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## Midbrain and brain stem mechanisms of conditioned inhibition of the rabbit's nictitating membrane response.

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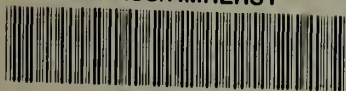
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MIDBRAIN AND BRAIN STEM MECHANISMS  
OF CONDITIONED INHIBITION OF THE RABBIT'S  
NICTITATING MEMBRANE RESPONSE

A Dissertation Presented

by

Frederick W. Mis

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

DOCTOR OF PHILOSOPHY

June, 1975

Psychology


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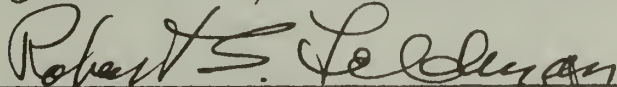
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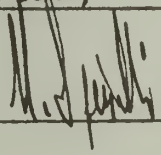
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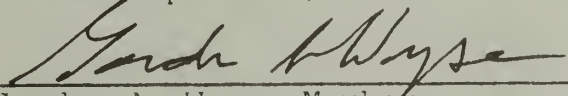
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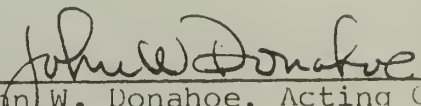
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## Abstract

A series of experiments were carried out in an effort to delineate the neural system responsible for the development of conditioned inhibition of the rabbit's NMR. In addition, an attempt was made to determine the precise nature of the inhibitory neural process associated with the development and manifestation of conditioned inhibition (Konorski, 1972).

Experiment 1 examined the modification in the amplitude of the unconditioned NMR produced by electrical stimulation of a variety of midbrain and brain stem loci. The results of this experiment suggested that stimulation at the level of the anterior oculomotor nucleus in the anesthetized rabbit augmented the amplitude of the unconditioned NMR, while stimulation at the level of the abducens nucleus reduced the amplitude of the unconditioned NMR.

Experiment 2 was designed to extend these findings to the conditioned NMR in the chronic unanesthetized rabbit. The results of this experiment indicated that the most dramatic reduction in the amplitude of the CR was produced by electrical stimulation of the nucleus of Darkschewitsch, the interstitial nucleus of Cajal, or the anterior Red Nucleus. Transection of the extraocular muscles did not eliminate the reduction in the amplitude of the CR produced by brain stimulation.

In Experiment 3, lesions placed in the nucleus of

Darkschewitsch, the interstitial nucleus of Cajal, the anterior end of the small-celled portion of the Red Nucleus and the tegmental reticular formation prevented the development of conditioned inhibition in Pavlov's conditioned inhibition paradigm. Lesions placed in the rootlets of the oculomotor nucleus, pontine reticular formation, posterior portions of the Red Nucleus, and small lesions placed in the dorsal tegmental nuclei did not prevent the development and manifestation of conditioned inhibition. In addition, transection of the extraocular muscles did not prevent the development and manifestation of conditioned inhibition.

It was concluded that a midbrain-brain stem circuit which originates in the midbrain area containing the nucleus of Darkschewitsch and the interstitial nucleus of Cajal was responsible for the development of conditioned inhibition of the rabbit's NMR. The inhibitory neural mechanism responsible for the development and manifestation of conditioned inhibition was either a descending inhibitory process specific to the extraocular motor system, or a reciprocal inhibitory process which arises in the midbrain-brain stem circuit to modulate the activity of the abducens nucleus.

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I wish to express my gratitude to Dr. John W. Moore for guidance and continuous assistance throughout my graduate career. I would also like to thank Drs. Robert S. Feldman, D. Nico Spinelli, and Gordon A. Wyse for their assistance and encouragement during the conduction of this series of experiments. In addition, the support staff of the Psychology Department deserve a special note of thanks, especially Al Williams, Charlie Gorman, Gaspar DiPasquale, Jonathan Carr, and Anita Pliner. As always, my parents deserve a note of thanks for their continued encouragement of my educational endeavors. I am also extremely grateful to Nancy L. Zygmunt, whose patience, understanding, and technical expertise facilitated the completion of this dissertation.

Lastly, I would like to thank my rabbits who gave their lives in the interest of science.

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Despite the fact that learned inhibition plays a central role in a number of theoretical formulations of the learning process (i.e., Pavlov, 1927; Hull, 1943; Rescorla & Wagner, 1972), no particular neural mechanism or system has been implicated unequivocally in any of its behavioral manifestations (cf. Boakes & Halliday, 1972). For example, Douglas (1972) proposes that the hippocampus is intimately involved in the development of learned inhibition while Dickinson (1972) focuses on the role the septal nucleus may play in this process. In addition, there have been numerous investigations of inhibitory pathways in the nervous system (cf. Eccles, 1969; Szentagothai, 1961), but none of these pathways (e.g., the fiber tracts in the hippocampus) have been directly related to the development of learned inhibition.

Recently, a number of behavioral investigations employing the nictitating membrane response (NMR) of the rabbit as the behavioral index of learning (i.e., Marchant, Mis, & Moore, 1971) have demonstrated that Pavlovian conditioned inhibition training leads to the development of a conditioned stimulus (CS) which unequivocally fulfills the stringent behavioral requirements for inhibition specified by Rescorla (1969). The present experiments were therefore designed in an effort to elucidate some of the neural mechanisms which might play a role in the development of learned inhibition of the rabbit NMR, and to help localize the neural structures associated with this inhibitory process.



