Conditioned Inhibition: Selective Response of Single Units

Abstract. *Single cell activity and local field potentials in parastriate cortex of cats and rabbits were studied during a Pavlovian discrimination procedure. Cell activity was selectively modified; conditioned changes occurred in response either to the reinforced stimulus or to the unreinforced one, but not to both. Cells exhibiting conditioned alteration in response to the unreinforced stimulus are thought to participate in specialized circuits mediating conditioned inhibition.*

Behavioral studies of conditioning have demonstrated that organisms can learn different contingencies or relationships between events in their environment (*1-3*). For example, just as they learn that a given stimulus (CS^+) will be followed by another (such as electric shock), they may also learn that a different stimulus (CS^-) will not be followed by shock. The latter does not constitute a neutral stimulus, but in Pavlovian terms, may be a conditioned inhibitor (or, in this example, a "safety signal" since it predicts the nonoccurrence of shock).

Early experiments on conditioning of electroencephalographic (EEG) rhythms revealed that the electrical response of local populations differed depending on whether the signal was a CS^+ (conditioned excitor) or a CS^- (conditioned inhibitor) (*4, 5*). For example, using a discrimination procedure, Morrell and Ross (*5*) demonstrated conditioned blocking of alpha rhythm in response to the CS^+ and augmentation of alpha rhythm in response to the CS^-.

In the experiment described herein we extended such electrophysiological analysis to the single-unit level and studied the behavior of individual elements that respond selectively to the unreinforced stimulus (CS^-). Other single-unit studies of classical conditioning have not explicitly addressed this issue. Our experiment indicates that cells exhibiting conditioned modification in response to the CS^- do not necessarily undergo alteration in response to other stimuli. To the extent that the CS^- may be identified as a conditioned inhibitor, these cells may be viewed as participating selectively in the process of conditioned inhibition. A discrimination procedure was used, since it has been demonstrated that in such procedures the CS^- becomes a conditioned inhibitor (*2, 3, 6*).

Tungsten microelectrodes (tip impedance at 1000 Hz, 5 to 15 megohms) were used to record simultaneously extracellular single and multiple unit activity and local field potentials in the parastriate cortex [areas 18 and 19, or visual areas II and III of Hubel and Wiesel (*7*)] of locally anesthetized, paralyzed cats and rabbits. Intubation of cats and tracheotomy in rabbits were performed under ether anesthesia. The animals were then paralyzed with Flaxedil and artificially respired. After infiltration of the scalp with Xylocaine, the skin was incised and reflected. Two burr holes (3 to 4 mm) were made over parastriate cortex. The dura was usually incised.

During the experiment, the animals were fixed in a modified head holder by cranial screws placed over the frontal sinus which allowed restraint without the discomfort of orbital or ear bars. They were positioned about 2 m from the tangent screen. The pupil contralateral to the cranial opening was dilated with 1 percent Neosynephrine. A contact lens was applied to correct refractive error and protect the cornea from drying. Supplemental doses of Flaxedil were given hourly and wound margins were repeatedly infiltrated with Xylocaine. [General anesthesia could not be used since it is known to abolish or markedly reduce the nonvisual responsiveness of visual cells (*8*).] Heart rate, pupillary diameter in the unfixed ipsilateral eye, and EEG were used in monitoring the general state of each animal. Carbon dioxide at the end of expiration was maintained at 3.5 to 4 percent.

