd, and V) were trained to discriminate between an outline figure I and an outline figure O obscured by a multiline grid mask that oscillated up and down across the figures (Fig. 2*D*). In two of the cats (Sd and V) the optic chiasm and the visual fibers in the corpus callosum were severed in the midline. Cooling probes were implanted in the MS sulcus to lie in contact with both its medial and lateral banks. The cats' ability to discriminate the two patterns was tested with MS cortex warm and active and with MS cortex cold and inactive.

Training. The procedures and displays used for preliminary training with static stimuli have been described (15). The number of intersecting oblique static and then moving (up and down, ≈1 cycle per sec) lines masking the stimulus figures was gradually increased up to five orthogonal pairs to form a grid pattern that obscured the figures. We have used a stationary grid in our previous studies (15), and when moved, it is a powerful stimulus for neurons in MS cortex (4). The luminances of the black and white parts of the televised stimulus were 22 candelas/m^2 and 96 candelas/m^2, respectively. Over 10,000 training trials were run by each cat.

Surgical Procedures. After completing training, cat S underwent one surgical procedure to implant cooling probes bilaterally in the MS sulcus, and cats Sd and V underwent three separate surgical procedures to (*i*) section the optic chiasm in the midline by a transbuccal approach to allow visual signals to be directed to the left hemisphere when the right eye was occluded, (*ii*) sever the visual fibers in the corpus callosum to eliminate possible direct interactions between visual regions in the two hemispheres (16–18), and (*iii*) install a cooling probe in the left MS sulcus. All three procedures were done by using antiseptic procedures and with the cats fully anesthetized with sodium pentobarbital (40 mg/kg i.p. and i.v. as necessary). During the recovery period the cats were given buprenorphine analgesic (0.01 mg/kg s.c.). All procedures were approved by the Animal Care and Use Committees at The Pennsylvania State University and at Boston University Medical Center.

Cooling Probes. Cryogenic probes were made with 23-gauge hypodermic tubing fashioned into a 9–11 mm × 3.5–4 mm loop, according to the methods described by Horel (19). This loop entered/exited from the sulcus between coronal levels anterior–posterior 0 and posterior 2, it was designed to run along most of the length of the MS sulcus, and it was in contact with the surface of areas PMLS and PLLS with some overlap into areas AMLS and ALLS. Attached to the loop was a copper–constantin microthermistor, which was used to

Abbreviations: MS, middle suprasylvian; ALLS, anterolateral lateral suprasylvian; AMLS, anteromedial lateral suprasylvian; MT, middle temporal; PLLS, posterolateral lateral suprasylvian; PMLS, posteromedial lateral suprasylvian; VS, fifth visual area.

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monitor the temperature of the cryode and insure that it stayed >0°C.

Cooling of Cortex. Cortex was cooled by circulating methanol, chilled to minus 78°C in a methanol/dry-ice bath through the hypodermic tubing of the probe. The actual temperature of the loop is determined by the interplay of three factors: (*i*) the methanol temperature when it reaches the loop in the probe, (*ii*) the velocity of methanol flow, and (*iii*) the ability of the cat's vascular system to resist a decrease in temperature. The microthermistor attached to the loop of the probe provided information on probe temperature that was used to make fine adjustments of methanol velocity. We maintained the probe temperature at 4–11 ± 0.2°C for 30 min or more, and it is reasonable to presume that the brain temperature was warmer.

