

ELECTROCORTICAL RECRUITING RESPONSES DURING CLASSICAL CONDITIONING¹

NORMAN M. WEINBERGER, PH.D.², KEN NAKAYAMA AND DONALD B. LINDSLEY, PH.D.

Department of Psychobiology, University of California, Irvine and Departments of Physiology and Psychology and the Brain Research Institute, University of California, Los Angeles, Calif. (U.S.A.)

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INTRODUCTION

The correlation between behavioral arousal and the EEG is well documented and constitutes an important index of brain-behavior relationships. In normal, waking animals, a gradual shift from low voltage fast waves to higher voltage slower rhythms accompanies the transition from an alerted state to one of sleep (Lindsley 1960). Recent findings suggest, however, that slight fluctuations in arousal level during the alerted state may not be accompanied by changes in the ongoing ECoG (Weinberger *et al.* 1967).

Searching for an index of arousal level more sensitive than the ECoG (Weinberger *et al.* 1965), it was found that recruiting responses (RRs) were attenuated by presentation of a conditioned stimulus (CS) which elicited a conditioned response (CR), whereas presentation of a non-reinforced differential stimulus (DS) produced neither a CR nor a decrement in RRs. The desynchronization of the ECoG, which often occurred during these contrasting stimuli and behaviors, was not distinguishably different. The present experiment was undertaken to replicate and extend these initial findings.

METHODS

Extensive and repeated observations of behavior and electrophysiological responses were made

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on three adult naive cats (3.0–3.8 kg). The cats were housed individually and had continuous access to food and water. Two to six weeks prior to the start of the experiment, electrodes were implanted under pentobarbital anesthesia. Electrode pairs consisted of two 0.010" diameter stainless steel wires, insulated and adjoined in side-by-side array with Epoxyite except for 0.5 mm at their tips (separated vertically by 1 mm). Cortical electrodes were positioned with the tip of the upper wire approximately 1 mm below the pial surface. RRs were differentiated from other repetitive potentials by their long latency (10–50 msec), incremental growth, amplitude modulation, and widespread distribution. Cortical loci of recruiting responses included the ipsilateral posterior sigmoid, mid-suprasylvian, mid-ectosylvian and posterior marginal gyri. Stainless steel wires, bared for 2 mm were sewn into the neck muscles, bilaterally, in two cats, and were used to apply high frequency shocks (100/sec) which served as the unconditioned stimulus (US). High frequency stimulation (100/sec) of the mesencephalic reticular formation served as the US for the third cat. All electrodes were wired to a sub-miniature connector (Amphenol Min-Rac.) and the whole assembly was secured to the skull and anchoring screws with dental cement.

The cats were tested in a chamber as previously described (Weinberger *et al.* 1967). Stimulation and recording were accomplished through a lightly suspended shielded cable (Tensolite) which did not interfere with movements. Background cortical activity was recorded on a Grass model III D electroencephalograph; RRs were amplified and recorded by means of Tektronix No. 122

pre-amplifiers (0.2 sec time constant), a Tektronix No. 502 oscilloscope, and a Grass C-4 camera. Thalamic and reticular stimulation and shock to the neck muscles were provided by Grass S-4 stimulators and SIU-4 isolation units. Stimulus presentation, brain stimulation, photography of RRs and inter-trial intervals were all automatically controlled by tape programmers, electronic timers, and relay circuits.

Procedure

An aversive, classical conditioning paradigm was employed. The CS was an "impure" tone (1000 c/sec clicks, of 0.1 msec duration), presented in 100 msec trains at the rate of 2/sec for 5 sec. The DS consisted of the same stimulus trains presented at a rate of 9/sec. The US (100/sec, 1 msec pulses) was applied to the neck muscle or the mesencephalic reticular formation with sufficient intensity to produce a characteristic unconditioned response (UR). US current never exceeded 4.5 mA. The CS (or DS) to US interval was 5 sec. The US was presented for 1 sec at CS offset. Inter-trial intervals averaged 2 min (range of 1–3 min) and were presented in a random sequence.

