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## Neuronal control of the classically conditioned nictitating membrane response in rabbit: a critical role for the dorsolateral pons.

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NEURONAL CONTROL OF THE CLASSICALLY CONDITIONED NICTITATING  
MEMBRANE RESPONSE IN RABBIT:  
A CRITICAL ROLE FOR THE DORSOLATERAL PONS.

A Thesis Presented

By

JOHN E. DESMOND

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of

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Department of Psychology

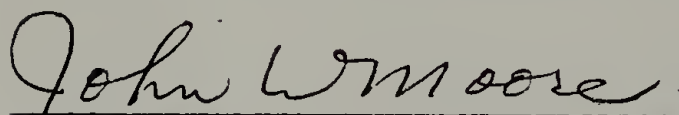
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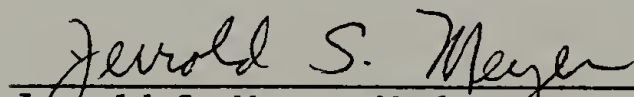
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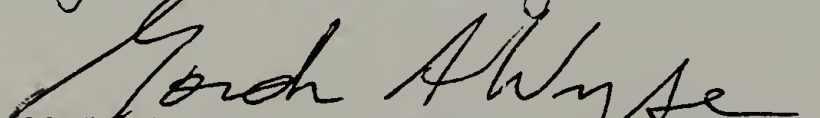
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## A B S T R A C T

The dorsolateral pontine brain stem was investigated as a possible locus of neural elements mediating the classically conditioned rabbit nictitating membrane response. Lesioning, recording, and stimulating techniques were employed for this purpose.

Radio-frequency lesions of the right dorsolateral pons severely impaired both acquisition and retention of the ipsilateral conditioned nictitating membrane response to visual, auditory, and tactile conditioned stimuli. Unconditioned responses were unaffected, and conditioning of the contralateral (left) eye was not impaired. These results suggested that disrupted conditioned responding was not due to sensory, motor, or attentional impairment. Thus, the neural control of the conditioned response is separate from that of the unconditioned reflex. Histological analyses indicated that the following structures were lesioned in disrupted animals and spared in the non-disrupted animals: locus subcoeruleus, the supratrigeminal region, brachium conjunctivum, and parabrachial nuclei.

Low-impedance tungsten monopolar electrodes were chronically implanted into the pontine brain stem. Multiple-unit recording during classical conditioning revealed a conditioned increase in multiple-unit activity which developed and extinguished concurrently with the acquisition and extinction of the behavioral conditioned response. Pseudo-conditioning and conditioned inhibition controls indicated that the

increase in multiple-unit activity was an associative learning phenomenon. Histology indicated that electrode tips recording the CR-associated electrical activity were located mostly adjacent or dorsal to the motor trigeminal nucleus.

In addition, periocular shock pulses elicited short latency evoked responses throughout most of the dorsolateral pons, suggesting that information concerning the unconditioned stimulus is relayed to this region. Furthermore, electrical stimulation to the dorsolateral pons produced a robust ipsilateral nictitating membrane response in a number of cases, suggesting that cells of the dorsolateral pons project to the motoneurons that produce membrane extension.

These data suggest that the dorsolateral pons contains pre-motor cells which mediate the conditioned but not unconditioned nictitating membrane response. Other interpretations, concerning fibers of passage through this region, are discussed.



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## CHAPTER I

### INTRODUCTION

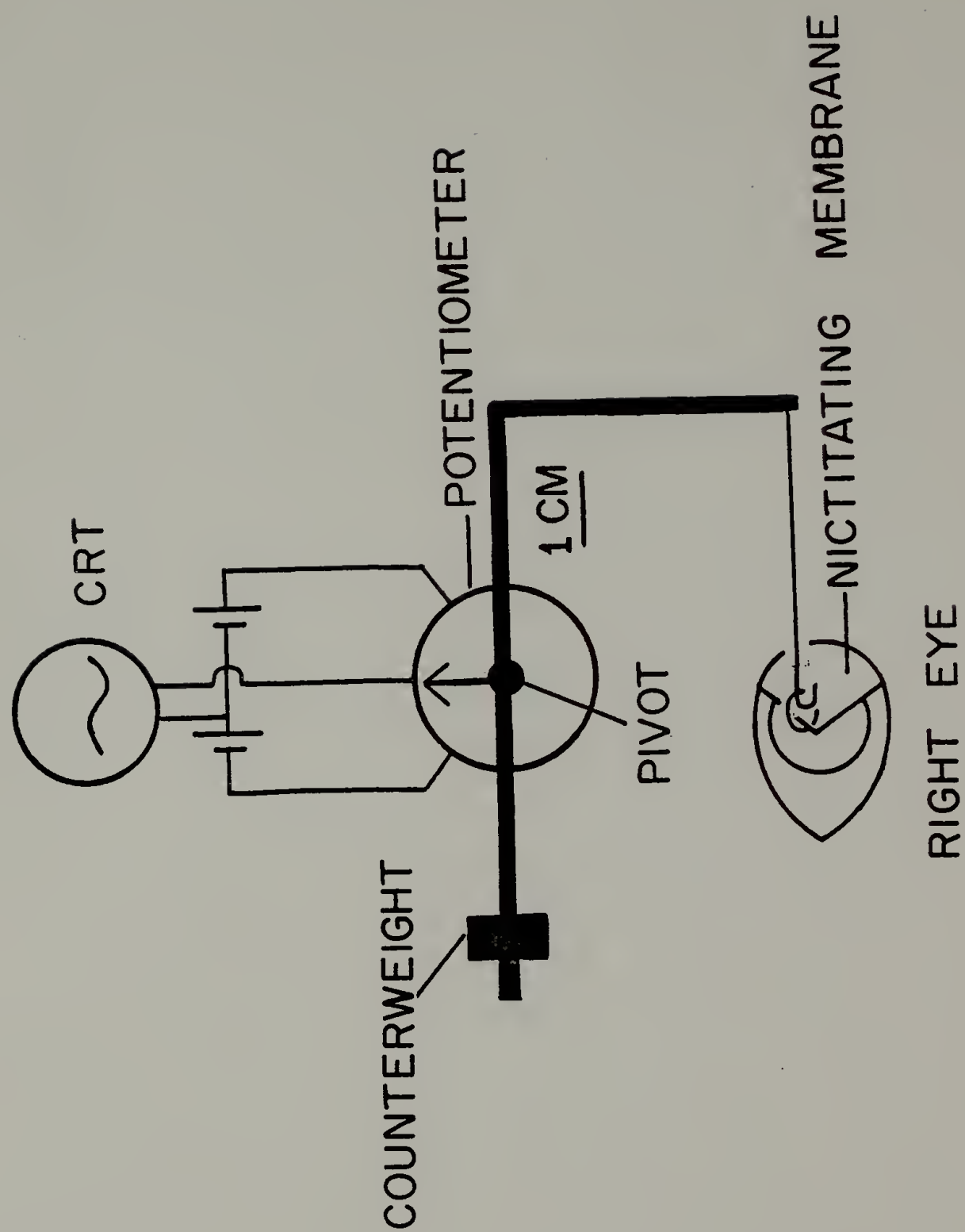
There are a number of animal preparations currently used to study the neural mechanisms of behavioral plasticity; these include habituation of the gill withdrawal response in *Aplysia* (Kandel, 1976), conditioned decrease in phototactic behavior in the marine mollusk *Hermisenda* (Alkon, 1979), and the retention of active and passive avoidance tasks in rats and mice (Allen, Allen, and Rake, 1974; Dismukes and Rake, 1972; McGaugh, 1966). The classically conditioned rabbit (and cat) nictitating membrane response (NMR), however, has become one of the preparations of choice for the investigation of the physiological mechanisms of associative learning in mammals. The reasons why this preparation is an attractive one for such an investigation have been elaborated by others (Moore, 1979; Thompson, Berger, Cegavske, Patterson, Roemer, Teyler, and Young, 1976). Briefly these reasons are: (a) Conditioning can be achieved within a single session, but a number of trials are required for acquisition; (b) An alpha response does not occur to the conditioned stimulus; (c) Pseudoconditioning or sensitization does not occur; (d) Stimulus parameters involved in the conditioning procedure (e.g., interstimulus interval) have been investigated; (e) The conditioned response is robust, discrete, and easily quantified in terms of latency and amplitude; (f) The efferent control of the NMR is known; (g) The preparation allows precise control of the stimuli; (h) The preparation does not depart dramatically from physiologically normal conditions; (i) The baseline response level is extremely low between trials;

and (j) The rabbit NMR preparation is of considerable importance for contemporary learning theorists.

The procedures for establishing conditioned responding in the NMR preparation have been described in detail elsewhere (Gormezano, 1966). The unconditioned stimulus (US) is either a periocular electric shock, or a puff of air, to the right eye. The former stimulus is preferred as a US in this laboratory for the following reasons: (a) Stimulus parameters, such as duration and intensity, are readily controlled with an electrical US. (b) There is greater control over the region of stimulation with an electrical US; air puff tends to stimulate more marginal tissue. (c) Eyelid retraction is necessary for air puff but not for shock; in this respect air puff is a less "physiological" stimulus than eye shock. (d) Direct contraction of eye muscles from shock has not been observed with current levels up to 10 mA, well in excess of normal levels used in conditioning experiments (1-3 mA).

The conditioned stimulus (CS) can be a variety of cues such as a light, a tone, or a mild backshock. The unconditioned and conditioned responses (URs and CRs) consist of an eyeblink and a nasotemporal extension of the nictitating membrane across the cornea; the response is detected via a potentiometer which is mounted on the animal's head and which is connected by thread and a small hook to a loop of nylon sutured to the nictitating membrane. Movement of the membrane creates a dc signal which is amplified and recorded on an ink-writing oscillograph. Figure 1 is a schematic representation of the recording apparatus.

Fig. 1. Schematic representation of method of recording the NMR.





### The Unconditioned Response: Neural Control

The following discussion is derived from literature on both rabbits and cats, and assumes that, with regard to the NMR preparation, the similarities of the two species outweigh the differences. Nevertheless, caution must be exercised, as much of the neural circuitry and physiology involved in the NMR reflex remain to be established for both species.

The sweep of the nictitating membrane appears to be a passive consequence of eyeball retraction (Berthier and Moore, 1980; Harrison and Cegavske, 1981; Motaïs, 1885, cited in Bach-y-Rita, 1971). Eyeball retraction is accomplished via the retractor bulbi muscles, which surround the optic nerve at the point where the nerve exits the globe; recent evidence indicates that the extraocular muscles also contribute to the NMR (Berthier and Moore, 1980). Following stimulation to the cornea or surrounding tissue, the nictitating membrane extends from its resting position in the nasal canthus across the cornea toward the temporal canthus, and then retracts to its resting position.

Stimulation of the abducens (VIth) nerve, but not the IIIrd, IVth, or VIIth nerves, or the superior cervical ganglion, causes extension of the nictitating membrane in rabbit (Cegavske, Thompson, Patterson, and Gormezano, 1976). This suggests that axons of retractor bulbi motoneurons innervate the muscles via the VIth nerve. Retractor bulbi motoneurons for rabbits and cats have been localized in ipsilateral abducens, oculomotor, and accessory abducens nuclei by horseradish peroxidase (HRP) procedures (Berthier and Moore, 1980; Disterhoft and Shipley, 1980; Grant, Gueritaud, Horcholle-Bossavit, and Tyc-Dumont, 1979; Gray, McMaster, Harvey, and Gormezano, 1980; Guegan, Gueritaud, Horcholle-

Bossavit, 1978; Spencer, Baker, and McCrea, 1980). The latter nucleus is located ventrolateral to the abducens nucleus, and axons of accessory abducens neurons in cats project toward the abducens nucleus and join the VIth nerve (Spencer et al., 1980). The following lines of evidence, however, suggest that accessory abducens motoneurons are chiefly responsible for defensive globe retraction and nictitating membrane extension:

(1) Lesioning the abducens nucleus in rabbit does not eliminate the ipsilateral NMR (Powell, Berthier, and Moore, 1979). The accessory abducens nucleus was spared in this study.

(2) Electrical stimulation of the IIIrd nerve in rabbit causes only retraction of the nictitating membrane toward the nasal canthus (Cegavske et al., 1976). This may be accomplished via the levator palpebrae superioris muscle (Harrison and Cegavske, 1981). In addition, Meredith, McClung and Goldberg (1981) observed contraction in cat retractor bulbi muscles when N.III was stimulated, but observed that N.III induced contractions were much weaker in tension than contractions induced by N.VI stimulation. Other mechanical differences were observed between N.III and N.VI induced contraction, and the authors concluded that the mechanical characteristics of retractor bulbi muscles responding to N.III stimulation were similar to those of extraocular muscles also controlled by N.III. They also concluded that the retractor bulbi muscles that are excited by N.III probably do not participate in globe retraction associated with the NMR, but probably do function in patterned eye movements.

(3) Accessory abducens neurons are antidromically (but not synaptically) activated by VIth nerve stimulation in both rabbit (Berthier and Moore, 1982) and cat (Baker et al., 1980). In both studies, extracellular field potentials were recorded at various depths through the accessory abducens nucleus.

(4) Using extracellular recording, negative field potentials (3-5 ms latency) were observed in accessory abducens neurons following electrical shock to the ipsilateral periorbital region of the rabbit eye (Berthier and Moore, 1982). Similarly, Baker et al. (1980), using intracellular recording of cat accessory abducens neurons, observed excitatory postsynaptic potentials (EPSPs) (1.6-2.0 ms latency) following electrical shock to the ipsilateral cornea.

(5) Berthier and Moore (1982) found no single unit responses to ipsilateral eye shock with extracellular recording in the rabbit abducens nucleus. Baker et al. (1980) observed only small amplitude EPSPs in cat abducens and oculomotor neurons following corneal or Vth nerve stimulation. The latencies of these responses (3-5 ms) were longer than those for accessory abducens cells. On the other hand, Baker et al. (1980) observed that abducens and oculomotor cells displayed strong excitatory responses at disynaptic latencies to vestibular nerve stimulation, whereas accessory abducens cells responded relatively weakly to vestibular input.