Testing. Within 2 days of probe implantation all three cats willingly started reconditioning, using the same procedures as used preoperatively, with the grids made up of five pairs of intersecting lines. Testing with the cooling probe in operation began between 13 and 20 days after their implantation. Each day there were two sessions, separated by 6–8 hr. In both sessions the cats each wore a harness and were tethered to a bar above the test apparatus. The length of the tether was sufficient to allow complete freedom of movement within the test apparatus. In one of these sessions, the tether supported cooling tubes, which were connected to the cooling probe(s). A cooling session consisted of a set of trials with the probe(s) at body temperature, a second set with the probe(s) at the cold temperature (cat S = 4–8°C; cat Sd = 6–10°C; cat V = 8–11°C), and a third set with the probe(s) rewarmed. A typical cooling session consisted of 40–100 trials. Cats Sd and V had an opaque occluder placed over the right eyes, so that visual signals would be directed only to the left hemispheres and to the cortices adjacent to the cryogenic probe. In sessions in which MS cortex was cooled, the cat handler was unaware of the exact temperature of the probe(s), which was recorded by a different investigator. Initial testing began with the moving stimuli used during reconditioning and continued with a number of stimulus variants not seen previously by the cat. The variants consisted of the same plaid pattern moved horizontally or kept static and an array of black rectangles of various sizes ranging from 0.3 × 0.3 cm^2 to 1.8 × 1.8 cm^2 that covered 16%, 36%, or 45% of the screen area (see Fig. 2*D*). At no time did the cats display any behavior indicating irritation by the implants.

Electrophysiology. Electrophysiological recording methods were used to verify cooling blockade of neuronal activity in cat S. Cat S was anesthetized with halothane and nitrous oxide, immobilized with gallamine triethiodide, and prepared for visual stimulation and electrophysiological recording by using standard procedures (20). Action potentials of a cluster of several neurons were recorded simultaneously, and the multiple neuron spike train was sorted by a microcomputer and related to stimulus and temperature timing marks embedded in the data stream by using described procedures (21, 22).

Tissue Fixation and Histology. After the last behavioral session for cats Sd and V and after the electrophysiology session for cat S, the cats were anesthetized with sodium pentobarbital (50 $mg \cdot kg^{-1}$) and their vascular systems were perfused first with saline and then with 10% (vol/vol) formal saline to fix the brain. The brain was removed and embedded in celloidin and sectioned in the coronal plane at 50 μm; the sections were then stained for Nissl substance and myelin to verify that the visual fibers crossing the midline had been severed and to demonstrate the integrity of MS cortex adjacent to the probe.

RESULTS

Anatomy. Fig. 1 shows the banks of the MS sulcus of cat V. Overall, there is little distortion of the cortical microstructure, neurons appear healthy, cortical layers are readily identifiable, and the composition of the layers matches descriptions for the region (23). Within the sulcus, three small impressions, indicative of contact points between the cryoprobe and brain surface, are evident in layer I. No damage due to physical contact or to tissue cooling is evident.