Midline and intralaminar nuclei within the thalamic recruiting system were stimulated for 2 sec at predetermined intervals during the training procedure. Stimulus parameters were 8/sec pulses (0.5–1.0 msec and 0.2–1.4 mA). A complex schedule of thalamic stimulation was superimposed upon the classical conditioning paradigm (Fig. 1). Stimulation for recruiting was always given in pairs of 2 sec trains, separated by a

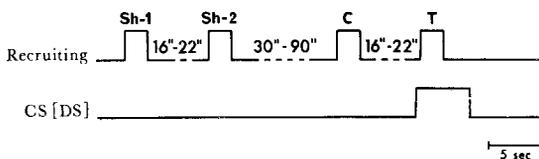


Fig. 1

Summary of interlocking schedules of thalamic stimulation ("Recruiting") and the classical conditioning paradigm (CS, DS). The US was presented during inter-trial intervals during sensitization (phase III) and at CS termination during conditioning and differentiation (phases IV and V). Abbreviations in this and subsequent figures: Sh, sham stimulation not associated with a trial; C, control stimulation preceding a test trial; T, test stimulation 0.7 sec following trial onset.

variable interval (16–22 sec). RRs elicited by the first 2 sec train of the pair (control or "C" stimulation) served as a control for the effects of the CS or DS which was presented 0.7 sec preceding the subsequent train of stimulation (test of "T" stimulation). This procedure was employed because the threshold for RRs may vary markedly within brief periods of time, even in the absence of observable behavioral changes.

Although stimulus intensities employed were insufficient to produce behavioral responses, RRs were also elicited (using a similar pair of stimulation trains) during inter-trial intervals in order to preclude the possible association of thalamic stimulation with the CS, DS or US. As a further precaution against possible conditioning between brain stimulation and stimulus presentation, RRs were elicited on only 6 of the 10 daily trials, the ongoing ECoG being recorded during the remainder.

The experiment was conducted in five phases:

I. *Initial stimulation and recording.* Initial recordings were made, and the electrode pair producing the most widespread RRs was chosen for future work. Optimal stimulus parameters were kept unaltered, except for the voltage, during the remainder of the experiment.

II. *Habituation to the CS.* The CS was presented according to the regular conditioning schedule, but the US was never presented. Ten trials were given daily. Thalamic stimulation was superimposed on this schedule during this and subsequent phases of the experiment.

III. *Sensitization.* This phase was not started until the CS produced neither orienting nor alteration of RRs during one session. Then phase II continued, with the addition of the US which was presented randomly during 10 inter-trial intervals. This phase continued until the CS caused no alteration of the RRs during one session.

IV. *Conditioning.* The regular conditioning paradigm was employed, the US being presented at CS offset. As during phases II and III, 10 daily trials were given. Conditioning was continued until the animal exhibited a CR to CS presentation on all daily trials.

V. *Differentiation.* Conditioning was continued, with the following changes. A total of 12 daily trials were given; 6 DS trials were interspersed with 6 CS trials, with the restriction that the first

and last trials were CS trials. The DS was never followed by the US. Thalamic stimulation was given during 4 CS and 4 DS trials. Differentiation was continued until the cat gave no CR to the DS during a session, and the DS did not alter the RRs.

In addition to the above, each daily session began with a few US presentations to establish UR threshold, and a few thalamic stimulation trains when the cat was lying quietly to insure that there had been no large change in RR threshold from the previous day. Thalamic stimulation current, ordinarily kept constant during a session, was always monitored, and changes in RR amplitude were never accompanied by changes in stimulation current.

At the termination of the experiment, the animals were sacrificed by an overdose of pentobarbital. A weak anodal DC current was passed through the electrodes. The brains were perfused with 10% formaldehyde and potassium ferrocyanide and sectioned at 80 μ . Recruiting sites were the nuclei centralis lateralis (2 cats) and parafascicularis (1 cat).