(6) Morphological features of cat accessory abducens neurons are different from those of abducens and oculomotor nuclei with regard to size, somata and dendritic synaptic densities, and orientation and extent of dendritic fields. Such morphological differences suggest

functional differences for these populations of neurons (Spencer et al., 1980). Coupled with the electrophysiology data, these results suggest that abducens and oculomotor nuclei are responsible for patterned eye movement, whereas accessory abducens neurons are responsible for eyeball retraction (Baker et al., 1980).

The sensory component of the NMR reflex is probably mediated via ophthalmic and maxillary branches of the Vth nerve, and it is possible that the mandibular branch is also involved. Berthier and Moore (1982) concluded that activation of accessory abducens neurons following eye stimulation was a disynaptic event in rabbit; Baker et al. (1980) made the same conclusion for cat. Hence, secondary fibers of sensory trigeminal nuclei are believed to project to accessory abducens neurons to form the reflex arc.

The precise location of the secondary trigeminal cells mediating the NMR is unknown. However, Berthier and Moore (1982) made transverse knife cuts of the rabbit brain stem caudal to the facial and accessory abducens nuclei and continued to observe efferent volleys from the VIth nerve in response to ipsilateral eye shock. This result suggests that nucleus interpolaris and nucleus caudalis of the sensory trigeminal complex are not necessary for the NMR reflex, and that nucleus oralis and the principal sensory nucleus of N.V are sufficient to produce the reflex. Given these results, data derived from electrophysiology experiments (Kruger and Michel, 1962; Wall and Taub, 1962) as well as results from transganglionic transport of HRP (Panneton and Burton, 1981) suggest that corneal and infraorbital sensory input (involved in the NMR) are represented in ventral portions of nucleus oralis and

the principal sensory nucleus. The studies mentioned above used cats, but similar research involving rabbits is in progress (Cegavske, personal communication). In addition, injections of HRP into the accessory abducens nucleus would be expected to produce labelling of trigeminal neurons involved in the NMR reflex; such experiments are also in progress in this and other laboratories.

Finally, the latency of the unconditioned NMR in unanesthetized rabbit as a function of periocular electro-stimulation parameters was investigated by Moore and Desmond (in press). The results showed that the latency of the NMR to a single shock pulse is primarily determined by current level (as opposed to pulse duration), and the function describing this relationship (for current intensities up to 10 mA) is  $L = 17 + 34\exp(-.62I)$ , where  $L$  = latency (ms) and  $I$  = stimulus current in mA. The function asymptotes at approximately 17 ms, and the authors suggested that the 17 ms can be decomposed as follows: 4 ms to fire accessory abducens motoneurons (Berthier and Moore, 1982), 9 ms for conduction to retractor bulbi muscles, synaptic transmission, and recruitment of retractor bulbi muscle fibers (Lennerstrand, 1974; Meredith et al., 1981), and 4 ms for the nictitating membrane to initiate its sweep after eyeball retraction (Cegavske et al., 1976).

#### The Conditioned Response: Neural Correlates

Attempts to demonstrate the involvement of particular brain regions in the generation of conditioned responses have employed lesioning, stimulation, and physiological recording techniques. Despite the fact that specific brain areas have been implicated in the generation of



CRs, demonstrations of causality between any particular structure and the CR have been lacking in most studies. The following is a summary of experiments relevant to the discussion of the neural control of the conditioned NMR.

Neocortex. Studies investigating the role of neocortex in eyeblink and NMR conditioning include that of Papsdorf, Longman and Gormezano (1965) who observed that potassium chloride application to the dura of the rabbit (producing a reversible functional ablation of the cortex and possibly other areas) eradicated conditioned nictitating membrane responding for 195 trials over a 254 minute period, during which time percent CRs gradually increased; unconditioned responding was not affected by the treatment. Other researchers observed that multiple (Woody, 1970) and single unit (Woody, Vassilevsky and Engel, 1973) activity of neurons in coronal-precruciate cortex of cat showed a CS-evoked increase in activity as a function of eyeblink conditioning (where CS was click stimulus and US was glabella tap), and that electrical stimulation of this cortical region could serve as a CS for eyeblink conditioning (Woody and Yarowski, 1972). A subsequent study was conducted in which lesions of this region, but not more caudal cortical regions, were found to permanently impair acquisition of conditioned responding (Woody, Yarowski, Owens, Black-Cleworth and Crow, 1974). Lesioned animals made less than 25% CRs, whereas non-lesioned cats reached asymptote at 60%. Lesioned animals showed significantly fewer spontaneous blinks and required a significantly greater force of glabella tap to produce a UR than non-lesioned conditioned cats, but were not

significantly different from non-lesioned naive animals in either measure.

Despite the results of Woody et al. (1974), there is evidence that acquisition of conditioned responding can proceed normally in decorticate rabbits (Moore, Yeo, Oakley and Russell, 1980; Oakley and Russell, 1972; see also Moore, 1979) and cats (Norman, Buchwald and Villablanca, 1977), and that retention of conditioned responding is similarly undisturbed. The major reported effect of decortication was an increase in CR onset latency with respect to control animals (Oakley and Russell, 1972; Oakley and Russell, 1977). Woody et al. point out that the type of stimuli employed as CSs and USs could determine the functional importance of cortex in conditioning. The CS and US in the Woody et al. study were click and glabella tap, respectively; the decortication studies discussed above used tone or white noise for the CS and periorcular shock for the US.

The explanation of the discrepancy between the results of Woody et al. (1974) and the decortication studies described above may be that the critical site(s) of learning is dependent upon the specific stimuli which are used as CS and US. On the other hand, the cortex may have provided some type of facilitation of sensory transmission necessary for conditioned responding in the Woody et al. paradigm, but not essential for conditioning in the decortication studies. Corticofugal modulation of sensory input is well documented, and physiological experiments indicate the presence of both excitatory and inhibitory influences of neocortex on sensory transmission (Carpenter, 1976). Another possible explanation for the discrepancy may lie in the fact that

Woody et al. observe short latency CRs in their preparation, whereas other researchers utilizing eyeshock observe relatively long latency CRs. Finally, it should be noted that Woody et al. employed a trace conditioning procedure (i.e., where there is an interval of time between CS offset and US onset; other studies discussed thus far employed a delay paradigm where CS offset is contiguous with US onset). However, decorticate rabbits were found not to be impaired in trace conditioning procedures relative to control animals (Moore, personal communication).

Hippocampus. The hippocampus has also been implicated in conditioning of the NMR. Multiple unit activity (MUA) in hippocampal areas CA1, CA3, CA4, and the granule cell layer of the dentate precedes the behavioral conditioned NMR by 25-35 ms and parallels its development during acquisition in both rabbit (Berger, Alger, and Thompson, 1976) and cat (Patterson, Berger, and Thompson, 1979). These observations were confirmed in rabbit by Moore, Desmond, and Berthier (1981). A single unit study (Berger and Thompson, 1978), investigating areas CA1 and CA3 identified the pyramidal cells as the units exhibiting the learning-dependent changes in activity. In addition, post-trial stimulation of the hippocampus disrupted acquisition of conditioned NMRs (Salafia, Romano, Tynan, and Host, 1977); furthermore, the disrupted acquisition was not due to hippocampal seizure activity (Salafia, Chiaia, and Ramirez, 1979).

Despite these compelling data, unimpaired acquisition (Solomon and Moore, 1975; Solomon, 1977), as well as facilitated acquisition (Schmaltz



and Theios, 1972), in hippocampectomized rabbits have been reported. These results suggest that the hippocampus is not causally involved in conditioning per se. However, the hippocampus evidently does have some role, especially in higher-order processes. For example, bilateral dorsal hippocampectomy results in a disruption of the latent inhibition phenomenon (Solomon and Moore, 1975). Furthermore, bilateral dorsal hippocampectomy impairs long-trace conditioning (Weisz, Solomon, and Thompson, 1980), and discrimination reversal (Orr and Berger, 1981).

Septal Nuclei. The septal nuclei have also been linked with NMR conditioning in rabbit. Berger and Thompson (1977) demonstrated that lateral septal nuclei, but not medial septal nuclei, show the same type of conditioned MUA that the hippocampus displays during NMR conditioning. Berry and Thompson (1979) disrupted acquisition of NMR conditioning by producing small medial septal lesions in rabbits. These lesions disrupted hippocampal theta rhythms and presumably spared hippocampal efferent pathways through the lateral septal nuclei, allowing disrupted hippocampal activity to propagate. Larger septal lesions, damaging in whole or in part both medial and lateral septal nuclei, however, failed to disrupt conditioning of the rabbit NMR in one study (Lockhart and Moore, 1975), and actually produced faster acquisition of rabbit eyeblink CRs, relative to controls, in another study (Maser, Dienst, and O'Neal, 1974).

Basal Ganglia. One experiment has been reported in which large bilateral lesions destroying 2/3 of the anterior portion of the head of the caudate nucleus severely disrupted the acquisition of rabbit eyeblink

conditioning (Powell, Mankowski, and Buchanan, 1978). Unilateral lesions were not disruptive. Control experiments indicated that conditioning deficits were not due to decreased sensitivity to the US, impairment of the UR, or deficits in general activity. It is not known whether or not large bilateral caudate lesions would disrupt well established conditioned responding. It is also not known if there are electrophysiological correlates of the CR in the caudate nucleus.

Mesencephalon and Metencephalon. The studies described thus far have failed to demonstrate that the more rostral brain regions are essential for conditioning. The results of Norman, Buchwald, and Villablanca (1977) strongly support this conclusion as they were able to observe eyeblink conditioning in cats that received brain stem transections at the level of the colliculi. The authors concluded that the experiment demonstrates ". . . the independent capacity of the brain stem to support a simple form of learning." Moore (1979) elaborates further on possible brain stem circuits involved in NMR learning, stressing the suitability of reticular formation neurons for mediating conditioning. The present experiments, described below, support the notion that the brain stem possesses the neural elements which support conditioning. Specifically, dorsolateral pontine (DLP) lesions eradicated both retention and acquisition of ipsilateral, but not contralateral, CRs. The URs were not affected by the lesions. The lesion data from these experiments have been documented in preliminary reports (Desmond, Berthier, and Moore, 1981 a, b; Moore, Desmond, and Berthier, 1981), and are currently in press (Desmond and Moore, in press). Furthermore,

recording electrodes in this region of the brain have yielded an increase in MUA paralleling the behavioral NMR. These data have also appeared in preliminary reports (Desmond, Berthier and Moore, 1981 a; Moore, Berthier and Desmond, 1981; Moore, Desmond, and Berthier, 1981).

It should be noted that evidence emerged after the completion of the present experiments implicating cerebellar nuclei with conditioning (McCormick, Lavond, Clark, Kettner, Rising, and Thompson, 1981). Specifically, results similar to those described above for DLP have also been described for interposed and dentate nuclei. The relationships, if any, among these cerebellar structures and the DLP structures involved in the present experiments are uncertain at this time, and various possibilities are elaborated in the discussion section.

## CHAPTER II

### EXPERIMENT ONE

#### Method

Animals. The animals in this experiment were 30 male and female New Zealand albino rabbits (Oryctolagus cuniculus) obtained from a licensed supplier. The animals were individually housed and were allowed ad lib. access to food and water. The rabbits weighed 2.5-3.0 kg.

Procedure. Two procedures were used to assess the effects of DLP lesions on CRs. For the first, acquisition test, there were two groups of animals; the first is designated Lesion-Acquisition (LA), in which naive animals were given a unilateral lesion to the right DLP and the effect of the lesion on the initial acquisition of learning was measured. The second group is designated Sham-Acquisition (SA). This group received the same treatment as group LA except that no current passed through the lesioning electrode. There were a total of 7 animals in the LA group, and 5 in the SA group.

For the second procedure, retention test, there were also two groups of animals. One is designated Lesion-Retention (LR). These subjects were given acquisition training prior to the lesion of the right DLP; post-lesion testing assessed the retention of learning of these animals. The other group is designated Sham-Retention (SR). This group was treated identically to LR except that no current passed through the electrode. A total of 23 animals were included in the LR group, and 6

in the SR group. All animals were unsystematically assigned to groups prior to any conditioning training.

On the day prior to the first training session, the subjects had their right nictitating membrane sutured and received 30 min. of habituation to the conditioning chamber. The conditioning chambers were constructed from ventilated, fire-proofed, and sound-attenuated filing cabinets. Four animals could be trained simultaneously in this apparatus. The conditioning chambers were located in a room adjacent to the oscillographs and programming equipment, and fans in the conditioning room provided background noise. A detailed description of the equipment is available in other published reports (e.g., O'Malley, Hupka, and Moore, 1969).