The electrode was lowered into the brain until a cell or cell cluster with sufficiently differentiable spikes was encountered. Receptive fields, preferred stimulus configuration, and orientation specificity were determined for each cell or cell group. Stimuli consisted of visual patterns that could be flashed on or off or presented as moving targets on the tangent screen; they were presented every 1.6 seconds. The duration of the flash stimuli was 40 msec, whereas that of the moving stimuli varied depending on cell preference for rate of movement. It was usually possible to define two stimulus configurations to which distinct responses occurred. The usual procedure followed was to pair one configuration (CS^+) 20 msec after its onset with a 20-msec shock (unconditioned stimulus, UCS) to a muscle in the contralateral hind leg. Shock intensity, ranging from

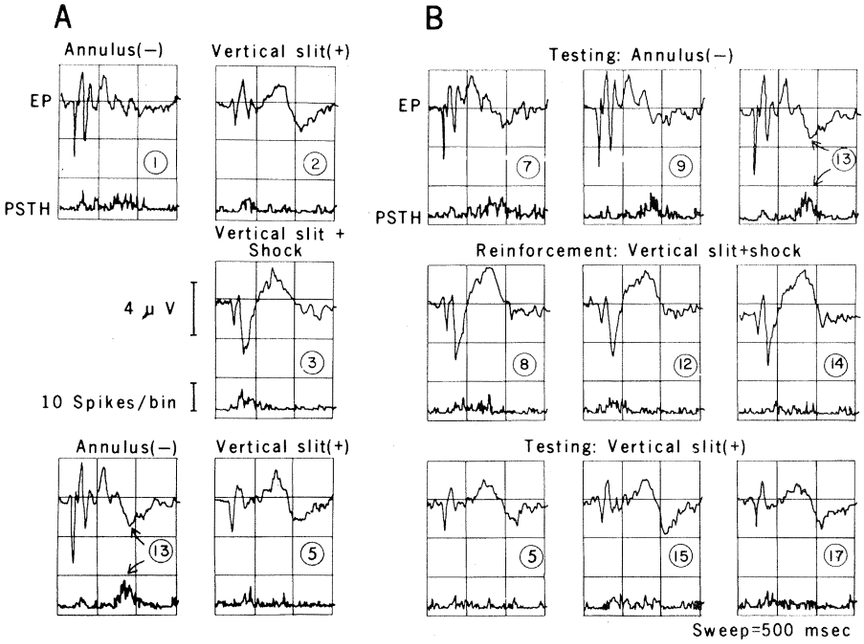

Fig. 1. The gradual development in a cell's firing pattern in response to CS^-. Encircled numbers, which are referred to as panel numbers, indicate the stimulus sequence (see text). (A) The overall change. Panels 1 and 2 show EP and PSTH to the two stimuli prior to conditioning. After conditioning, a new peak developed in the cell's response to CS^- (see arrow) which corresponded with a similar change in EP. (B) Panels 7, 9, and 13 demonstrate the gradual development of the change in EP and PSTH to CS^-. Panel 5 in (A) was the first 50 and panel 5 in (B) was the second 50 testing trials of the CS^+. The differences illustrate the degree of normal variability. CS^+ + UCS stimulus blocks, 4, 6, 11, and 16 and CS^- stimulus block 10 are not illustrated. Unless otherwise indicated, in the case of stimulus blocks which included 100 trials, the PSTH and EP are based on the first 50 presentations. Bin width for PSTH, 4 msec; CS duration, 40 msec; a 10-msec UCS was presented 15 msec after termination of the CS^+.

10 to 20 V, was adjusted to produce a minimal muscle twitch. The other configuration served as the unreinforced stimulus (CS^-). In the case of some cells for which the flash stimuli were used, however, a 40-msec CS^+ was followed 15 msec after its offset by a 10 msec shock UCS.

The general procedure consisted of first using blocks of 100 presentations of each of the two stimuli as a pretest to determine the unit response and average evoked response to each prior to conditioning. (In the figures, these are labeled 1 and 2, respectively.) Two-hundred paired presentations of the CS^+ and shock (divided into two blocks of 100 presentations, labeled 3 and 4, respectively) were then followed by a postconditioning test consisting of 100 presentations of CS^+ alone (that is, an extinction procedure labeled 5) [see (*9*)]. This was followed by a reconditioning and discrimination procedure during which many irregular alternations of CS^+ and shock pairings and of the CS^- were used to maximize the possibility of differential conditioning. During this phase, the following sequence of stimulus presentations was used: CS^+ + UCS, CS^-, CS^+ + UCS, CS^-, CS^-, CS^+ + UCS, CS^+ + UCS, CS^-, CS^+ +UCS, CS^+ (labeled 6 to 15, respectively). In some cases, an additional final CS^+ + UCS and CS^+ test sequence was added (labeled 16 and 17). Each stimulus block in this phase of the experiment consisted of 50 trials, except for the last CS^+ test which was always 100 trials.