RESULTS

The behavior of all three cats was essentially the same, differing mainly in the number of sessions required to meet criteria for advancing from phase to phase of the experiment. Stimulus loci, thresholds, and the distribution of RRs differed

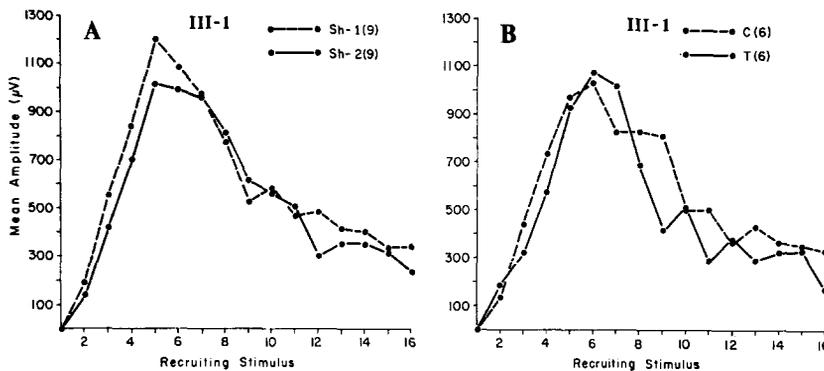


Fig. 2

Mean recruiting "envelopes" for one cat during the first day of sensitization (phase III-1) following habituation. The figures in parentheses indicate the number of 2 sec trains of stimuli contributing to the means. In this and subsequent graphs, the abscissa denotes the serial number of the thalamic recruiting stimulus in a train (8/sec for 2 sec). *A*: Sham recruiting; *B*: Recruiting during CS presentation (T); there were no significant alterations.

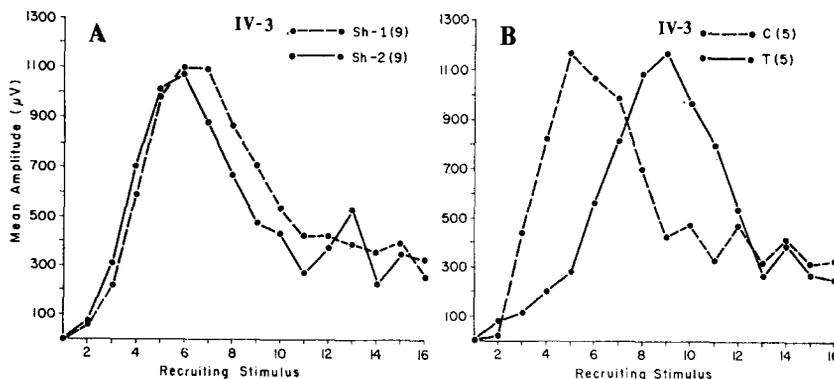


Fig. 3

Mean recruiting "envelopes" of one cat for the third day of conditioning (phase IV-3), which was the first day that the cat gave a CR. *A*: Sham RRs. *B*: Recruiting before and during test trial onset. Note that in the control situation peak amplitude is reached at the fifth stimulus and at the ninth stimulus during the CS test trial.