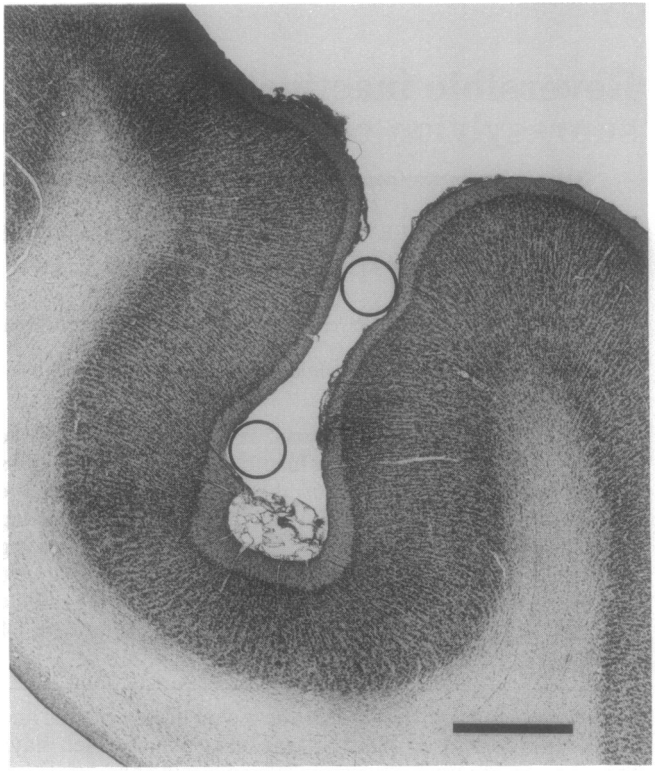

FIG. 1. Photomicrograph of a cresyl violet-stained coronal section to show the microstructure of the medial (at left) and lateral (at right) banks of MS sulcus of cat V. The impressions formed on the cortical surface by the cooling probe are evident. Overall the distortion is minimal, and cortical layers are readily identifiable. Circles represent the size and position of the cooling probe in cross section. No mechanical or cooling damage is evident. (Scale bar = 1 mm.)

Cooling Blockade of Behaviors. For all three cats, cooling eliminated the ability to discriminate between the I and the O when the plaid masking pattern was in *motion* in the standard directions of up and down. Qualitatively the data collected both from cat S and from the left hemispheres of cats V and Sd were very similar (Fig. 2 *A–C*). Results from the most extensively studied cat, cat S, are described in detail, and data from cats Sd and V are described when they differ from those collected from cat S. Fig. 2*A* shows that before cooling (days 1–10) cat S made the correct choice between 70% and 82% of the time (mean = 77 ± 1%), which is reliably above chance (50%) for each comparison (*t* test, $P < 0.05$). On days 11–17 cooling inactivation was applied, and this treatment reduced the correct number of choices to between 45% and 66% (mean = 55 ± 3%) level, which differed reliably from performance immediately before (mean = 84 ± 3%) and immediately after (mean = 80 ± 4%) cooling during the same session. Control data collected on days 18–21 show that inactivation of MS cortex had no significant effect on the ability of cat S to identify the I and the O when the standard masking plaid pattern was *stationary* (warm: 77 ± 3%; cold: 74 ± 1%). This result agrees with other studies (24, 25) that report that ablation of MS cortex does not impair discrimination of static figures and shows that the impairment induced by cooling is specific to the moving plaid pattern.

Additional data were collected with an oscillating horizontal moving mask on days 22 and 23 and with nonsystemati-