Neuroelectric activity was fed through a cathode follower and capacity-coupled amplifiers (frequency response 350 Hz to 10 kHz for spikes and 1 to 100 Hz for slow waves), to oscilloscopes, pulse-height discriminators, f-m tape, and a Nicolet Med 80 computer. Computed poststimulus time histograms (PSTH) of unit response were compared with averaged evoked potentials (EP) obtained from the same microelectrode. The PSTH and EP were averages of 50 trials.

Our electrodes often yielded records of the activity of several cells. Such records were only accepted for analysis if the signal-to-noise ratio was at least 5:1 for the largest spike. Thus, the activity of at least one unit, and often two, were unambiguously and separately discriminable by amplitude window settings from noise or competing biopotentials. Discriminator output and raw records were oscillographically monitored to assure that the same element was being identified throughout the experiment. Cells were included in the study only if they showed stable responses for 15 to 20 minutes in the pretest period. In the absence of a generally acceptable statistical criterion by which to evaluate changes in firing pattern, our definition of a conditioned change required that amplitude alterations be greater than 50 percent or that shifts in the timing (latency) of peaks occur. In either case, we required that the changes exhibit stability over trial blocks or a systematic relationship to reinforcement contingency.

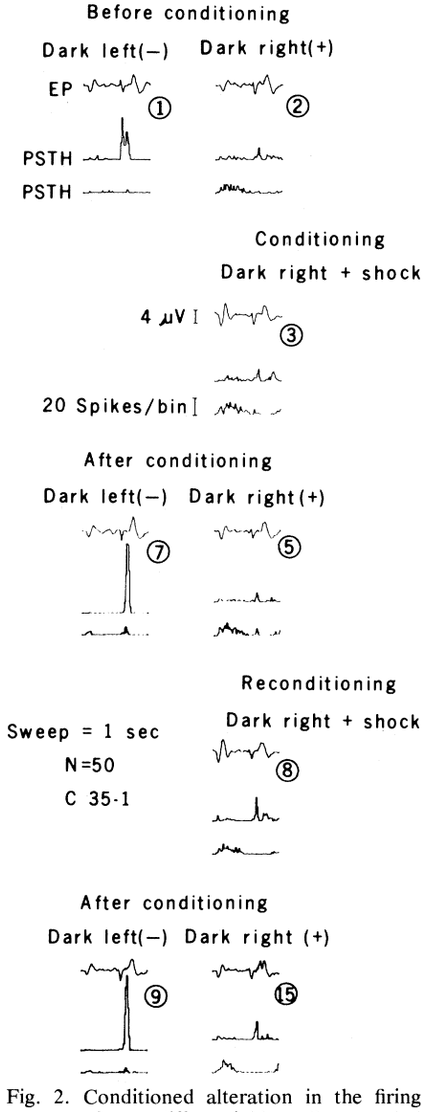

Fig. 2. Conditioned alteration in the firing pattern of two differentiable cells recorded through the same microelectrode. Encircled numbers, referred to as panels, indicate the stimulus sequence. Responses to CS^- are shown in panels 1, 7, and 9 and those to CS^+ alone in panels 2, 5, and 15. The cell depicted in the upper histogram exhibited conditioned modification to CS^-, whereas the one in the lower histogram showed a slowly developing change to CS^+. CS^+ + UCS stimulus blocks 4, 6, 11, 12 and 14 and CS^- stimulus blocks 10 and 13 are not illustrated. For stimulus blocks which included 100 trials, the PSTH and EP were based on the first 50 presentations. Bin width for PSTH, 8 msec. The CS's were moving stimuli; a 20-msec UCS was presented 20 msec after CS^+ onset.