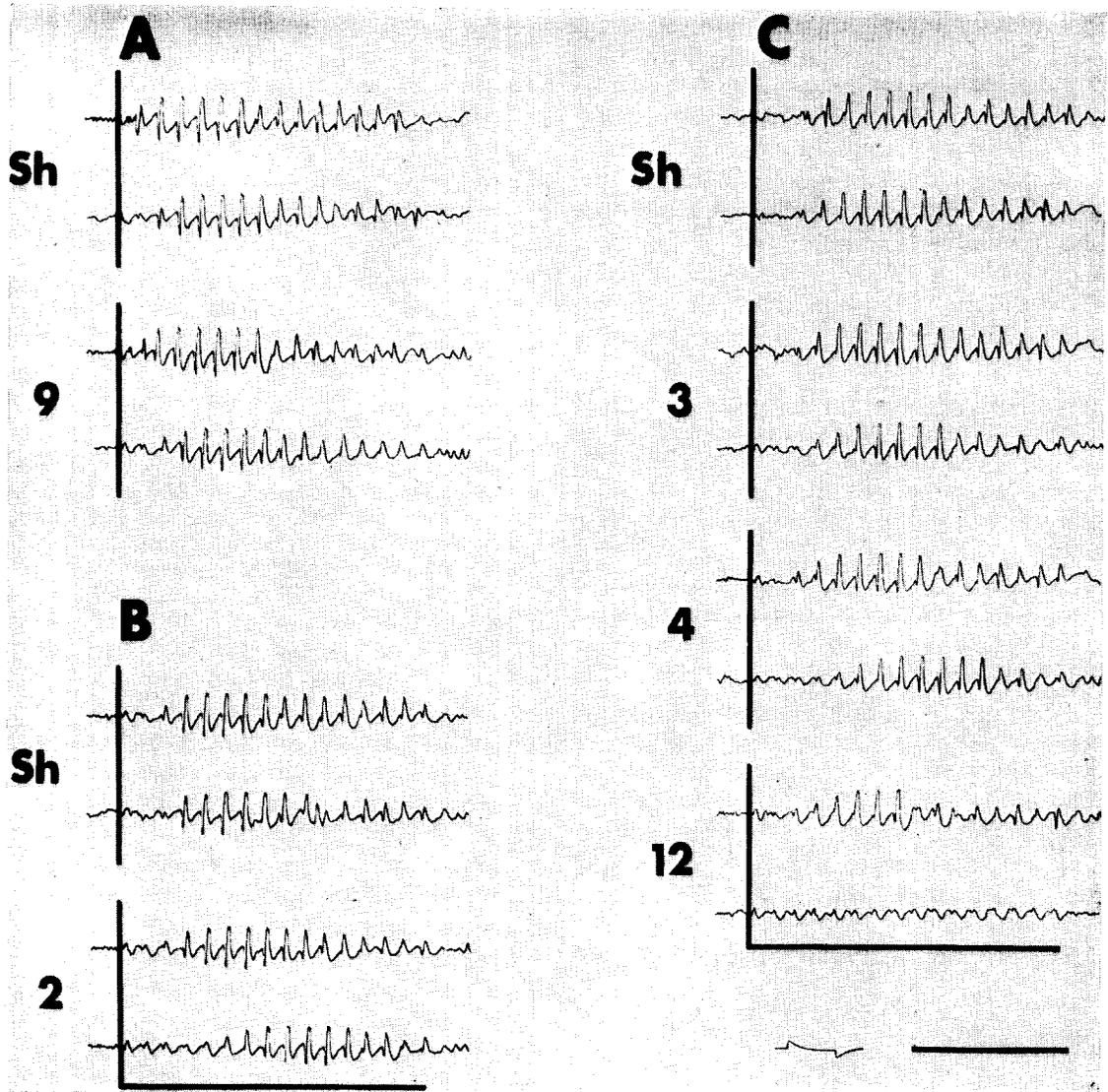


Fig. 4

Selected recruiting responses of the cat whose recruiting envelopes appear in Fig. 2, 3, 5 and 6. "Sh" refers to sham 1 and 2, the numbers refer to test trials, the vertical lines denote trial onset, and the horizontal lines indicate the duration of thalamic recruiting stimulation (8/sec, 1.0 msec, 3-5 V, 0.2-0.7 mA). Records are presented in pairs denoting the first and second trains of stimulation respectively, for sham and test trial conditions. *A: Sensitization* (phase III-1): recruiting elicited during CS presentation was not altered to an extent greater than was sham 2 recruiting. *B: Conditioning* (phase IV-3): RRs evoked during the CS which elicited a CR were initially blocked, and delayed in attaining maximum peak amplitude. Sham 2 RRs were not affected. *C: Early differentiation* (phase V-4): the cat gave CRs to both the CS and DS on all trials; 3, a DS trial depicting a slight delay in recruiting; 4, a CS trial illustrating a long delay in recruiting; 12, a DS trial showing almost complete blockade of recruiting associated with an intense CR. Calibrations: 200 μ V and 1 sec.

among the animals, but there were no essential differences in the effect of the experimental procedures upon such responses. The particular data illustrated are from one animal but are

representative of the other two animals.