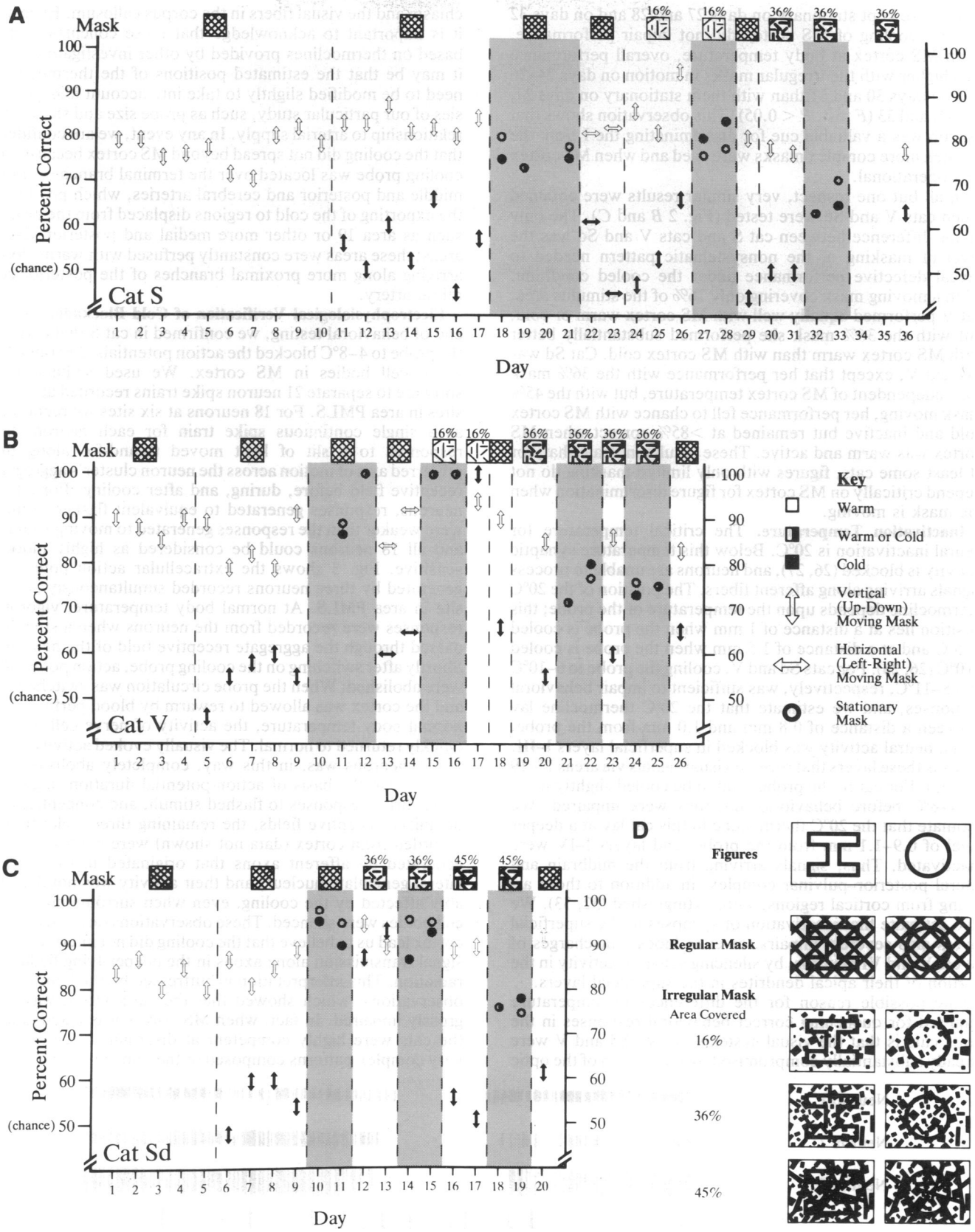

FIG. 2. (*A*) Ability of cat S to discriminate the I figure from the O figure when masked by different moving (arrows) and stationary (circles) masks. Data were not collected on days 20, 34, and 35. See key for code (*B Right*). (*B*) Performance data from cat V. Data were not collected on day 10. (*C*) Data from cat Sd. (*D*) Unmasked and masked stimuli drawn to scale. Regular and irregular masks could be moved in front of the I (reinforced) and O (unreinforced) patterns.

cally arrayed masking elements on days 24–26 (16% of screen) and days 30, 31, and 36 (36% of screen). For all of these masks, inactivation of MS cortex impaired performance. However, when the nonsystematically arrayed pat-

terns were kept stationary on days 27 and 28 and on days 32 and 33, cooling of MS cortex did not impair performance. With MS cortex at body temperature, overall performance was higher with the irregular masks in motion on days 24–26 and on days 30 and 31 than with them stationary on days 27, 28, 32, and 33 (F test, $P < 0.05$). This observation shows that motion was a valuable cue for discriminating the I from the O when more complex masks were used and when MS cortex was operational.

In all but one respect, very similar results were obtained when cats V and Sd were tested (Fig. 2 *B* and *C*). The only major difference between cat S and cats V and Sd was the level of masking of the nonsystematic pattern needed to reveal defective performance under the cooled condition. With a moving mask covering only 16% of the stimulus area, cat V performed equally well with MS cortex warm or cold, but with the 36% mask she performed substantially better with MS cortex warm than with MS cortex cold. Cat Sd was like cat V, except that her performance with the 36% mask was independent of MS cortex temperature, but with the 45% mask moving, her performance fell to chance with MS cortex cold and inactive but remained at >85% correct when MS cortex was warm and active. These results indicate that, for at least some cats, figures with only limited masking do not depend critically on MS cortex for figure discrimination when the mask is moving.