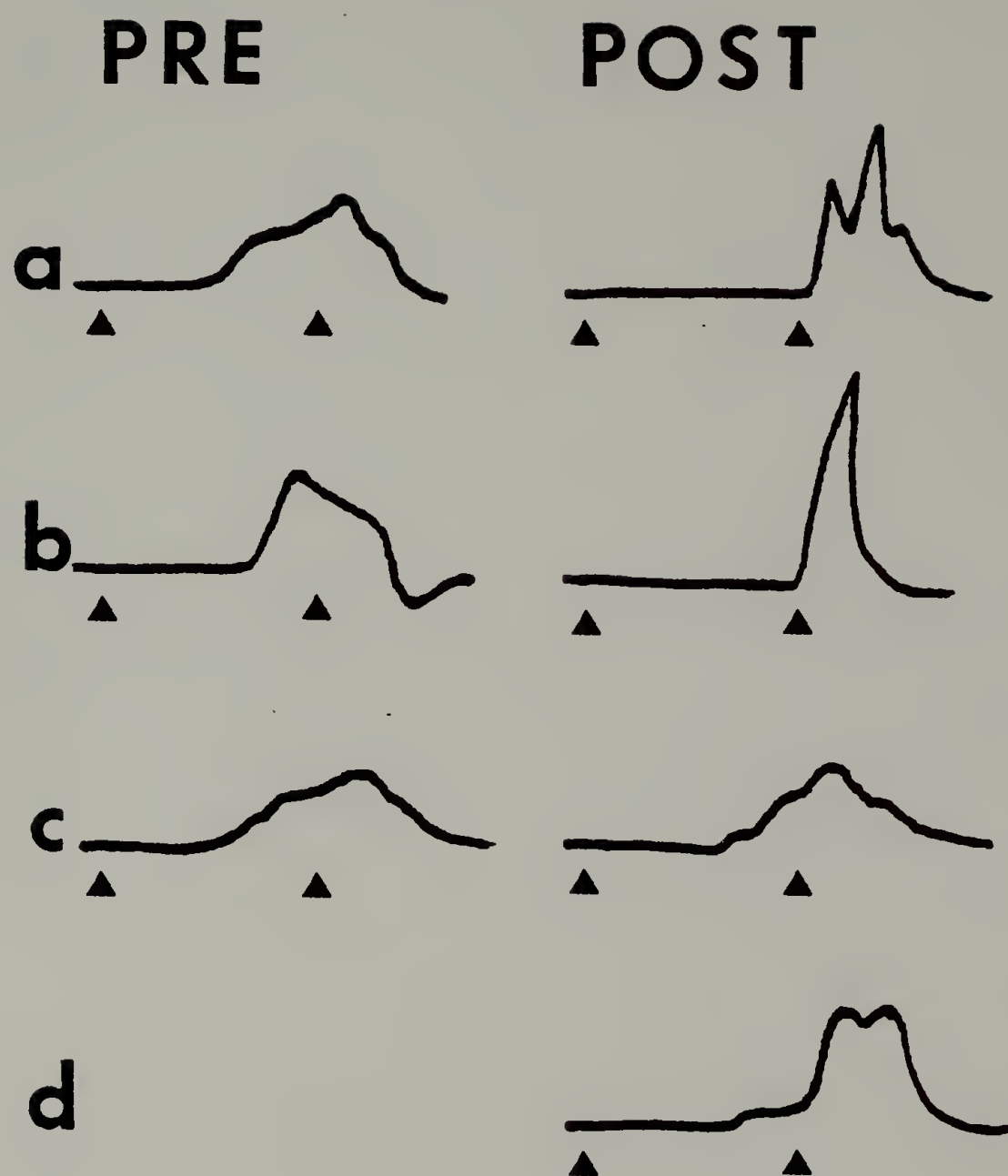
Classical conditioning procedures were standard (Gormezano, 1966). The CSs were either light (L = two 4.5 V incandescent panel lights) or tone (T = 1200 Hz sinusoidal tone of 85 dB SPL presented through a speaker mounted in front of the animal). The US was electric shock (60 Hz ac, 2 mA, 50 ms duration) to the periocular region of the right eye. Stimulation was applied via 9-mm stainless steel wound clips to which alligator leads from the stimulus source were attached. These electrodes were crimped in to the marginal region of the eye: One located in an inferior position within 3 mm of the margin and the other in a posterior position, also within 3 mm of the margin. The CS-US interval was 0.5 sec (CS and US terminating together in a forward delay conditioning procedure), and the intertrial interval (ITI) was 15 sec. Training always consisted of 100 trials per day; 50 reinforced L (L+) and 50 reinforced T (T+) were presented in a random sequence, but not



more than 2 consecutive presentations of the same stimulus were allowed. (This training procedure will be referred to as L+/T+.) Both CRs and URs were recorded by an electromechanical transducer and ink-writing oscillograph. An upward pen deflection of 1 mm corresponded to a 0.5 mm extension of the nictitating membrane, and an extension greater than or equal to this amount, occurring after CS onset but before US onset, was the criterion for a CR. Figure 2 illustrates some oscillographic tracings of CRs and URs. For each day of training, the percent of CRs to L and T CSs were computed. Typically, naive rabbits give CRs on 80-100% of the trials within only 1-3 days of training.

Acquisition animals (LA and SA) were classified as CR-disrupted (D) or CR-nondisrupted (ND) after 5 days of post-surgical acquisition training; the percent CRs to L and T were averaged for each animal over the 5 days so that an animal's performance could be described by a single score. Retention animals (LR and SR) were allowed 5-7 days of training before surgery such that 80-100% CRs to both L and T were obtained for 2 consecutive days before surgery. Following surgery, animals were classified as D or ND after 5 days of retention testing. A pre-surgical score was computed for each animal, and this was based upon the average percent CRs to L and T over the last 3 days prior to surgery. Post-surgical scores for retention animals were based upon the percent CRs averaged over 5 days of testing. (It should be noted that 8 of the LR animals received only L+ training before and after surgery, and this accounts for unequal sample sizes when L and T percent CRs are reported.) The classification of D or ND was easily accomplished due to the virtual all-or-none effect of the lesions.

Fig. 2. Representative NMRs from lesion experiments. Left panel: For each tracing the onset of the CS is marked by the left-hand triangle, and the onset of the US is marked by the right-hand triangle. Upward deflections occurring after CS onset but before US onset are CRs. Those occurring after US onset are URs. PRE refers to a pre-lesioning trial. POST refers to a post-lesioning trial. a. Representative NMRs to L for Animal 7 in the D group. b. Representative NMRs to T for the same animal. c. Representative NMRs to L for Animal 17 of the ND group. d. An NMR to T for a D animal (Animal 2) lesioned prior to training. This tracing shows a rare CR on the fourth session of training.





It was recognized that an absence of CRs subsequent to brain destruction could indicate sensory or motor deficits rather than deficits in learning or memory. Thus, the following control procedures were employed to assess the contribution of these factors:

(1) A neurological examination was performed on all animals before and after surgery. This examination consisted of a test of pupillary and eyeblink reflexes to a bright penlight, a test of equilibrium and locomotion, and a test of the UR. The latter test consisted of a mild tactile stimulus to the cornea and surrounding regions, using a cotton-tipped applicator, and measuring the amount of extension of the nictitating membrane in mm.

(2) Four D animals (along with 10 ND and 11 Sham controls) received variations in the CS-US interval. Two values were used, 0.25 sec and 0.75 sec. (2 days of L+/T+, 100 trials/day, for each value). This control procedure tested two possibilities. The first is that the CR-disrupting lesion somehow affects the optimal temporal arrangement of CS and US necessary for the production of a CR. The second possibility is that the lesion slows the reaction time of the animal, and thus, it takes longer for the animal to produce a CR after CS onset.

(3) All D animals (and a random sample of ND and Sham animals) received a single session (80-100 trials of L+ and T+) of contralateral conditioning training. That is, the left nictitating membrane was sutured, the eye shock was switched to the left eye, and CRs were recorded from both eyes. Because CRs occur independently in the right and left eyes of the rabbit (Stickney and Donahoe, submitted for publication), the absence of CRs in the eye contralateral to the side of the lesion

might indicate that the animal is not perceiving the CS, or is experiencing attentional deficits. On the other hand, the occurrence of CRs in the contralateral eye would tend to preclude these possibilities.

(4) Two D animals, 1 ND animal, and 4 Shams were given some conditioning training using mild backshock (B+) as a CS. This stimulus consisted of 60-Hz ac electrical stimulation of 5-7 V delivered across safety-pin electrodes implanted subcutaneously on the animal's back, one on each side of the spine in the thoracic region, and 5-10 cm apart. The purpose of this procedure was to test whether or not CR-disruption would be evident when a tactile modality CS was employed. If disruption from the lesion was observed with a tactile CS, as well as visual and auditory CSs, then it would be extremely unlikely that the disruption was due to interruption of afferent sensory information.

Surgical procedures. Animals were given an initial injection of chlorpromazine (either 4 mg/kg, i.m. one hour before sodium pentobarbital, or 3 mg/kg, i.v., immediately before sodium pentobarbital), which tends to potentiate the anesthetic action of sodium pentobarbital. Sodium pentobarbital was administered via lateral ear vein (20-25 mg/kg), and this was followed by an i.m. injection of atropine (0.10 mg/kg). The animal's head was then shaved, Xylocaine was applied to the scalp, and the animal was positioned in a Kopf model 900 stereotaxic instrument equipped with a rabbit head holder. A midline incision was made in the scalp, the skull was exposed, and a trephine hole was made 10.0-14.5 mm posterior to Bregma (13.5-14.5 mm was optimal for disruptive DLP lesions). The skull was positioned such that Lambda was  $1.5 \pm 0.1$

mm lower than Bregma, and an electrode, made from a size 00 insect pin and insulated with enamel (except for 0.25-1.5 mm of tip), was lowered 13.0-14.5 mm ventral to the dura and 2.0-3.0 mm lateral to the midline on the animal's right side. The lesion was made with a Grass Model LM4 radio frequency lesion maker by increasing the current from 0 to 20 mA (about 30 V) over a 30 sec period. Control animals (SR and SA) were treated exactly the same, except that no current was passed through the electrode. The skull hole was then packed with Gel Foam, the scalp was sutured with 11 mm wound clips, and the animal was allowed 2-4 days of post-operative recovery before commencing with acquisition or retention training.

Deaths. Three lesioned and three sham-operated animals died shortly after surgery; their data are not reported. Another animal (Animal 12, LR) died after 4 days of post-surgical testing; the data for this animal were included in this report and the mean post-surgical percent CRs was based upon 4 (instead of 5) days.

Histology. When all behavioral tests were completed, the animals were perfused transcardially under deep sodium pentobarbital anesthesia. Isotonic saline was gravity fed into the heart, followed by 10% formaldehyde. The brain was immediately removed from the animal and stored in 10% formaldehyde for 3-4 days, followed by a 30% sucrose/formaldehyde solution for 7-10 days (until brain sank). The brains were then frozen sectioned in the coronal plane (40  $\mu$ m thickness), and every other section was mounted on slides using a gelatin mounting solution. The

sections were stained with cresyl violet acetate (0.5%); tissue coagulation and surrounding gliosis were used to delimit the boundaries of the lesion.

Most animals were sacrificed within 11-21 days of surgery. The following animals were allowed longer survival periods: Animal 3 (41 days), Animal 4 (58 days), Animal 10 (61 days), and Animal 26 (41 days).

### Results

The results of the acquisition and retention procedures, as well as the results of the contralateral conditioning session are summarized in Table 1. For the acquisition procedure, conditioning of the 5 sham-operated animals resulted in an average of 62 percent CRs to L+ and 67 percent to T+. Of the lesioned animals, 4 were classified as CR-Disrupted (D) (Animals 2, 4, 8, 10), and 3 as Nondisrupted (ND) (Animals 13, 15, 16). The ND animals were similar to Shams, giving 58 percent CRs to L+ and 70 percent to T+. However, the D animals gave 0 percent CRs to L+ and only 3 percent to T+. Note also in Table 1 the complete lack of overlap of the D animal distribution of scores with those of the ND and Sham animals as indicated by the ranges of the 3 groups. In addition, 2 of the D animals (Animals 4 and 10) were then allowed 5 more weeks of recovery, followed by additional L+/T+ training; these animals remained at close to 0 percent CRs. Although it remains to be systematically demonstrated whether or not recovery of CRs occurs over time, these observations suggest that recovery does not occur over a 60 day post-surgical period.

In the retention procedure, 6 of the 23 lesioned animals were

TABLE 1  
 PERCENTAGE CRs FOR DISRUPTED, NON-DISRUPTED, AND SHAM  
 ANIMALS IN ACQUISITION AND RETENTION EXPERIMENTS

	<u>D</u>		<u>ND</u>		<u>S</u>	
	T	L	T	L	T	L
ACQ						
Post $\bar{X}$	3	0	70	58	67	62
Range	0-8	0	46-85	20-82	48-78	32-80
SE	2	0	12	19	6	8
N	4	4	3	3	5	5
RET						
Pre $\bar{X}$	97	89	99	93	95	92
Range	94-100	71-97	99-100	83-100	92-100	81-99
SE	1	4	0	1	1	3
N	4	6	11	17	6	6
Post $\bar{X}$	4	3	93	91	96	93
Range	0-10	0-8	76-100	56-99	92-99	83-98
SE	3	1	3	3	1	2
N	4	6	11	17	6	6
CON						
$\bar{X}$	93	85	96	86	95	93
Range	82-100	60-100	74-100	40-100	84-100	84-100
SE	2	4	3	6	3	3
N	10	10	10	10	7	7

Abbreviations: D = Disrupted; ND = Non-Disrupted; S = Sham; T = Tone; L = Light; ACQ = Acquisition; RET = Retention; CON = Contralateral; SE = Standard Error. Tabled entries have been rounded off to whole percentages.



classified as D (Animals 1, 3, 5, 6, 7, 9), giving means of 3 percent CRs to L+ and 4 percent to T+ in post-surgical testing. The remaining 17 lesioned animals (Animals 11, 12, 14, 17-30) were ND with 91 percent CRs to L+ and 93 percent CRs to T+. The 6 Sham animals gave 93 percent to L+ and 96 percent to T+. Again note in Table 1 that the distribution of post-surgical scores of D animals does not overlap those of ND and Sham animals. In contrast to post-surgical scores, the pre-surgical response levels were quite similar for the 3 groups: D animals had 89% to L+ and 97% to T+, ND animals gave 93% to L+ and 99% to T+, and Shams gave 92% to L+ and 95% to T+. The pre-surgical scores were subjected to an arc sin transformation (Myers, 1972), and an analysis of variance of the pre-surgical scores indicated no differences among the groups in response to L+,  $F(2, 26) < 1$ . There were differences among the groups in response to T+,  $F(2, 18) = 4.67$ ,  $p < .025$ , but post-hoc tests indicated that the differences were due to a higher response level of ND animals compared to Sham animals,  $t(18) = 3.05$ ,  $p < .01$ .

The daily percent CR scores for animals in the acquisition and retention procedures are reported in Table 2 and Table 3, respectively. Table 2 shows percent CRs to L+ and T+ for lesioned and sham-operated animals over 5 days of training. Table 3 lists percent CRs for the last 3 days prior to surgery as well as 5 post-surgical test days. These tables illustrate that animals classified as disrupted displayed a consistently low level of conditioned responding.