I. Initial stimulation and recording. The animals were initially hyperactive when first placed in the experimental chamber. RRs could be elicited

ed only inconsistently, and were not well developed. The animals continued to be active for a few minutes when first placed in the chamber throughout the duration of the experiment. RR thresholds were determined during quiet wakefulness when the cats were either sitting or lying down with eyes open and head erect. RR development and amplitude might vary within a brief period (*e.g.*, 20 sec) in the absence of gross changes in the ECoG or behavior.

II. *Habituation to the CS.* Presentation of the CS initially provoked orientation toward the loudspeaker. During CS presentation, RRs were attenuated or delayed in appearance. Both behavioral orientation and the alteration of the RRs became habituated over the course of this phase of the experiment (see also Everts and Magoun 1957).

III. *Sensitization.* The US produced a UR characterized by rapid head withdrawal and shoulder hunching, eye closing, and often crouching. Immediately following the US, the RRs were irregular or greatly attenuated (amplifier blockade precluded quantification of the effect). Introduction of the US (unpaired with the CS) did not thereafter cause the CS to alter RRs for two cats. The third cat exhibited a sensitization effect for three sessions, but prior to the start of conditioning the CS did not alter RRs for any cats (Fig. 2; 4, A).

IV. *Conditioning.* Pairing of the CS and US

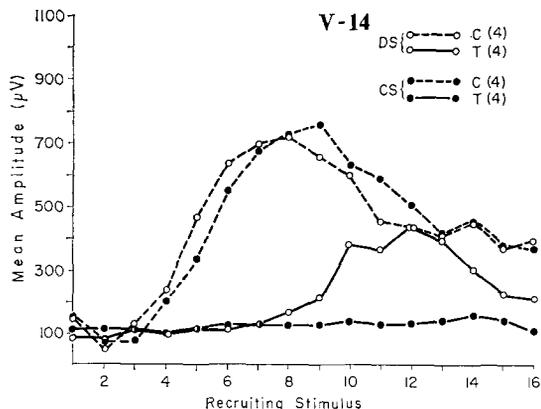


Fig. 5

Mean recruiting "envelopes" for one cat during differentiation (phase V-14) following discrimination. At this point, the cat gave a CR to the CS but not to the DS. The CS produced almost complete blockade, and the DS caused severe attenuation and delay of recruiting.

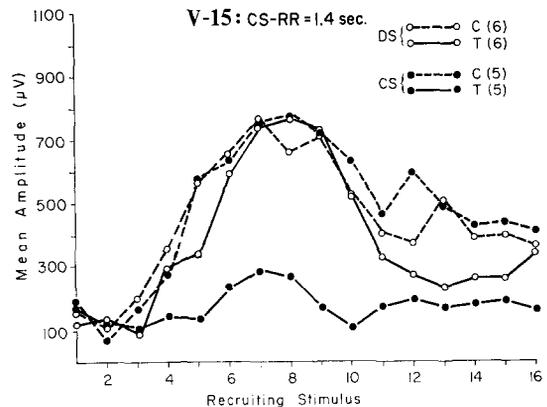


Fig. 6

Mean recruiting "envelopes" during differentiation (phase V-15); the interval between CS (or DS) onset and the start of thalamic stimulation had been increased from 0.7 sec to 1.4 sec. The CS caused marked attenuation but the DS had little effect upon recruiting responses.

produced a CR after from 21 to 46 trials. The CR was similar to the UR, but more variable in intensity. Following initial appearance of the CRs, the CS consistently altered RRs. As found during the habituation phase, two effects were noted: (1) RRs were delayed in onset, but reached a peak amplitude equal to that attained during control (C) stimulation (Fig. 3; 4, B); (2) RRs might be "blocked"; *i.e.*, fail to exhibit characteristic amplitude increments during thalamic stimulation (Fig. 5). Both effects occurred within a single session while using the same stimulus current. Blocking was associated with intense CRs. It was possible to increase the probability of obtaining RR delay at the expense of blocking by slightly increasing the thalamic stimulation current; conversely, blocking could replace delay by decreasing the current.