Inactivation Temperature. The critical temperature for neural inactivation is 20°C. Below this temperature synaptic activity is blocked (26, 27), and neurons are unable to process signals arriving along afferent fibers. The position of the 20°C thermocline depends upon the temperature of the probe; this position lies at a distance of 1 mm when the probe is cooled to 5°C and at a distance of 1.5 mm when the probe is cooled to 0°C (26, 28). For cats Sd and V, cooling the probe to 6–10°C and 8–11°C, respectively, was sufficient to impair behavioral responses, and we estimate that the 20°C thermocline lay between a distance of 0.8 mm and 1.0 mm from the probe. Thus, neural activity was blocked in superficial layers I–III, and it is these layers that receive visual signals via areas 17–19 (29–31). For cat S, the probes had to be cooled slightly more to 4–8°C before behavioral functions were impaired. We estimate that the 20°C thermocline in this cat lay at a deeper level of 0.9–1.1 mm from the probe, and layers I–IV were inactivated. Thus, signals arriving from the midbrain and lateral posterior–pulvinar complex, in addition to those arriving from cortical regions, were extinguished (32, 33). We also estimate that inactivation of synapses in the superficial layers also severely impairs, or even blocks, discharges of layers V and VI neurons, by silencing synaptic activity in the portion of their apical dendrites in the superficial layers.

One possible reason for the differences in temperature required for quenching correct behavioral responses in the three cats is that the visual systems of cats Sd and V were already substantially compromised by the section of the optic chiasm and the visual fibers in the corpus callosum. Even so, it is important to acknowledge that these conclusions are based on thermoclines provided by other investigators, and it may be that the estimated positions of the thermoclines need to be modified slightly to take into account idiosyncrasies of our particular study, such as probe size and shape and relationship to arterial supply. In any event, we are confident that the cooling did not spread beyond MS cortex because the cooling probe was located over the terminal branches of the middle and posterior and cerebral arteries, which precludes the exporting of the cold to regions displaced from the probe, such as area 19 or other more medial and posterior visual areas; these areas were constantly perfused with warm blood arriving along more proximal branches of the posterior cerebral artery.

Electrophysiological Verification of Cold Blockade. At the end of behavioral testing, we confirmed in cat S that cooling the probe to 4–8°C blocked the action potentials generated by nerve cell bodies in MS cortex. We used spike-sorting software to separate 21 neuron spike trains recorded at seven sites in area PMLS. For 18 neurons at six sites we recorded, as a single continuous spike train for each neuron, the responses to a slit of light moved to-and-fro along the preferred axis of motion across the neuron cluster's aggregate receptive field before, during, and after cooling. For all 18 neurons, responses generated to equivalent flashed stimuli were weaker than the responses generated to moving stimuli, and all 18 neurons could be considered as highly motion sensitive. Fig. 3 shows the extracellular action potentials generated by three neurons recorded simultaneously at one site in area PMLS. At normal body temperature, vigorous responses were recorded from the neurons when a stimulus passed through the aggregate receptive field of the neurons. Shortly after switching on the cooling probe, action potentials were abolished. When the probe circulation was switched off and the cortex was allowed to rewarm by blood perfusion at normal body temperature, the activity of nerve cell bodies quickly returned to normal. The visually evoked activity of 15 of the neurons was, in this way, completely abolished by cooling. On the basis of action-potential duration, high activity, brisk responses to flashed stimuli, and concentrically organized receptive fields, the remaining three spike trains recorded from cortex (data not shown) were believed to be generated by afferent axons that originated in the dorsal lateral geniculate nucleus, and their activity was not detectably affected by the cooling, even when surrounding nerve cell bodies were silenced. These observations on axons in the cortex lead us to believe that the cooling did not interfere with signal transmission along axons in the deeper-lying thalamic radiation. This interpretation is buttressed by the behavioral observations, which showed that the cat's vision was not grossly impaired. In fact, when MS cortex was inactivated, the cats were highly competent at discriminating between very complex patterns composed of the I and O and partially