Although CRs were nearly eradicated in the D animals, there were no cases in which URs of the D animals (or ND or Sham animals) were impaired. This is exemplified in Figure 2 (rows a and b) by the upward

TABLE 2  
DAILY PERCENT CRs FOR ACQUISITION (LA AND SA) ANIMALS

		POST-SURGICAL									
Animal		<u>L</u>	<u>T</u>	<u>L</u>	<u>T</u>	<u>L</u>	<u>T</u>	<u>L</u>	<u>T</u>	<u>L</u>	<u>T</u>
2	LA	0	0	0	22	0	0	0	12	0	6
4	LA	0	8	0	0	0	0	0	0	0	0
8	LA	0	0	0	0	0	0	0	0	0	0
10	LA	0	0	0	2	0	0	0	0	0	0
13	LA	0	4	100	94	98	100	92	100	74	100
15	LA	24	30	92	100	96	100	100	100	98	96
16	LA	0	0	4	2	0	32	14	96	84	100
55	SA	0	0	86	80	70	94	78	92	96	100
59	SA	0	2	2	8	94	94	98	98	100	96
61	SA	0	0	0	2	10	46	56	94	92	100
79	SA	10	4	92	94	98	96	100	98	100	100
80	SA	8	4	90	94	72	96	98	96	98	100

Abbreviations: LA = Lesion-Acquisition, SA = Sham-Acquisition,

L = Light, T = Tone.

TABLE 3  
DAILY PERCENT CRs FOR RETENTION (LR AND SR) ANIMALS

Animal	PRE-SURGICAL						POST-SURGICAL											
	L		T		L		L		T		L		T		L		T	
	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T
1 LR	70	96	86	100	86	96	0	0	22	30	14	22	0	0	0	0	0	0
3 LR	100	100	96	100	96	100	0	0	2	4	2	4	0	0	0	0	0	2
5 LR	94		100		97		0	0	0		0		2		0			
6 LR	93		99		93		0	1	1		3		7		20			
7 LR	54	92	76	96	84	94	0	0	0	0	0	0	0	0	4	0		0
9 LR	100	98	96	98	80	98	2	8	0	2	0	2	0	0	0	0	2	2
11 LR	90	100	92	100	86	98	100	98	100	98	100	100	92	100	100	96		96
12 LR	88	100	98	100	94	100	50	36	86	96	88	92	86	80	-	-		-
14 LR	90	100	88	96	100	100	96	94	98	100	100	98	94	96	94	96		96
17 LR	96		91		83		18	80	80		86		93		100			
18 LR	93		82		99		100		93		99		99		100			
19 LR	84	100	84	100	84	100	2	20	40	96	86	94	78	94	72	60		60
20 LR	96	100	100	98	92	100	90	100	100	100	100	100	100	100	94	100		100
21 LR	94	98	90	96	96	98	98	100	100	100	100	100	94	100	98	94		94
22 LR	100	100	90	100	96	98	98	100	98	98	98	98	96	96	100	100		100
23 LR	98		98		97		100		100		100		97		100			
24 LR	88	100	84	100	100	98	94	76	84	88	86	90	94	96	98	94		94
25 LR	92	98	94	98	98	100	96	96	100	100	100	98	98	100	100	100		100
26 LR	100	100	100	100	96	96	98	100	86	100	92	100	100	100	100	100		100
27 LR	100	98	100	100	100	100	92	96	100	98	100	98	96	96	100	100		100
28 LR	83		98		99		100		100		99		89		100			
29 LR	70		91		88		60		61		96		94		96			96
30 LR	100		99		88		94		98		98		98		100			100
54 SR	90	94	88	98	84	80	96	98	98	98	98	98	98	96	90	98		98
63 SR	66	88	86	96	90	94	90	82	82	98	92	94	64	98	86	96		96
64 SR	94	94	96	90	88	100	96	96	90	100	96	100	92	100	88	94		94
74 SR	98	100	92	100	96	100	88	90	100	98	100	98	98	100	100	96		96
75 SR	98	92	100	100	98	100	96	100	92	76	94	98	84	88	98	100		100
76 SR	90	82	98	94	100	100	100	100	96	100	100	100	98	98	94	96		96

LR = Lesion Retention    SR = Sham Retention    L = Light CS    T = Tone CS

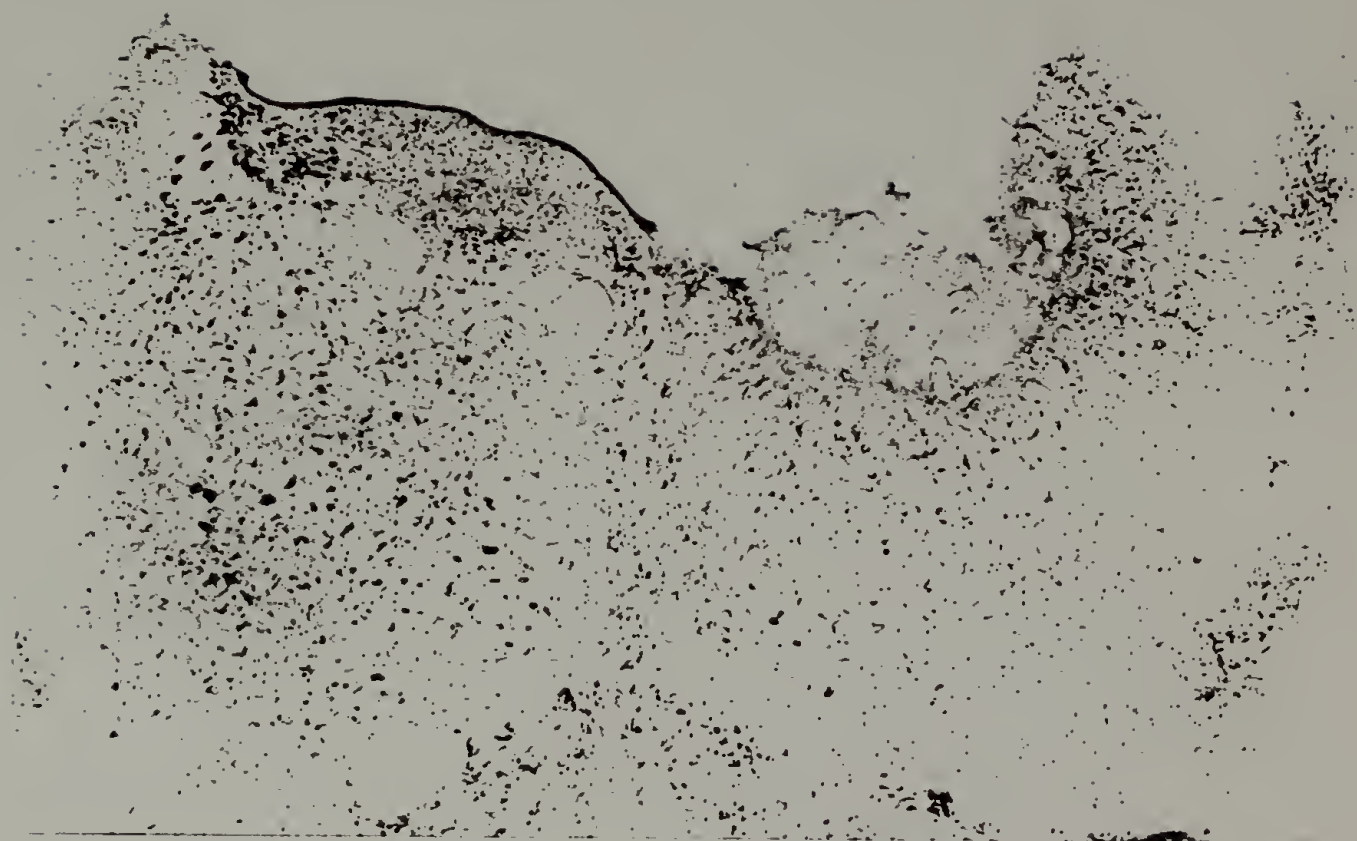


pen deflection that occurs after the US onset in the column marked POST. Note that the slight enhancement of post-lesion URs, seen in this figure, was not typically observed in D or ND animals. A second test of the UR was performed in which a mild tactile stimulus (using a cotton-tipped applicator) was applied to the cornea and/or surrounding regions; a full and robust extension of the nictitating membrane was observed in all animals. Moreover, a neurological examination of D animals indicated normal pupillary and eyeblink reflexes to a bright light, and normal equilibrium and locomotion. The animals gained weight normally, appeared healthy at all times, and were behaviorally indistinguishable from ND and Sham rabbits.

Figure 2 depicts representative oscillograph tracings of NMRs of D and ND animals before and after lesioning. Rows a and b are tracings from a D animal (Animal 7); note the presence of CRs before lesioning and the absence of post-lesioning CRs. The lesion for this animal is shown on the lower portion of Figure 3. Row c of Figure 2 shows tracings from an ND animal (Animal 17). The lesion for this animal is shown on the upper portion of Figure 3. The striking propinquity between disruptive and nondisruptive lesions is evident from the two photographs of Figure 3, which were taken at the same level in the brain stem. Row d of Figure 2 shows a tracing from a D animal in the LA group (Animal 2). A rare CR is evident in this tracing, but note the low amplitude and long latency of the response. CR topographies similar to this one were common for the D animals.

Thus, a total of 10 D animals from groups LA and LR displayed a profound impairment in their ability to produce CRs from the eye

Fig. 3. Photomicrographs of Nissl-stained transverse sections, both taken at the level of the parabrachial nuclei. The top photograph is from Animal 17 (ND group). The lesion (right side) includes central gray, and portions of the medial longitudinal fasciculus, locus coeruleus, and mesencephalic nucleus of N.V. The bottom photograph is from Animal 7 (D group). The lesion includes brachium conjunctivum, parabrachial nuclei, portions of locus coeruleus, and mesencephalic nucleus of N.V. The field of view for each photograph is 5 mm x 7 mm.



ipsilateral to the lesions (right eye). However, when the shock was switched to the left eye and CRs were recorded from both eyes, all 10 of the D animals showed robust conditioning of the left eye, as indicated in Table 1 (the right eye remained impaired); these animals gave 85 percent CRs to L+ and 93 percent to T+. A random sample of 10 ND animals were similarly tested and they responded at 86 percent to L+ and 96 percent to T+, and 7 Shams similarly tested gave 93 percent to L+ and 95 percent to T+. An analysis of variance of these data (subjected to arc sin transformation) failed to detect significant differences among these groups to both L+,  $F(2, 24) < 1$ , and T+,  $F(2, 24) < 1$ .

Note that robust conditioning of the left eye occurred in a single test session, even though shock had previously been administered only to the right eye. This contralateral transfer phenomenon has been observed by other researchers (Pearce, Montgomery, and Dickinson, 1981). Thus, despite the fact that CRs from the eye contralateral to the US are rare or absent during training of the ipsilateral eye, there is apparently some sort of plasticity occurring in brain regions mediating the contralateral CR, and this plasticity is responsible for the transfer of training effect.

Variations in the CS-US interval, using values of .25 sec. and .75 sec., did not appreciably affect the percentage of CRs in any of the groups. The results are summarized in Table 4. In addition, training with backshock as the CS was found to be ineffective in eliciting ipsilateral, but not contralateral, CRs from the D animals that were tested (Animals 4 and 10); in contrast, the ND animal (Animal 14) and the Shams (Animals 64, 74-76) that were given B+ training showed appreciable

TABLE 4  
 PERCENTAGE CRs IN DISRUPTED, NON-DISRUPTED, AND SHAM ANIMALS  
 USING CS-US INTERVALS OF 0.25 AND 0.75 SECONDS

	0.25 sec		0.75 sec	
	T	L	T	L
DISRUPTED				
$\bar{X}$	0	0	1	1
Range	0	0	0-4	0-2
SE	0	0	1	1
N	4	4	4	4
NONDISRUPTED				
$\bar{X}$	96	93	99	96
Range	88-100	73-99	95-100	81-100
SE	1	2	1	2
N	10	10	10	10
SHAM				
$\bar{X}$	93	88	96	93
Range	85-97	67-97	93-100	70-100
SE	1	3	1	3
N	11	11	11	11

Abbreviations: T = Tone, L = Light, SE = Standard Error. Tabled entries have been rounded off to whole percentages. Animals tested were as follows: Disrupted, Animals 4, 8-10; Non-Disrupted, Animals 11, 13-16, 20-22, 25, 27; Shams, all.



conditioning to this CS. The results are summarized in Table 5.

The histological reconstructions for D and ND animals are presented in Figure 4, and Table 6 describes anatomical landmarks present at each section level for this and subsequent figures. The histology for the acquisition and retention procedures is pooled in this figure because the locus of CR-disruption appeared to be identical for all D animals (e.g., compare LA Animal 4 with LR Animal 9). The relative volumes of lesioned tissue in the D and ND animal brains were estimated by cutting out the lesioned area from drawings of the brain sections, and then multiplying the total weight of the lesion cut-outs by the average distance between sections (Wolf and Gollob, 1980). The data obtained were positively skewed, so a log transformation was used. A point bi-serial correlation showed that disruption was positively correlated with lesion volume,  $r_{pb}=.52$ , and the correlation was found to be significant,  $t(28)=3.21$ ,  $p < .01$ . However, another measurement, the maximum lateral distance of the lesion from the midline, was also determined for each animal. Disruption was also found to be positively correlated with the lateral extent of the lesion,  $r_{pb}=.58$ , and this was significant,  $t(28)=3.76$ ,  $p < .01$ . Furthermore, lesion volume was positively correlated with lateral extent of the lesion,  $r=.60$ , and this too was significant,  $t(28)=3.97$ ,  $p < .01$ . Close inspection of Figure 4 reveals that there are some cases where disruption resulted from relatively small lesions (Animals 3, 4, and 9). Hence, it is quite possible that disruption is correlated with lesion volume simply because larger lesions tended to encompass critical lateral brain structures.

The latter possibility was evaluated by looking at various brain

TABLE 5  
 PERCENTAGE CRs FOR DISRUPTED, NON-DISRUPTED,  
 AND SHAM ANIMALS USING A BACKSHOCK CS.