V. *Differentiation.* The CS continued to elicit CRs and the afore-mentioned effects on the RRs (Fig. 4, C4). CRs were initially produced also by the DS, with concomitant delay or blockade of RRs (Fig. 4, C3 and C12). Continued training resulted in loss of the ability of the DS to produce conditioned responses. Paradoxically, the DS still altered recruiting, causing an initial blockade with later RR amplitude increments which usually failed to reach control peak amplitudes; at the same time, the CS effect remained potent (Fig. 5). The continued effect of the DS on RRs was puzzling until it was noted that the onsets of the CS

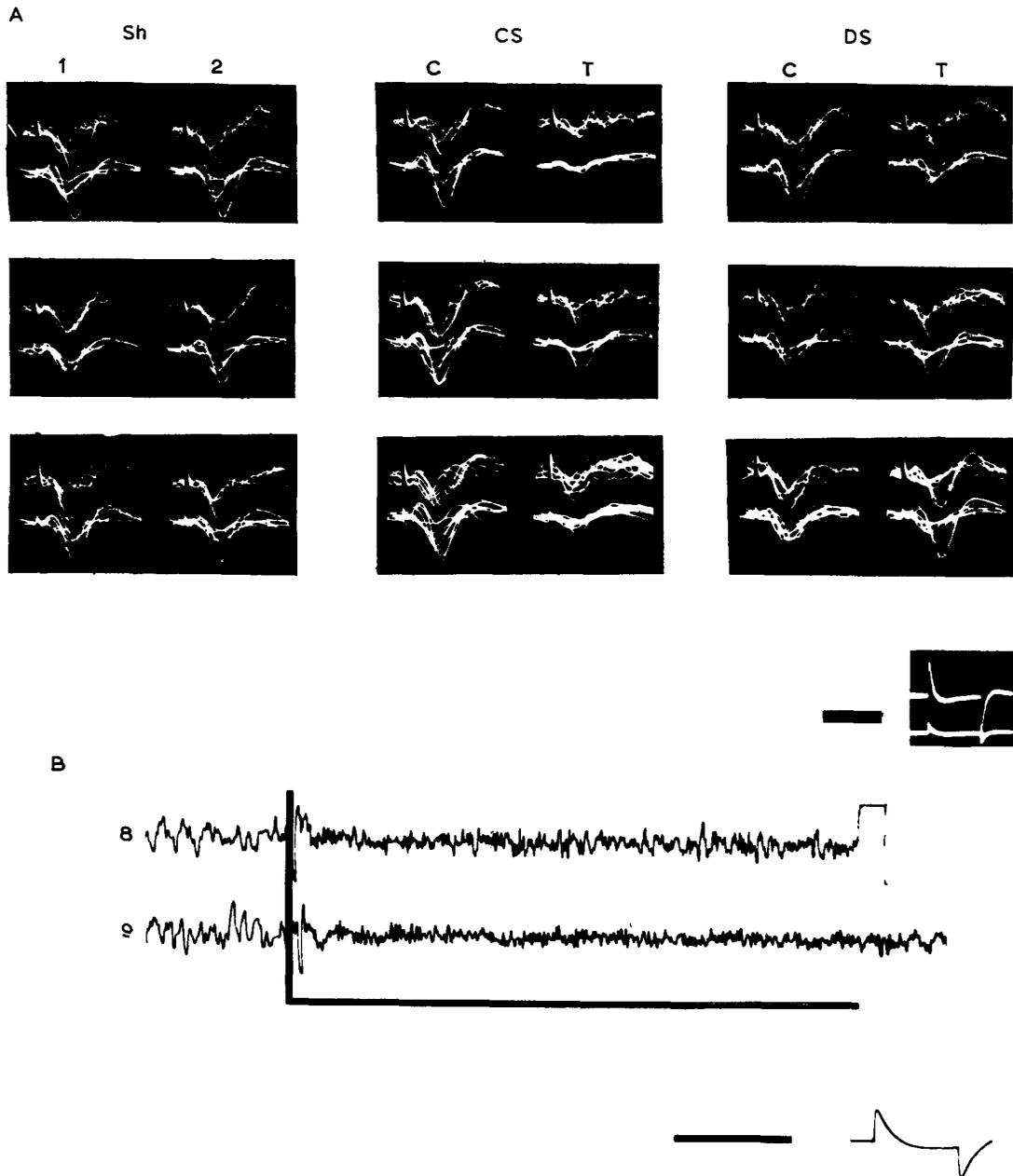


Fig. 7

Selected records from the last differentiation session for one cat illustrating the differential effects of the CS and DS upon recruiting, compared with the ECoG. The interval between onset of the CS (or DS) and thalamic stimulation remained at 1.4 sec. *A*: Detail of recruiting responses during "sham", CS and DS trials which were accompanied by thalamic stimulation. *Top trace*: right ectosylvian gyrus; *bottom trace*: right posterior sigmoid gyrus. Alternate sweeps were photographed. Recruiting was suppressed only during CS presentation. Calibrations: 50 msec, 200 μ V. *B*: ECoGs from the right posterior sigmoid gyrus during non-stimulation trials. The vertical line denotes trial onset, the horizontal line indicating trial duration. Both the CS (trial 8) and the DS (trial 9) produced desynchronization although only the CS elicited a CR. Calibrations: 1 sec, 200 μ V.

and DS were identical, both stimuli consisting of tone pips differing only in their rate of presentation. Thus, DS onset might be expected to produce a phasic increase in arousal level until it had been discriminated from the CS. This hypothesis was tested by increasing the interval between stimulus onset and thalamic stimulation from 0.7 sec to 1.4 sec. This procedure clearly eliminated the DS attenuation of RRs, while the CS continued to disrupt recruiting (Fig. 6; 7, A).

ECoGs recorded on non-stimulation trials throughout training failed to reflect the differential effects of the CS and DS as clearly as development and amplitude of RRs. During behavioral inhibition to the DS, the ECoG was variable, often being more synchronous than during response to the CS, but desynchronization often accompanied both response to the CS and inhibition of response to the DS (Fig. 7, B). In contrast, RRs were always more severely attenuated during responding than during inhibition.