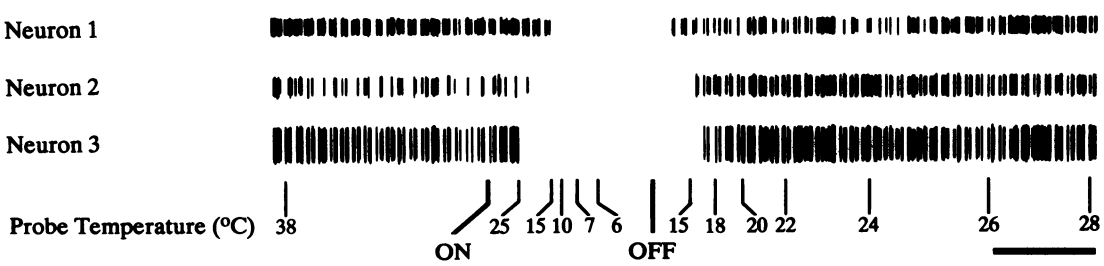

FIG. 3. Action potentials of three neurons before, during, and after cooling of the cortex adjacent to the probe. Before cooling, action potentials were generated to short bars moving to-and-fro across the receptive fields of the neurons. The bursts are synchronously linked to the to-and-fro motion of the stimulus. When the probe was switched on, the numbers of evoked action potentials diminished until no activity was evoked; at this point the neurons in MS cortex were silent. When the probe was switched off, neuronal activity rapidly returned and reached control levels within ≈1 min. (Bar = 30 sec.)

obscuring static plaid masks. In our opinion, this high level of competence argues convincingly against widespread depression of neural activity and direct interference with signal transmission in the thalamic radiation.

CONCLUSIONS AND IMPLICATIONS

Three technical and three experimental conclusions that can be drawn on the effects of reversible inactivation of MS cortex in the behaving cat are as follows: (*i*) Reversible inactivation by cooling is a powerful, yet inexpensive, technique for investigating cerebral cortical function in the behaving animal. The power lies in the ability of *each* experimental animal to repeatedly serve as its own control and the flexibility the experimental paradigm provides in allowing test stimuli to be chosen, or even developed, as a study progresses. (*ii*) Repeated cooling over a period of 3 to 4 weeks does not alter neuronal responsiveness or animal behavior in a permanent way. (*iii*) Cooling produces equivalent results, regardless of whether both hemispheres are intact or whether only one hemisphere is able to receive and process the visual signals.

Three conclusions concern the role of MS cortex in the segregation of figure and mask through differential motion: (*i*) Processing of a moving, obscuring mask interacts with the processing of a static figure, and in the presence of a functioning MS cortex these two types of signal can be segregated. When MS cortex is inactivated, the processing of combined static and motion signals is blocked. We conclude that MS cortex is important for segregating differentially moving patterns. This conclusion supports the observations of Kiefer *et al.* (24). However, we note that the locus or loci where signals about static and moving patterns interact are unknown, and whether MS cortex is involved in using motion cues to construct a representation of figure and ground or whether it is merely a processor in a complex circuit remains to be determined. (*ii*) Cooling of MS cortex does not interfere with the identification of a figure when the figure is obscured by a static, nonmoving mask. This result suggests that MS cortex does not contribute significantly to the processing of static images and is not essential for figure–ground segregation in the absence of differential motion. This observation confirms results obtained by others (24, 25) and conforms to the evidence implicating the posterior suprasylvian cortex as a major processor of complex static patterns (34, 35). (*iii*) The differences in performance level exhibited to the static and moving, 36% and 45% nonsystematically arrayed masks show that differential motion can be a useful cue for simplifying the identification of heavily masked figures, which are more difficult to identify when both figure and mask are stationary.

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