<u>ANIMAL</u>	<u>TOTAL R. EYE TRIALS</u>	<u>% CRs</u>	<u>TOTAL L. EYE TRIALS</u>	<u>% CRs</u>
4 (D)	600	2	200	70
10 (D)	250	0	100	97
14 (ND)	250	95		
64 (S)	100	100		
74 (S)	100	90		
75 (S)	100	98		
76 (S)	200	59		

Abbreviations: D = Disrupted, ND = Non-Disrupted, S = Sham, R = Right,  
 L = Left.

Fig. 4. Histological reconstructions of lesions for animals in Disrupted and Nondisrupted groups. Selected transverse sections through the brain stem are depicted, and capital letters correspond to section levels. The distance of each section level rostral to the level of N.VI (measured along the neural axis) is given in parentheses. Letters with a prime symbol designate brain sections from Disrupted animals (these animals were numbered 1-10); letters without the prime designate Nondisrupted animals (these animals were numbered 11-30). Below are individual animal numbers corresponding to the brain sections (going from left to right), listed by section level:

A (9.8mm): 11, 12, 13  
 B (8.6mm): 11, 12, 13, 14  
 C (7.7mm): 11, 12, 14, 15, 16  
 D (6.9mm): 12, 15, 16  
 E (6.1mm): 12, 15, 16  
 F (5.2mm): 16  
 G (4.2mm): 16, 17, 18, 19, 20, 21, 22  
 H (2.8mm): 17, 18, 19, 20, 21, 22, 23, 24, 25, 27  
 I (2.2mm): 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30  
 J (1.5mm): 17, 18, 19, 21, 23, 24, 25, 27, 29, 30  
 K (0.7mm): 17, 19, 24, 25, 26, 27, 29, 30  
 L (0 mm): 26, 29, 30  
 E': 1, 2, 3, 4  
 F': 1, 2, 3, 4, 5, 6  
 G': 1, 2, 3, 4, 5, 6, 7, 8, 9, 10  
 H': 1, 2, 3, 4, 5, 6, 7, 8, 9, 10  
 I': 1, 2, 3, 5, 6, 7, 8, 9, 10  
 J': 2, 5, 6, 7, 8, 10  
 K': 2, 7





TABLE 6  
ROSTRAL DISTANCE AND LEVEL OF TRANSVERSE SECTIONS  
THROUGH BRAIN STEM FOR FIGURES 4, 5, AND 7.

<u>SECTION</u>	<u>DISTANCE (mm)</u>	<u>LEVEL</u>
1	0	N.VI
2	0.7	Caudal pole of motor nucleus of N.VI
3	1.5	N.V
4	2.2	Rostral pole of motor nucleus of N.V
5	2.8	Parabrachial nuclei
6	4.2	Caudal pole of ventral tegmental nucleus
7	5.2	Rostral pole of ventral tegmental nucleus
8	6.1	Caudal pole of nucleus of N.IV
9	6.9	Rostral pole of dorsal raphe nucleus
10	7.7	Caudal pole of red nucleus
11	8.6	Rostral pole of nucleus of N.III
12	9.8	Caudal pole of nucleus of Cajal

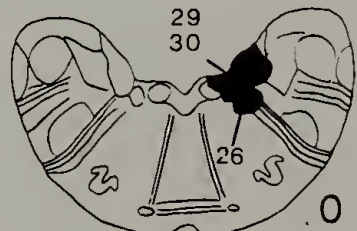
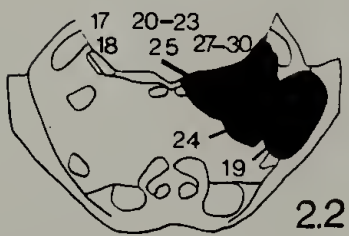
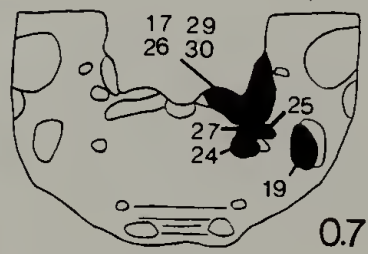
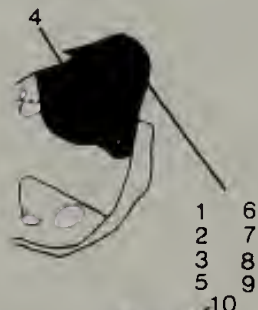
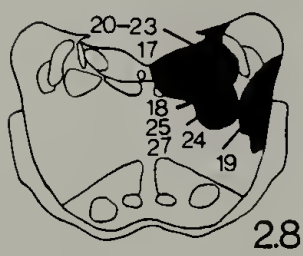
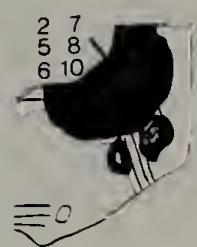
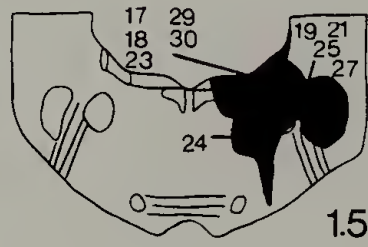
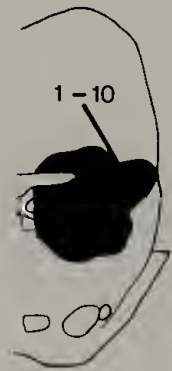
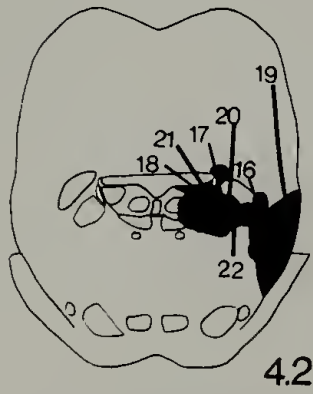
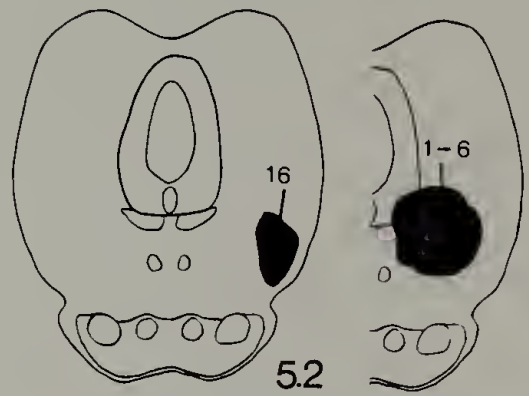
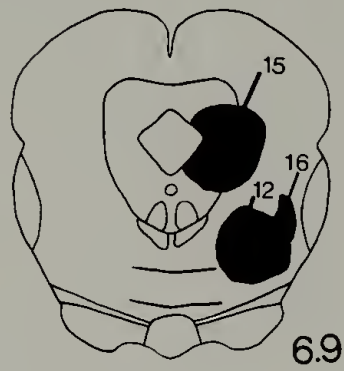
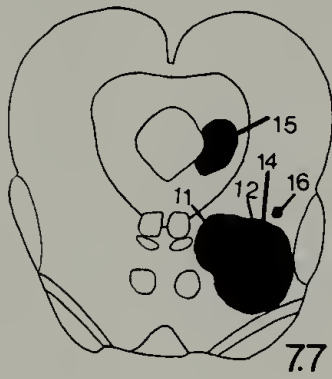
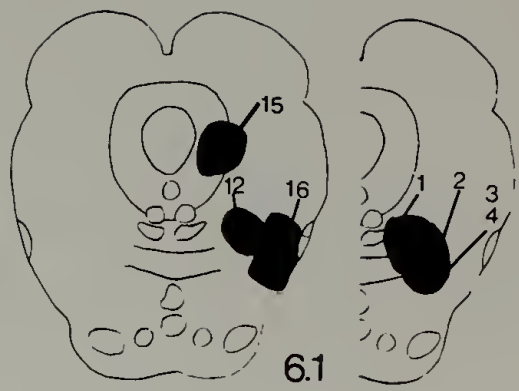
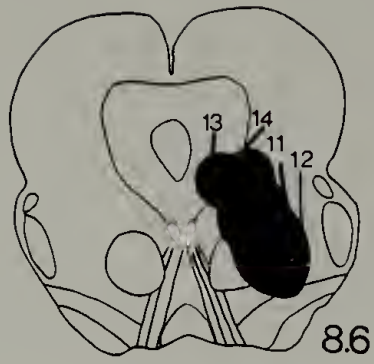
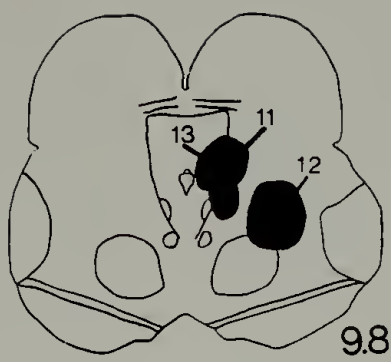
regions for each D and ND animal, and noting whether or not the lesion encroached upon these regions. A Fisher exact probability test (Siegel, 1956) was performed for each region. The results of this procedure indicated that the following structures tended to be lesioned in the D animals and spared in the ND animals: the brachium conjunctivum ( $p < .001$ ), locus subcoeruleus ( $p < .001$ ), lateral parabrachial nuclei ( $p < .001$ ), medial parabrachial nuclei ( $p < .001$ ), and the supratrigeminal region (Mizuno, 1970) ( $p = .005$ ). A significant difference was not found in the proportion of D and ND animals that were lesioned in other adjacent structures ( $p > .05$ ); these structures included periaqueductal gray, ventral tegmental nucleus, dorsal tegmental nucleus, nucleus reticularis pontis oralis, mesencephalic nucleus of N.V, locus coeruleus, periventricular gray, the medial longitudinal fasciculus, the lateral lemniscus, the motor nucleus of N.V, the principal sensory nucleus of N.V, nucleus reticularis pontis caudalis, and the medial vestibular nucleus.

Figure 5 depicts the union of ND animal lesions on whole sections and the union of D animal lesions on adjacent hemisections. This figure reveals the lateral portion of the brain stem common to D animals, particularly in the sections representing the brain stem at 1.5-4.2 mm rostral to N. VI. Note in this figure (as well as in Figure 4) that the most rostral point where D animal lesions are found is about 6.1 mm rostral to N. VI, and the most caudal point is about 0.7 mm rostral to N. VI.

### Discussion

To summarize, ipsilateral lesions of the dorsolateral brain stem,

Fig. 5. Selected transverse sections through the brain stem showing pooled area of tissue loss following lesions of the right side of the brain stem. Nondisrupted cases are depicted on whole sections. Disrupted cases are depicted on hemi-sections. Numbers to the lower right-hand portion of whole sections refer to distance (mm) rostral to the level of the abducens nerve. Other numbers designate individual animals.



1mm



while not affecting URs, eradicated CRs to visual, auditory, and tactile CSs in the ipsilateral, but not contralateral eye. These data suggest that disruption is not a result of motor or sensory deficits involved in either responding to or perceiving the relevant stimuli. In addition, contralateral conditioning tends to preclude attentional deficits as an explanation of the effect. Furthermore, variations in the CS-US interval failed to ameliorate response levels in D animals, suggesting that disruption is not dependent upon any one specific temporal arrangement of the CS and the US, nor is it a result of an increase in the reaction time of the animal.

It therefore, seems reasonable to conclude that the neural information which is involved in the production of the conditioned NMR perhaps originates in the DLP or is relayed through it (e.g., from cerebellum via brachium conjunctivum), and that the disruptive lesions interrupted this information. However, one should keep in mind the limitations of the lesion technique in evaluating this conclusion. Specifically, a number of secondary effects can result from lesions, including vascular disruption and ischemia, transneuronal degeneration, alteration of neurochemical pools, denervation supersensitivity, sprouting, and diaschisis (Schoenfeld and Hamilton, 1977). Thus, secondary changes remote to the actual site of the lesion may in fact be responsible for the observed behavioral results.

The contributions, if any, of these secondary changes to the results of the present study are largely unknown. One might speculate that transneuronal degeneration was not a factor because disruption occurred immediately after lesioning and the time course of transneuronal



degeneration is probably weeks (Schoenfeld and Hamilton, 1977); however, the phenomenon is not adequately understood, so such reasoning may be inappropriate. Hence, supporting evidence was sought to evaluate whether or not the DLP contained neural elements essential for the CR. In Experiment Two, low impedance tungsten electrodes were chronically implanted into the brain stem of rabbits and multiple-unit activity (MUA) was recorded during the conditioning procedure. The rationale for this experiment was that a region of the brain critical for CR production might display changes in baseline electrical activity when the behavioral CR occurred.

## CHAPTER III

### EXPERIMENT TWO

#### Method

Animals. The animals in this experiment were 20 male and female New Zealand albino rabbits obtained from a local supplier. They were housed and fed as described in Experiment One.

Procedure. The surgical procedures employed were identical to those in Experiment One except that instead of giving the animals a lesion, Epoxylite-coated tungsten monopolar electrodes (Frederick Haer & Co.) were implanted into the right brain stem (7 rabbits, Animals 109, 112, 115, 119, 123, 125, and 126, were implanted bilaterally in the brain stem. Thus, data from 27 electrodes are reported.). The electrodes were of low impedance (80-100 K $\Omega$ ) and had tips 25-60  $\mu$  m in length and 5-10  $\mu$  m in diameter. Three jewelers' screws were fastened into the skull, one of which served as the indifferent electrode while the other 2 served as anchors. Dental cement was applied to the skull to hold the electrode in place. When the cement hardened, the scalp was sutured with 00 nylon suture; the electrode and indifferent wires protruded externally from the animal's head. The animals were allowed 2-5 days of recovery from surgery before training.