DISCUSSION

These results demonstrate the depression of RRs by a sensory stimulus which produced heightened arousal; *i.e.*, which served as a novel or conditioned stimulus (see also Evarts and Magoun 1957; Angyán *et al.* 1964). It appears that the consistent delay or attenuation of RRs following habituation was due to the conditioning properties acquired by the CS as a consequence of its having been followed by the US (shock). These effects cannot be explained by depression of thalamic excitability due to prior (16–22 sec) stimulation because RRs elicited during sham 2 stimulation were not altered as were RRs elicited during the CS. Neither is it likely that C stimulation served as a signal or second-order conditioned stimulus for subsequent CS–US complexes, because thalamic stimulation did not accompany all trials, and was presented as often during inter-trial intervals as during CS (or DS) presentation. Finally, the CS had a more marked effect than the DS on RRs. Thus, this study replicates and extends the results of previous studies which have shown an inverse relationship between behavioral arousal level and RR amplitude (Evarts and Magoun 1957; Yamaguchi *et al.* 1964; Weinberger *et al.* 1965).

During differentiation, when the cats were well trained and the DS did not produce a CR, the DS still had depressive effects upon the RRs, which were eliminated by increasing the interval between the DS and test stimulus train. The minimum interval which would have produced this same lack of DS depression of RRs was not studied systematically. Presumably, this would provide a sensitive index of the time required for correct discrimination of the DS from the CS. For the present study and stimuli used, we can only state that this discrimination required an interval of more than 0.7 but less than 1.4 sec.

The ECoG was variable during DS trials accompanied by inhibition (no CR). These results are consistent with those of a previous study (Weinberger *et al.* 1967) in which visual stimuli were used in a classical appetitive situation. In the present study, RRs consistently were differentially affected by the CS and DS after discrimination training, and so appear to be more sensitive than the ECoG (at least with visual analysis) to slight changes in arousal level alert animals. Amygdaloid fast activity (Pagano and Gault 1964) and cortical DC shifts (Rowland and Goldstone 1963) also appear to be more sensitive indices.