Of the 86 cells or cell clusters analyzed in this study, 31 exhibited evidence of modifiability, with 13 clearly identified single elements showing an alteration in firing pattern which was selective only to the nonreinforced stimulus. Conditioned responses to CS^- were manifested by several types of alteration in firing pattern, such as a shift in the latency of discharge (2 out of 13), the appearance of a new peak or a series of peaks (7 out of 13), or the loss of an activity cluster (4 out of 13).

Figure 1 illustrates an example of a conditioned change in response to CS^-. Figure 1A shows the overall result of conditioning. Panels 1 and 2 show the EP and PSTH to two stimuli prior to conditioning. After conditioning, there was a new peak in the cell's response to CS^- (annulus) which corresponded with a similar change in EP (see arrows, panel 13 and compare with panel 1). In contrast, the conditioning procedure did not result in any change in EP or PSTH to CS^+ (compare panel 2 with panel 5).

These modifications in response to CS^- developed gradually in the course of discrimination training, as shown in Fig. 1B, panels 7, 9, and 13. Equally clear was the lack of any systematic change in response to CS^+ + shock (panels 8, 12, and 14) or to CS^+ alone (panels 5, 15, and 17). This progressive emergence of a change in CS^--elicited cell activity is similar to the acquisition pattern observed in behavioral studies of conditioned inhibition (*1*).

In one instance where the activities of two closely adjacent cells were recorded through the same microelectrode, one cell showed a clear-cut change in response to CS^- and the other to CS^+ (Fig. 2). After conditioning, the firing pattern of the cell displayed in Fig. 2, panels 1 and 2, underwent a shift from a two-peaked to a one-peaked configuration in response to CS^-. There developed also a gradual and marked augmentation in the amplitude of the response (compare panel 1 with panels 7 and 9). There was little change in activity of the same cell to CS^+ (compare panel 2 with panels 5 and 15).

The activity of the cell depicted in the lower histogram showed a slowly developing change to CS^+. There was an augmentation in amplitude and shortening of latency of the peak followed by an interval of cell silence (compare panel 2 with panels 5 and 15). This cell exhibited no systematic or sustained change to the CS^- (compare panel 1 with panel 9).

These examples illustrate the kinds of conditioned alterations exhibited by single units in response to the unreinforced stimulus. With respect to the CS^+, our findings of conditioned modification of firing pattern in single elements of parastriate cortex are similar to those previously reported (*10, 11*). The unique feature of the present study was the demonstration of conditioned responses to the unreinforced stimulus. Cells exhibited modification which was selective for reinforcement contingency; that is, they changed either to the CS^+ or to the CS^-. Thus far, we have not observed any single cell that has exhibited conditioned alteration to both CS^+ and CS^-. The number of cells that showed selective change to CS^- (13 out of 86) was approximately equal to the number exhibiting modification to CS^+ (10 out of 86) (*12*).

Differences in the conditions necessary for producing conditioning were also apparent between cells which exhibited modification in response to either the CS^+ or CS^-. In general, a modification to CS^+ did not take place unless there was a distinct cellular response to shock. Such was not the case for cells selectively responsive to CS^-. The latter often did not respond to the UCS directly, although clear-cut changes in the local field potentials indicated that the UCS did influence other elements of the same population (Fig. 1, panels 3, 8, 12, and 14).

We think that these results demonstrate a possible neural substrate of conditioned inhibition (*13*). The existence of single units exhibiting selective conditioned modification to the CS^- suggests that the circuitry mediating conditioned inhibition contains elements not involved in conditional excitatory processes, at least at the level of parastriate cortex. A more definitive demonstration of such functional distinctiveness would require that in a test where reinforcement contingencies are reversed, a cell showing modification in response to the CS^- originally would again be modified in response to the new CS^-. A cell showing conditioned alteration to CS^+ would be expected to exhibit similar faithfulness to its original reinforcement contingency. Yet, even without this most persuasive test, such qualitative specificity as we have described provides a further example of cell-specific behavior in learning comparable to the precise tuning to other aspects of environment and experience which appears to be characteristic of the sensory systems in general (*14*).