All conditioning and testing was conducted one animal at a time in a sound-attenuated and electrically shielded chamber which contained 2 Grass P15 low noise, battery powered differential ac preamplifiers (so

that two brain regions could be recorded simultaneously), a Grass SIU8 stimulus isolation unit, a Wagner ground circuit, and a Grass CCU1 constant current unit. The SIU and CCU units were used in conjunction with a Grass S88 stimulator to deliver dc shock pulses to the periocular region of the rabbit's eye during conditioning training. The forward panel of the chamber contained 2 loud speakers and a light source to provide CSs and continuous masking noise. All cables going into or out of the chamber were shielded and grounded to the screening of the chamber. The animal and metal components of the recording arrangement were similarly grounded. Programming equipment for conditioning was located in an adjacent room. Neural activity was filtered from 300 Hz to 10 kHz, amplified, and displayed on a Tektronix 502A oscilloscope. Photographs of neural activity were taken with a Tektronix oscilloscope camera C-27 and Polaroid 3000 speed black and white Land pack film. A parallel circuit from each P15 preamplifier provided input to Grass 5P3 integrator preamplifiers (calibration:  $200 \mu\text{V}/\text{cm}$ ) so that neural activity could be displayed on a polygraph along with the NMRs.

Prior to conditioning, each animal received a very mild stimulus to the periocular region of the right and left eyes in order to determine if an evoked response was elicited in the brain stem. This was accomplished with square wave single-pulse electrical stimuli of 20-40 V dc and .1 - .2 ms duration provided by a Grass S88 stimulator, an SIU, and a Wagner ground circuit (see Becker et al., 1961). The stimulus was delivered via wound clips attached to the tissue as described previously. The polarity of stimulation was reversed to insure that responses observed were not artifactual. The stimulation was sufficient to produce

a twitch or slight blink of the eye. The presence of shock artifact necessitated the use of brief, low voltage pulses, and the Wagner ground circuit was also useful in reducing the stimulus artifact. Evoked responses were displayed on the oscilloscope and photographed for subsequent analysis.

The animals were then given L+/T+ conditioning as in Experiment One. The only differences in procedure were as follows: (a) An ITI of 30 sec instead of 15 sec was used; (b) White masking noise of 65 dB SPL was constantly presented from a speaker directly in front of the animal; (c) The nictitating membranes of both eyes were sutured so that CRs could be recorded from both; and (d) The US consisted of a 50 ms train of dc square wave pulses (60 pulses per sec, pulse duration = 8.3 ms, 1.0 - 2.5 mA) to the periocular region. The US was generated by a Grass S88 stimulator and associated stimulus isolation and constant current units.

In general, each animal received a sufficient number of L+/T+ trials, with the US applied to the right eye, to produce robust and consistent CRs. The presence or absence of an increase in MUA concurrent with the production of a CR was noted. A range of 100-500 L+/T+ trials ( $\bar{X}$  = 275) was required for this portion of the experiment. The animals which displayed an increase in MUA during CRs were then given extinction trials (L-/T-) to determine if the MUA extinguished along with the behavior. A range of 20-245 L-/T- trials ( $\bar{X}$  = 97) were required to satisfactorily make this determination. Animals which had bilateral implants were then given L+/T+ training to the contralateral eye to determine if conditioned MUA occurred in the contralateral electrode.

There were two exceptions to this general procedure. First, Animals 34 and 126 were not naive when the electrodes were implanted. They had previously been trained on L+/T+. Second, Animals 41-44, which had only one electrode implanted in the right brain stem, were given L+/T+ with the US administered initially to the left eye. This variation was introduced to determine if left eye CRs resulted in conditioned MUA in the right brain stem. These animals were then switched to L+/T+ with the US applied to the right eye, followed by extinction if conditioned MUA was observed. The 7 bilaterally implanted rabbits were also used to determine if unilateral CRs resulted in contralateral conditioned MUA.

Two types of control procedures were employed:

(1) Pseudoconditioning. Animals 109, 112, 115, 119, 123, and 125 received 300 pseudoconditioning trials (100 trials/day) prior to any L+/T+ training. Pseudoconditioning trials consisted of presentations of eyeshock alone every 30 sec with L- or T- occurring 10, 15, or 20 sec after each eyeshock. The sequence of light and tone was random, but not more than 2 consecutive presentations of the same stimulus were allowed. The same provisions were true of the sequence of shock-stimulus intervals, i.e., L- or T- could follow eyeshock after either 10, 15 or 20 sec, but the same time value could not occur more than twice in a row. Thus, the pseudoconditioning procedure employed was one where the CSs and USs were explicitly unpaired. The US was always administered to the eye that was to be reinforced in subsequent L+/T+ training. One non-naive animal, Animal 126, received 20 L+/T+ trials to establish the fact that it was conditioned, and then it received 100 pseudocondi-



tioning trials. The purpose of this variation was to test whether or not CRs and concomitant MUA would persist under nonassociative presentations of the CS and the US.

(2) Conditioned inhibition. Animals 43-45, 94 and 109 received 300-1400 ( $\bar{X} = 1008$ ) conditioned inhibition (CI) trials subsequent to L+/T+ training and extinction. Conditioned inhibition consisted of light trials paired with the US (L+) and compound light and tone trials not paired with the US (LT-; thus CI is also abbreviated L+/LT-). The two trial types were presented in a random order and, again, the same stimulus was not allowed to occur more than twice in a row. A sufficient number of CI trials were presented so that differential conditioned responding developed, i.e., presence of CRs on L+ trials and absence of CRs on LT- trials. The animals typically received two 100 trial sessions of CI training per day, with each session separated by 3-6 hours. This procedure has been found to be efficient in developing CI (e.g., Allan, Desmond, Stockman, Romano, Moore, Yeo, and Steele-Russell, 1980). Conditioned inhibition requires the animal to suppress CRs to a compound CS, so it provides an alternative control procedure for assessing any nonassociative factors contributing to the MUA.

After conditioning, extinction, and CI training was completed, the animals were given monopolar electrical stimulation to the brain (ESB) using the recording electrode as the cathode and the indifferent electrode (jeweler's screw) as the anode. Stimulation was provided by a Grass S88 stimulator along with stimulus isolation and constant current units located outside of the conditioning chamber. Stimulation



consisted of a 50 ms train of pulses presented at a frequency of 500 Hz. Pulse duration was 0.1 or 0.25 ms and current ranged from 30-700  $\mu$ A. The SIU was capacity coupled during stimulation to reduce electrode polarization. The initial current was always 30  $\mu$ A, and this was increased until either some behavioral effect was observed, or until current surpassed 700  $\mu$ A. The behavioral effect of ESB was noted for each animal, and, if an NMR was elicited, the results were recorded by polygraph or photographed off the oscilloscope for the purpose of estimating latency of response.

After completion of all experiments, the animals were transcardially perfused as described in Experiment One. The electrodes were removed from the brains by first carefully cutting the skull surrounding the dental cement pedestal, and then lifting the pedestal and electrodes out of the brain. Histology was performed as described for Experiment One. Electrode tip positions were easily located because the electrodes were in the brain for at least 1-4 weeks prior to sacrifice, and dark-staining gliosis formed around the tip.

### Results

Three characteristics were assessed for each electrode implant. These were: (a) The presence or absence of an evoked response when an ipsilateral eyeshock pulse was delivered; (b) The presence or absence of a conditioned increase in MUA during the production of a behavioral CR; and (c) The presence or absence of an ipsilateral NMR in response to electrical stimulation through the electrode.

Evoked Response. A total of 19 electrodes recorded evoked-responses

in the pontine brain stem resulting from single-pulse shock to the peri-ocular region. Response latencies were measured from stimulus onset to peak amplitude of the response. Figure 6 illustrates evoked responses obtained from Animal 109; this figure shows simultaneous recordings from the right and left brain stem following brief eyeshock pulses, and arrows indicate the earliest response peaks. The latency of response ranged from 1.4-33 ms ( $\bar{X}$  = 6.53, S.D. = 7.43, N=19); the results are given in Table 7. In most cases, stimulation of the eye contralateral to the recording electrode also resulted in an evoked response; latencies ranged from 1.5-35 ms ( $\bar{X}$  = 6.16, S.D. = 7.97, N=18). These data are also presented in Table 7. Of interest is the fact that in some cases the response latency from contralateral stimulation was less than that resulting from ipsilateral stimulation.

Figure 7 depicts the position of the electrodes as reconstructed from Nissl-stained sections. The presence of an "r" on the left side of the animal number indicated that an evoked response was recorded from the electrode corresponding to that number. The data from Animal 115 (from both right and left DLP electrodes) were excluded from analysis because of an evident lack of independence between the waveforms from the two electrodes. Thus, a question mark appears on the left side of the animal number in Figure 7.

Conditioned MUA. Each animal was trained on L+/T+ until consistent and robust CRs to both CSs were observed. When this was accomplished, each electrode was classified as either a "hit" or a "miss." A hit was defined by the occurrence of an increase in MUA that paralleled the

Fig. 6. Evoked responses, recorded from brain stem electrodes in Animal 109, to single-pulse periocular stimulation of 0.1 ms duration and 25 V. Top trace of each pair is from the electrode in the left side of the brain stem, and bottom trace is from the right side electrode. In column 1, periocular stimulation was to the left eye, and in column 2, to the right eye. Row B is the same as row A, except that the polarity of the eyeshock leads was reversed. Arrows indicate the first evoked response for each trace. Note the absence of an evoked response from the left brain stem in column 2; a response was subsequently evoked at a higher stimulation voltage (not depicted). Vertical calibration: 100  $\mu$ V/cm; horizontal calibration: 1 ms/cm. Negative is up.

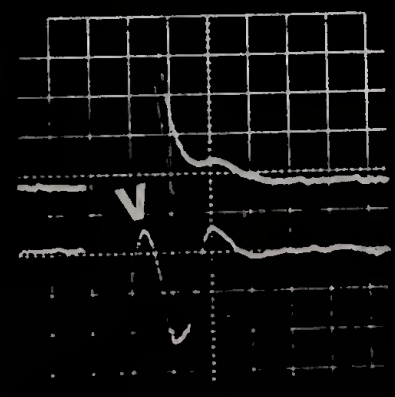
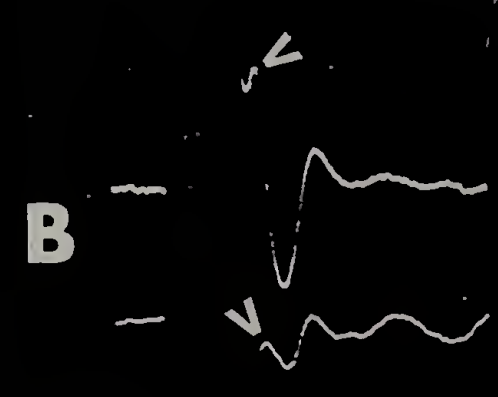
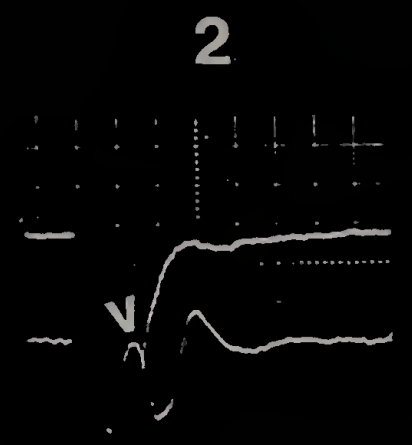
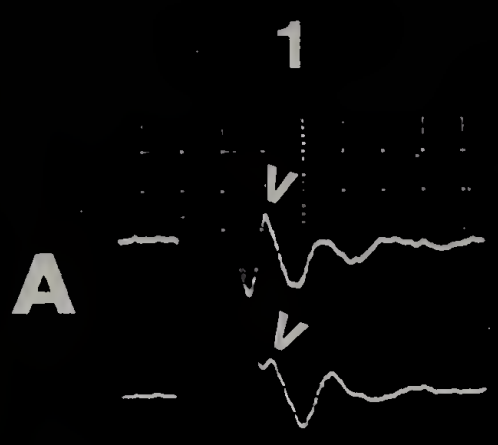