Recruiting blockade and cortical desynchronization can be produced by high frequency stimulation of both the reticular formation (RF) and the thalamic recruiting system (TRS) (Moruzzi and Magoun 1949), which lends credence to the belief that the latter is a rostral extension of the former (Jasper 1958). Although the issue is still controversial (Krupp and Monnier 1966), there is support for the position that these TRS-initiated activating effects are not independent of the RF (see Schlag *et al.* 1961; Schlag and Chaillet 1963; Weinberger *et al.* 1965). In addition, RF lesions have been reported to attenuate or abolish recruiting blockade elicited by high frequency stimulation of several forebrain sites and also the sciatic nerve (Velasco *et al.* 1965).

At the intracellular level, recruiting blockade produced by brain stimulation is associated with attenuation of the prolonged inhibitory postsynaptic potentials characteristic of many thalamic elements during recruitment; increased excitatory synaptic drives resulting in sustained depolarization inactivation are seen in other

thalamic cells (Purpura and Cohen 1962; Purpura and Shofer 1963; Purpura *et al.* 1966). It is not yet known whether similar events occur during recruiting attenuation or blockade produced by sensory stimulation such as has been demonstrated in the present study.

SUMMARY

The relationship between cortical recruiting responses (RRs) and arousal level was investigated using three cats trained in a classical conditioning situation. A complex schedule of 8/sec thalamic stimulation (eliciting RRs) was superimposed upon an aversive, differential discrimination paradigm to assess the effects of presentation of a novel and conditioned stimulus (CS) and differential stimulus (DS) upon RRs. ECoGs were recorded on trials unaccompanied by thalamic stimulation.

RRs were depressed initially during presentation of the CS when it provoked orientation as a novel stimulus, the effect habituating with continued CS presentation. These depressive effects were reinstated by pairing the CS with shock, but only after the CS produced a conditioned response (CR). Attenuation of RRs consisted of slight or severe delay of amplitude growth with or without the final attainment of control peak amplitudes, or complete blockade.

The DS initially also caused depression of RRs, even when this stimulus no longer evoked CRs. Attenuation of RRs by the DS was abolished by increasing the interval between stimulus (CS or DS) onset and subsequent recruiting stimulation from 0.7 to 1.4 sec; the CS continued to disrupt recruiting. ECoGs during non-recruiting trials appeared to be less sensitive indices of behavioral arousal than RR development and amplitude.

RÉSUMÉ

LES RÉPONSES RECRUTANTES CORTICALES PENDANT LE CONDITIONNEMENT CLASSIQUE

La relation entre les réponses recrutantes corticales (RRs) et le niveau d'éveil a été étudiée chez trois chats soumis à un conditionnement classique. Un programme complexe de stimulation thalamique de fréquence 8/sec, qui entraîne la RR, a été associé à un conditionnement discriminatif avec aversion pour évaluer les

effets de la présentation d'un stimulus nouveau et conditionnel (CS) et un stimulus différentiel (DS) sur la RR. L'ECoG a été enregistré lors des essais non accompagnés de stimulation thalamique.

Les RRs étaient d'abord diminuées pendant la présentation du CS quand il provoquait le phénomène d'orientation comme un nouveau stimulus et l'effet d'habituation a lieu lorsque le CS continue à être présenté. Ces effets dépressifs étaient rétablis en associant le CS avec un choc, mais seulement après que le CS ait produit le CR. L'atténuation des RRs consistait soit en un blocage complet, soit en un délai léger important de l'augmentation d'amplitude avec ou sans l'acquisition finale des amplitudes initiales.

Le DS initialement cause aussi une dépression des RRs même quand ce stimulus n'évoque plus de CRs. L'atténuation des RRs par le DS a été abolie en augmentant l'intervalle entre le début du stimulus (CS ou DS) et la stimulation thalamique recrutante qui la suit, de 0,7 à 1,4 sec; le CS continue à diminuer ou supprimer la RR. L'ECoG pendant les essais non recrutants est apparu d'être un indice moins sensible d'éveil que le développement et l'amplitude de la RR.

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