The occurrence of anatomically distinct elements subserving conditioned excitation and inhibition would not necessarily be predicted from behavioral research. Yet the latter deals only with outcomes, that is, with the final motor end product. At an earlier stage in the encoding of experience, it appears that conditioned inhibitory and excitatory processes involve separate neuronal systems. Further analysis of the cellular constituents of these separate systems may provide direct information on the time-course and kinetics of these processes at the neuronal level.

LEYLA DE TOLEDO-MORRELL*
Department of Psychology, University of Toronto, Toronto, Ontario, Canada M5S 1A1
THOMAS J. HOEPPNER
Department of Neurological Sciences, Rush Medical College, Chicago, Illinois 60612
FRANK MORRELL
Department of Neurological Sciences, Rush Medical College, and *Marine Biological Laboratory, Woods Hole, Massachusetts 02543*

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9. This "extinction procedure" did not ordinarily result in actual extinction since the latter requires more trials than we employed [see (*10*)], especially in view of the relatively large number of reinforced trials previously delivered. Therefore, the extinction procedure served as a test of conditioning.
10. F. Morrell, in *The Neurosciences: A Study Program*, G. C. Quarton, T. Melnechuk, F. O. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967), p. 452; in *The Brain and Human Behavior*, A. G. Karczmar and J. C. Eccles, Eds. (Springer-Verlag, New York, 1972), p. 259.
11. Further details on CS^+ responses will be described elsewhere (F. Morrell, T. J. Hoeppner, L. de Toledo-Morrell, in preparation).
12. The other cases (8 out of 86) where modifiability in cell activity was observed consisted either of populations with two or three undifferentiable cells or of units which showed the same response pattern to the two stimuli prior to conditioning. In these examples, a modification occurred in response to both CS^+ and CS^-. In the case of populations, it may very well be that separate elements were responsible for each type of change. Elements not distinguishing CS^+ from CS^- initially showed the same change to both stimuli after conditioning.
13. Specific tests such as summation or retardation which allow one to designate a CS^- as a conditioned inhibitor could not be used since the same elements did not show conditioning to both CS's. However, since measurable alterations in the pattern of neural activity could be induced by either stimulus, these tests were not regarded as critical.
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* Present address: Departments of Neurological Sciences and of Psychology, Rush Medical College, Chicago, Ill. 60612.

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Commissural Transmission in Humans

An important and intriguing question of human neurobiology concerns the relationship between the two cerebral hemispheres. Salamy has observed that "latency differences between ipsilateral and contralateral somatosensory evoked potentials show maturational trends in keeping with the myelogenic timetable and development of the corpus callosum" (*1*, p. 1409). If these differences reflect a maturation of the corpus callosum, this work is of great interest. We believe, however, that the activity of only the faster callosal axons is likely to be measured by this technique. If we assume that only one synapse is involved, and thereby subtract 0.5 msec from the adult values given by Salamy, the estimated interhemispheric conduction time of the P_2, P_1, and N_1 components of the evoked potential may be considered to be 3.0, 6.5, and 7.5 msec, respectively.

Given an interhemispheric conduction distance of approximately 100 mm, the axonal conduction velocity of impulses mediating such information is approximately 13 to 33 m/sec. Such axon conduction velocities might be expected to be mediated by myelinated axons 2.4 to 6.0 μm in diameter (*2*). Yet Tomasch reports fewer than 10 percent of human callosal axons to be more than 2.5 μm in diameter (*3*). Most myelinated axons are less than 1.5 μm in diameter, and fully 40 percent of callosal axons were found to be unmyelinated. In the macaque, electron microscopy (*4*) reveals such unmyelinated axons to be 0.08 to 0.5 μm in diameter. Such axons would

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