TABLE 7

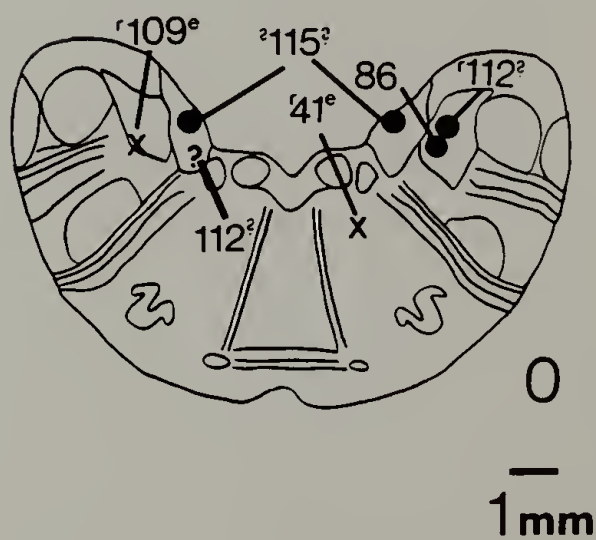
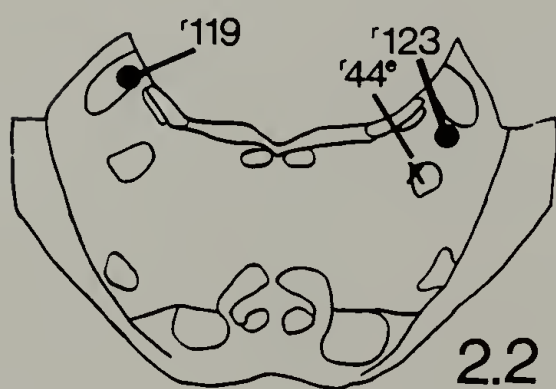
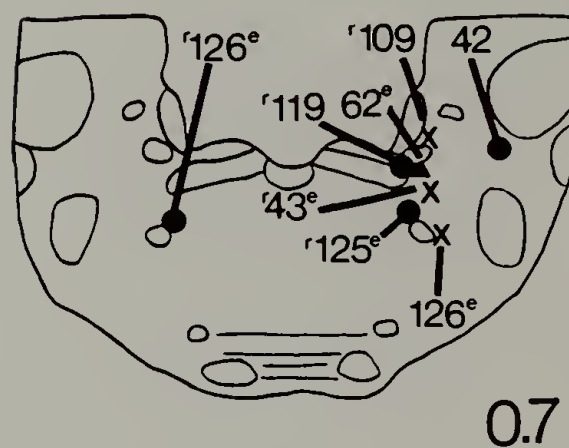
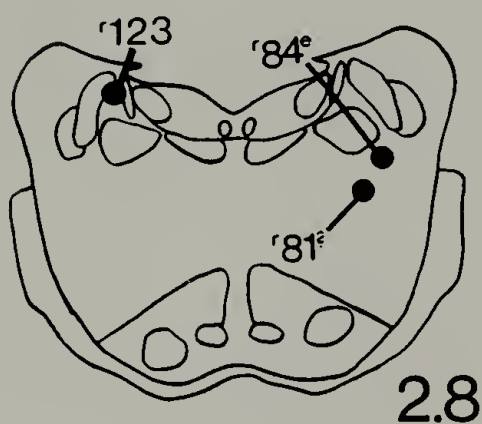
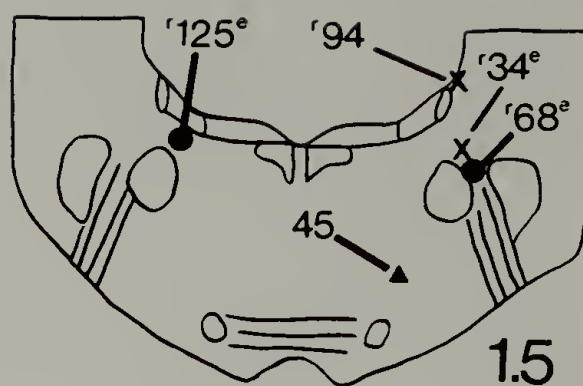
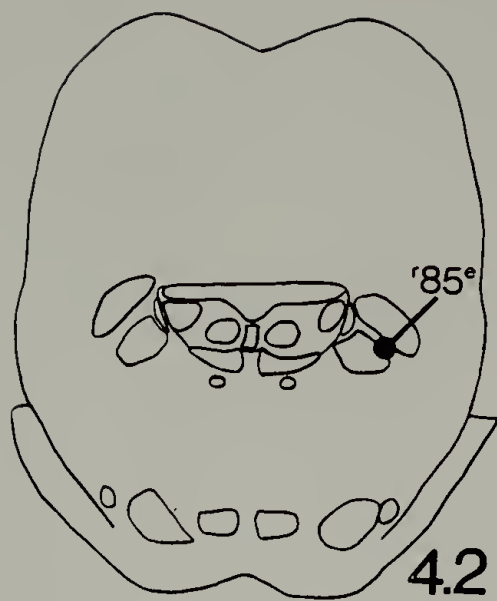
ELECTRODE CLASSIFICATIONS, ELECTRICAL BRAIN STIMULATION CURRENTS  
PRODUCING AN NMR, AND LATENCIES OF EVOKED RESPONSE TO  
PERIOCLAR STIMULATION FOR ALL ELECTRODES IMPLANTED

<u>ANIMAL</u>	<u>ELECTRODE</u>	<u>CLASS</u>	<u>MIN ESB</u>	<u>ER LAT (IPSI)</u>	<u>ER LAT(CONTRA)</u>
34	RBS	H	500 $\mu$ A	1.4 ms	1.5 ms
41	RBS	H	30	14.4	---
43	RBS	H	30	2.1	1.5
44	RBS	H	700	2.3	1.4
45	RBS	WH	---	---	NA
62	RBS	WH	30	---	NA
94	RBS	H	---	6.6	8.5
109	RBS	H	---	1.5	2.4
109	LBS	H	30	2.1	2.5
126	RBS	H	385	---	13.9
42	RBS	M	---	---	---
68	RBS	M	110	10.3	7.5
81	RBS	M	NA	3.5	2.1
84	RBS	M	30	6.6	10.3
85	RBS	M	30	2.5	2.5
86	RBS	M	---	---	---
112	RBS	M	NA	33	---
112	LBS	NA	NA	---	35
115	RBS	M	NA	NA	NA
115	LBS	M	NA	NA	NA
119	RBS	M	---	3.1	2.3
119	LBS	M	---	3.9	2.0
123	RBS	M	---	2.6	2.8
123	LBS	M	---	2.0	1.6
125	RBS	M	30	2.7	2.0
125	LBS	M	30	9	11
126	LBS	M	210	14.4	---

Abbreviations: RBS = Right Brain Stem; LBS = Left Brain Stem; H = Hit; WH = Weak Hit; M = Miss; Min ESB = Minimum electrical brain stimulation current that elicited an NMR response; ER Lat (IPSI) = Latency of first evoked response to ipsilateral periocular stimulation; ER Lat (CONTRA) = Latency of first evoked response to contralateral periocular stimulation; NA = Not available.

Fig. 7. Selected transverse sections through the brain stem showing location of recording electrode tips. Hits are designated by "X"'s, and misses are designated by filled circles. Two of the hits were weak hits, and these are designated by filled triangles. Numbers to the lower right-hand corner of brain stem sections refer to distance (mm) rostral to the level of N.VI. Other numbers designate individual animals. A lower-case "r" to the left of an animal number indicates that an evoked response to an eyeshock pulse was recorded from the electrode. A lower-case "e" to the right of an animal number indicates that ESB through the electrode elicited eyeblink, globe retraction, and a nictitating membrane response.



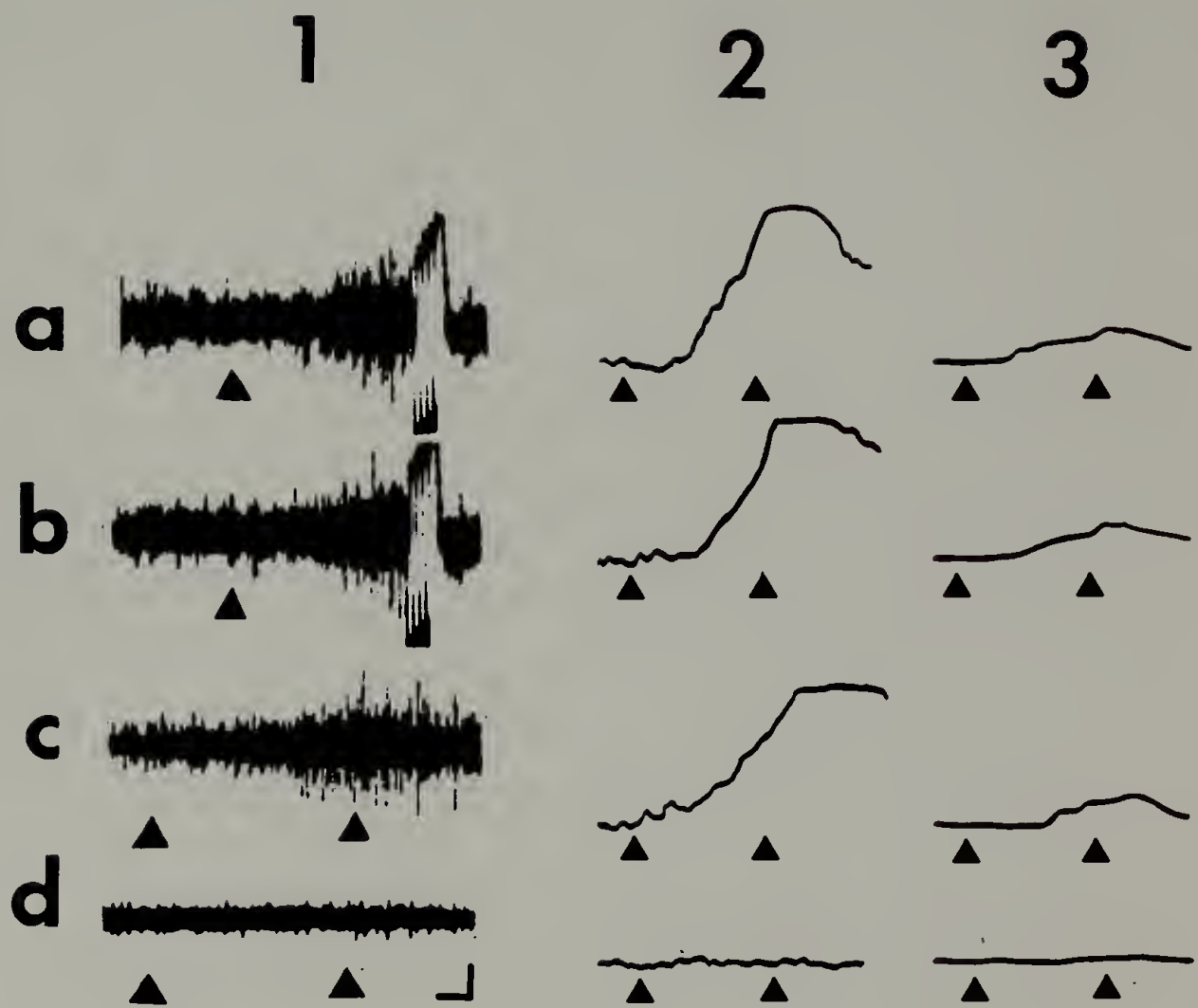


ipsilateral CR. This increase in MUA, moreover, had to be observed for both L+ and T+ CRs. For each electrode, the peak to peak amplitude of baseline MUA just prior to CS onset, and the amplitude of MUA during the production of a CR were measured from representative photographs of raw MUA. The formula,  $(CMUA - BMUA)/BMUA \times 100$ , where CMUA = the peak to peak amplitude of conditioned MUA during the production of a CR, and BMUA = the peak to peak amplitude of baseline MUA, yielded a percent increase in MUA resulting from conditioning. Electrodes classified as hits recorded a range of 50-200 percent increase in MUA during CRs. Misses were defined as electrodes which recorded a 0 percent increase in MUA during CRs.

Of the 27 electrodes implanted into left or right brain stem, 10 of the electrodes were classified as hits. These electrodes recorded a mean increase in MUA of 121% (S.D. = 56%) during the presence of CRs. Two of these hits displayed only a 50% increase in MUA, so these are referred to as weak hits. Furthermore, the conditioned increase in MUA extinguished simultaneously with the behavioral CRs. An illustration of typical oscilloscope tracings of MUA, along with the integrated MUA and the NMR, during conditioning is shown in Figure 8. The data in this figure were taken from Animal 34. Note the significant increase in activity that occurs to both L+ and T+ trials and how the activity extinguishes with the behavior. This is evident in both the raw and the integrated MUA.

Inspection of the polygraph records indicated that the onset of the increase in MUA preceded the CR onset by 10-40 ms for all the electrodes classified as hits, except for Animal 94. This animal displayed

Fig. 8. Multiple unit activity and NMR tracings for Animal 34. Column 1 illustrates typical raw multiple unit activity for Animal 34. A conditioned increase in neural activity can be observed for a T+ trial (a), and an L+ trial (b). Early (c) and later (d) extinction training to L- shows gradual diminution of activity. Columns 2 and 3 represent integrated multiple unit activity and the NMR, respectively, for the same trials as those in column 1. The first triangle always denotes CS onset. In columns 2 and 3, the second triangle denotes US onset. In column 1, US onset is indicated by shock artifact in a and b; the second triangle in c and d denotes CS termination. Calibration for column 1 only is 100 ms, 20  $\mu$ V.



conditioned MUA that was mostly concurrent with or lagging behind the CR. The latency measurements were estimated by measuring the points of pen deflection for both the NMR and the integrated MUA, using a mm ruler, and then converting these measurements into time (chart speed of the polygraph was 100 mm/sec). There was considerable variation in the lead of the MUA over the CR, even within a single conditioning session. On some trials there even appeared to be no lead, or the MUA lagged behind the CR; however, this was not consistent except in the case of Animal 94.

There were a total of 4 animals (Animals 43, 44, 109, 126) which supplied data addressing the question of whether or not conditioned MUA is observed when CRs contralateral to the electrode are produced. The following was observed in Animals 43, 44, and 109 (right and left side): (a) CRs were made on the side contralateral to the electrode, but not on the ipsilateral side, yet the electrode recorded conditioned MUA; (b) When CRs were made on the ipsilateral side (by switching US to the ipsilateral side) conditioned MUA was also observed, and it was of greater amplitude than that obtained from contralateral CRs.

Animal 126 was an interesting case. This animal first received right eye L+/T+ training; conditioned MUA was observed in both right and left brain stem electrodes (greater amplitude observed in right brain stem), but only right CRs were observed. When the shock was switched to the left eye, there were some trials on which a left CR was present and a right CR was absent, and on these trials conditioned MUA was not observed in either electrode. Hence changes in electrical activity were coupled only to the right eye CR for both electrodes.

The 6 animals which received pseudoconditioning training prior to L+/T+ did not display pseudoconditioned CRs nor did they show any consistent changes in MUA during the presentation of the CS. Only Animal 109 was classified as having a hit electrode (in both the right and left brain stem) when subsequent L+/T+ training was administered. In addition, Animal 126 received pseudoconditioning training after it was already established that the animal had a hit electrode. The results of this procedure were that CRs and conditioned MUA extinguished within 25 pseudoconditioning trials.

The 5 animals (Animals 43-45, 94, and 109 -- all with hit electrodes) which received CI training learned to produce a CR in the presence of L+ and to inhibit a CR in the presence of LT-. The conditioned MUA followed the same pattern as the CRs, i.e., MUA increased on L+ trials and did not increase on LT- trials.

Figure 7 shows the location of the 10 hit and 16 miss electrodes (the left DLP electrode for Animal 112 was not classified because the electrode wire broke off before conditioning was obtained). The hit electrodes, indicated by "X"s, were located no more than about 2.2 mm rostral to N. VI and about 3 mm lateral to the midline. Many of the hit electrodes were clustered either around or dorsal to the motor trigeminal nucleus. Four miss electrodes were located in lateral and medial vestibular nuclei (Animals 86, 112, 115). Other miss electrodes were clustered around more medial regions of the motor trigeminal nucleus (Animals 119 right, 125 left and right, 126 left) and in and around the brachium conjunctivum (Animal 84, 85, 119 left, and 123 left and right).



ESB. The administration of ESB through 22 implanted electrodes resulted in the elicitation of ipsilateral eyeblink, globe retraction, and extension of the nictitating membrane in 13 cases: Animals 34, 41, 43, 44, 62, 68, 84, 85, 125 left and right, and 126 left and right; ESB to the left side electrode of Animal 109 produced bilateral eyeblink, globe retraction, and NMR. There were 6 electrodes which, when stimulated, produced no observable effect on the animal (Animals 94, 109 right, 119 left and right, and 123 left and right). One animal, Animal 42, displayed bilateral eyeblinking and NMRs, kicking, and head movement. Two animals did not display any eye reflexes but did show other behaviors: Animal 45 displayed forceful kicking of the back legs and tensing of back muscles, and Animal 86 displayed lateral head movement. There were 5 electrodes in which data were unobtainable (Animals 115 left and right, Animal 112 left and right, and Animal 81). It should be noted that ESB produced slight head movement in Animals 43 and 44, some lower jaw movement in Animal 44, and slight upper lip movement in Animal 84.

An estimate of the latency of NMR onset, measured from the time of ESB onset, was available for 12 of the 13 animals. The measurements were obtained either from polygraph records or from photographs from the oscilloscope. The mean latency of NMR onset was 51 ms (S.D. = 13 ms, range = 25-70 ms).

The presence of a lower case "e" on the right side of the animal number in Figure 7 indicates that ESB elicited an NMR from the electrode. Animal 42 was not designated as such because the eye reflexes were accompanied by a considerable amount of gross movement.

In addition, Table 7 displays the minimum current necessary to elicit the NMR. ESB was effective in eliciting an NMR in regions of the brain stem as much as 4.2 mm rostral to N.VI, and effective sites appear to be around the motor trigeminal nucleus and near locus subcoeruleus.

### Discussion

Single-pulse stimulation to the periocular region resulted in fluctuations of field potentials in the vicinity of the recording electrode in a number of cases. These fluctuations, which were time-locked to the occurrence of the eliciting stimulus, are referred to as the evoked response to the stimulus (John, 1973). Such field potentials are produced by current sources and current sinks occurring in the vicinity of the recording electrode. Each individual source or sink is referred to as generator. In the analysis of field potentials, a principle of superposition holds. This refers to the fact that the potential observed at any point due to a population of generators is equal to the algebraic sum of the contributions from each individual generator. These generators consist of familiar phenomena such as pre- or post-synaptic depolarizations (or hyperpolarization) and propagating action potentials (Nicholson, 1979).

Thus, from a fixed recording electrode, the information available is the sum of the generator potentials, and from this information alone it is impossible to deduce the individual components. Additional information is required, such as the geometry of the neural elements under study, along with depth recordings through the relevant structures. Hence, limited information is available from the evoked response data,

presented above, with regard to identifying the precise location(s) of the generator(s) responsible for the field potentials.

However, what can be said about these data is that neural information concerning the US is relayed to dorsolateral regions of the brain stem, regions where conditioned MUA was detected. Such information concerning the US would be necessary for cells involved in conditioning. In many cases the latency of the evoked response was within 4 ms, and from this latency one can infer that the neural information is relayed to the recording site in 2-4 synapses. Perhaps the most thorough investigation of field potentials is attainable through current-source density analysis, in which an array of electrodes can essentially determine whether or not sources or sinks exist within a small circumscribed brain region. Remote sources and sinks do not contribute to such an analysis, whereas they do contribute in simple measurements of field potentials. However, identification of the precise nature of these sources and sinks still requires additional information concerning cell geometry (Nicholson, 1979).

Multiple-unit recording from the pontine brain stem during classical conditioning revealed a zone approximately 3 mm from midline, approximately 0.7-2.2 mm rostral to N.VI, and primarily dorsal to the motor trigeminal nucleus, where a conditioned increase in MUA was observed during the presence of a CR. The acquisition of the conditioned MUA developed with the acquisition of the CR, and extinction of the CR and conditioned MUA was concurrent. Furthermore, two control procedures, pseudoconditioning and conditioned inhibition, provided evidence that the increase in MUA was an associative learning phenomenon

reflecting pairing of the CS and US.

One possible explanation of the conditioned increase in MUA is that cells of the DLP receive converging neural information from the CS and the US. In addition, DLP cells are hypothesized to drive the motoneurons. Prior to conditioning, presentations of the CS do not change the activity of DLP cells. However, pairing of the CS and the US results in a change in the response of DLP cells to the CS such that the CS now produces an increase in neural activity, resulting in a CR. The plasticity that develops could be due to postsynaptic changes, e.g., alterations in membrane conductance of DLP cell dendrites, or to presynaptic changes, e.g., alterations in amount of transmitter released from presynaptic terminals (see Kandel, 1976).

Although it was sometimes difficult to precisely determine the onset of the CR with respect to the onset of the conditioned MUA, the latter tended to lead the CR by 10-40 ms on the average. There was often considerable variability in the latency relationship between the conditioned MUA and the CR over trials within an individual animal. For example, in a random sample of 20 trials from a session in which robust CRs were displayed, the onset of integrated conditioned MUA lead the onset of the CR by a mean of 21 ms for the right side electrode of Animal 109 (S.D. = 17 ms, range = -30 to 45 ms). Similar measurements were obtained from Animal 44 ( $\bar{X}$  = 16 ms, S.D. = 22 ms, range = -20 to 70 ms), and Animal 45 ( $\bar{X}$  = 32 ms, S.D. = 41 ms, range = -70 to 80 ms).

Such trial by trial variability could be due to any of a number of factors. One factor is that the electrode was not entirely within the population of cells responsible for the conditioned MUA. In this

case, the rate at which discharges from cells were recruited would affect the latency relationship of the conditioned MUA and the CR. A second possibility is that the electrode was in a mixed population of cells in which activity in one subset of cells (Set A) was responsible for the CR, and activity in another subset of cells (Set B) was not. If Set-B cells fired in the presence of the CS, but inconsistently, then the onset of the conditioned MUA with respect to the onset of the CR would vary. This would be particularly true if Set-B cells had lower discharge thresholds than Set-A cells. Variations in the momentary state of the organism could determine whether or not Set-B cells discharged in the presence of the CS.

Evidence from 3 animals suggest that conditioned MUA occurred not only in brain stem regions ipsilateral to conditioned eye, but in the contralateral brain stem as well. In addition the amplitude of conditioned MUA obtained from contralateral conditioning in these cases was less than that obtained from ipsilateral conditioning. The presence of contralateral conditioned MUA may constitute a physiological substrate for the transfer of training effect discussed in Experiment One. Thus, a left eye CR brought about by pairing the CS with a left eye shock (US), could produce conditioned MUA in the right brain stem such that the increased activity is not sufficient to drive a right eye CR, but is sufficient to facilitate subsequent conditioning of the right eye when the US is switched to the right eye.

Animal 126 was interesting because the electrode on the left side of the animal's brain, medial to the motor nucleus of N.V, displayed conditioned MUA only to the contralateral CR. This electrode may have



been located at a point where conditioning information is relayed from the right side of the brain to the left side.

ESB administered via recording electrodes produced, in many cases, a robust ipsilateral NMR. It is not likely that accessory abducens neurons were directly stimulated because 30  $\mu$ A (the mode ESB current) pulses stimulate fast-conducting fibers only within a 500  $\mu$ m radius of the electrode tip, and stimulate slow-conducting fibers and cell bodies only within a 125  $\mu$ m radius of the tip (Ranck, 1975). These radius estimates were derived from studies employing stimulus parameters very similar to the parameters employed in Experiment Two. Given these estimates, inspection of Figure 7 indicates that the NMR-eliciting sites for ESB were too far from the accessory abducens region to produce direct stimulation of the motoneurons. In all cases, with the possible exception of Animal 41, the electrodes were located outside the 500  $\mu$ m stimulation radius. The ESB results are, thus, consistent with a hypothesis that conditioning takes place in pontine brain cells rostral to N.VI, and that these pre-motor cells drive accessory abducens cells to produce conditioned responding.



## C H A P T E R     I V

### GENERAL DISCUSSION

Experiment One and Experiment Two provided evidence that the pontine brain stem contains cells which mediate the classically conditioned rabbit NMR. The evidence consisted of lesioning, recording, and stimulating this brain region.

Lesioning the DLP eradicated the ipsilateral CR, but did not affect the UR. Robust conditioning of the contralateral eye argues against attentional deficits, or deficits in perceiving the stimuli, as causes of disruption. The fact that disruption occurred to CSs of three different sensory modalities also tends to preclude sensory deficits as the cause of disruption. The neural control of the CR, therefore, is separate from the neural circuit for the UR. Hence, the cells which mediate the CR, which will be referred to as "critical cells," are pre-motor cells.

Classical conditioning of the NMR produced a conditioned increase of MUA in pontine brain stem regions. This conditioned activity developed concurrently with the behavioral CR, extinguished simultaneously with the extinction of the CR, and did not show pseudoconditioning. Thus, some cells of the pontine brain stem display alterations in response characteristics to the CS as a result of pairing the CS and US. Results such as these are expected if the brain stem contains critical cells. However, additional evidence should be obtained from single-unit studies. Unfortunately, single-unit recording might prove difficult

if critical cells tend to be small. Multiple-unit recording techniques sample both small and large cells, presumably resulting in a less biased picture of the neural activity within a given brain region (Buchwald, Holstein, and Weber, 1973).

In a number of cases, ESB administered to the brain stem resulted in eyeblink, globe retraction, and extension of the nictitating membrane. This suggests that the regions stimulated have projections to the accessory abducens nucleus. Such projections would be necessary to generate robust globe retraction (Berthier & Moore, 1980).

Although the results of Experiments One and Two suggest that the pons contains critical cells, other interpretations are possible. Specifically, the evidence presented thus far do not rule out the possibility that neurons in the critical region of the pons receive afferents from critical cells located elsewhere and relay CR information to accessory abducens motoneurons. Another possibility is that the pons contains fibers of passage projecting from distant critical cells enroute to accessory abducens cells.

In Chapter I, various possible loci of critical cells were discussed. Some of these, such as neocortex, hippocampus, and the septal nuclei, were regarded as unlikely candidates because destruction of these regions failed to disrupt CRs. Furthermore, the brain stem transections of Norman et al. (1977), which failed to disrupt conditioning of cat eyeblink, argues against the existence of critical cells rostral to the midbrain. Perhaps the most viable candidate for a source of critical cells, other than the pons, is the cerebellum. Specifically, McCormick et al. (1981) observed disruption of the ipsilateral, but not contra-

lateral, rabbit conditioned NMR following unilateral destruction of the cerebellum. The URs were not affected. The authors describe the lesions as "localized to the dentate nucleus and vicinity." In addition, multiple-unit recording from the dentate nucleus revealed an increase in MUA during the presence of a CR. Some relevant questions remain to be answered, however. For example the authors report no pseudoconditioning data. In addition, they did not look at the course of acquisition of the conditioned MUA (they implanted non-naive animals), nor did they report any data concerning extinction of the conditioned MUA. Furthermore, no stimulation data were reported. With regard to the lesions, it is not known whether dentate lesions disrupt acquisition as well as retention, for only the latter was tested.

Despite these criticisms, the results of McCormick et al. (1981) suggest that dentate cells may be critical cells. If this is true, then the pons could be a second source of critical cells, and both the pons and the cerebellum could function together to produce conditioned responding. Alternatively, one of the regions could be the genuine locus of critical cells and the other region could be merely a relay point or a site where axons from critical neurons are densely packed.

The latter possibility deserves attention because all the disruptive pontine lesions in Experiment One produced at least partial damage to the brachium conjunctivum, a massive fiber tract containing efferents from dentate and interposed nuclei. Dentate efferents in the brachium conjunctivum decussate at caudal midbrain levels and form the crossed ascending and descending limbs. The ascending fibers proceed to or through the red nucleus, and then terminate in the ventral lateral and

centromedian nuclei of the thalamus. The descending fibers terminate heavily within a portion of the reticulotegmental nucleus and nearby regions of the trapezoid body. There are also a few projections to the ipsilateral vestibular nuclei and adjacent bulbar reticular formation; these appear to come from the caudal portions of the ipsilateral brachium conjunctivum and restiform body (Cohen, Chambers, and Sprague, 1958).

The pontine lesions of Experiment One were generally rostral to the ipsilateral dentate projections, so it is far more likely that the decussating dentate fibers were interrupted rather than the uncrossed fibers. This implies that if dentate cells are responsible for the ipsilateral CR, then the conditioning "circuit" would involve two decussations, one at the decussation of the brachium conjunctivum, and the second at some other point so that the ipsilateral accessory abducens nuclei would ultimately be driven. Of significance to the dentate hypothesis, however, is that recording electrodes in and around the brachium conjunctivum were all classified as misses in Experiment Two (see Figure 7, especially the left side electrode of Animal 119).

Thus, it is uncertain at this time whether cells of the cerebellum or the pons (or both) are responsible for the CR. Various techniques could be applied to help resolve the uncertainties. For example, critical cells should receive information concerning both the CS and the US. Therefore, anatomical pathways originating from sensory neurons pertaining to the CS and US, and projecting to putative critical cells, should be identified. In addition, anatomical pathways from the sus-

pected critical cells to the accessory abducens motoneurons should also exist. Fiber-tracing anatomical techniques could be used for these purposes. However, the presence of a large number of interneurons intercalated between critical cells and motoneurons, or between sensory cells and critical cells, would make pathway identification more difficult.

Electrophysiological techniques could also be applied to investigate dentate and pontine cells. For example, stimulation of critical cells in either region should excite accessory abducens motoneurons. In addition single-unit recording techniques could be employed to test whether or not suspected critical cells respond to both CS and US presentations, or to electrical stimulation of CS and US sensory nuclei. In Experiment Two, evoked responses were observed to US presentations, suggesting that US information projects to brain stem sites; however, the interpretation of these field potentials is ambiguous for reasons which were previously discussed.

In sum, the results of the present experiments and those of McCormick et al. (1981) are promising developments toward understanding the physiological substrates of conditioning. Although it is premature to suggest that an engram has been localized, future research may ultimately identify a discrete population of cells responsible for the CR. From this population of cells an investigation of the cellular mechanisms of mammalian learning can begin.



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