### Nictitating Membrane Response Preparation

In a recent review of the neurophysiology of learning, Thompson, Patterson and Teyler (1972) argue for the adoption of a model biological system in which to investigate the neural processes involved in the acquisition of a new response. One possible model system may be the conditioned NMR of the rabbit. Until recently, most of the investigations which have employed the NMR of the rabbit as an index of learning have not been concerned with ascertaining the physiological mechanisms responsible for learning but had focused on the basic parameters or peripheral stimulus alterations which affected the elaboration of the conditioned response (see Gormezano & Moore, 1969, for a review). In addition to this large collection of behavioral data available to the neuroscientist concerned with establishing the conditioned NMR, the preparation has a number of additional features which may facilitate investigations attempting to delineate the physiological (anatomical, bio-electrical or biochemical) substrates of learning. First, the NMR is discrete and not made up of a long chain of complex motor responses. Second, the organism is basically immobile during the conditioning session, eliminating problems associated with a moving organism. The importance of this is illustrated by the findings of Worden, Marsh, Abraham and Whittesey (1964) who demonstrated that head position in the sound field determines the effective sound intensity reaching the auditory transducers, and CS intensity is

known to affect the acquisition of a conditioned response (cf. Gormezano & Moore, 1969). Third, the central nervous system (CNS) structure which contains the neurons directly controlling the NMR is known. Specifically, Thompson, Cegavske, & Patterson (1973) have confirmed that, as in the cat (cf. Hopkins, 1916; Rosenblueth, & Bard, 1932), the abducens nerve contains the axons of the motoneurons which control eyeball retraction. Postmortem examination of the orbit of the rabbit suggests that the NMR is a passive component of this eyeball retraction.

#### Pavlovian Conditioned Inhibition

The Pavlovian conditioned inhibition paradigm is especially interesting because it places those cues which are associated with the execution of the learned response in direct competition with those cues associated with the inhibition of that learned response. The paradigm typically involves the following stages: first, a conditioned response is established to two separate and distinct stimuli,  $CS_1$  and  $CS_2$ . Following this, a special type of differential conditioning is initiated in which  $CS_1$  continues to be reinforced while the compound consisting of  $CS_1$  and a third separate stimulus,  $CS_3$ , is not. Given that the nonreinforced cue is a compound consisting of  $CS_1$  and another cue, the subject gradually forms the appropriate discrimination between  $CS_1$  and the compound. In order to test for the acquired inhibitory properties of  $CS_3$  (see

Rescorla, 1969)  $CS_3$  and  $CS_2$  are compounded (summation testing) during extinction, and the degree of suppression in responding to  $CS_2$  is compared with suitable controls. An additional test for the inhibitory properties of  $CS_3$  involves determining if subsequent excitatory conditioning to  $CS_3$  is impeded (retardation testing) relative to appropriate controls as a result of the previous conditioned inhibition training.

### Mechanisms of Inhibition

In discussing the physiological mechanisms which might be involved in learning not to respond in a particular situation, Konorski (1972) categorizes these processes into the following four cases: (a) central reciprocal inhibition; (b) inhibition resulting from an antagonistic system overriding the peripheral components of the excitatory process (i.e., the muscle groups associated with the performance of the response); (c) a higher level descending inhibitory process, and (d) lateral inhibition. Although the physiological process of lateral inhibition plays a major role in regulating sensory information (cf. Bridgeman, 1971), its potential for accounting for the development of learned inhibition appears to be rather limited. However, the other three proposed physiological mechanisms of inhibition may account for the development of learned inhibition of the rabbit NMR. For example, the development of inhibition in the NMR preparation may be the result of the interaction between two reciprocally

inhibitory nervous centers. One center forms the relationship between  $CS_1$  and the unconditioned stimulus while the other center forms the relationship between  $CS_3$  and no unconditioned stimulus. This center then activates the system for the execution of the appropriate response while inhibiting the other neural system. In the conditioned inhibition paradigm, the compound consisting of  $CS_1$  and  $CS_3$  activates both neural systems and would require that the center not correlated with responding develop sufficient strength to inhibit the center which governs the execution of the response. In addition to this reciprocal inhibition between discrete neural systems, there is the possibility that reciprocal inhibition occurs within the particular motor system governing a response. For example, there may be inhibitory interneurons in the motor system which are inhibited during the performance of a response controlled by the motoneurons. These same interneurons might then tend to suppress the activity of the motoneurons within the system during the performance of responses not involving the particular muscle groups.

If an antagonistic mechanism were responsible for the manifestation of inhibition during the presentation of CS- there should be a correlated increase in the activity of an antagonistic system which would prevent the execution of the conditioned response. As described by Konorski, this inhibitory neural mechanism requires that the effector system governing the response also be activated by the cue which signals



"do not respond." Three lines of evidence suggest that Konorski's notion of an antagonistic inhibitory process may provide a reasonable explanation for the development of learned inhibition in the rabbit NMR preparation. First, studies from the instrumental literature indicate that a CS- in an appetitive key-peck situation induces activity in pigeons which is antagonistic to the performance of the conditioned response (e.g., Terrace, 1966). Second, Dabrowski (1971) has observed that dogs trained in a go-no go discrimination (leg flexion versus inhibition of leg flexion) to obtain food actively resist performance of the flexion response by engaging in antagonistic movements during the presentation of the stimulus which signals "do not respond". Finally, Thompson (1973) reported that electrical stimulation in the anterior portion of the oculomotor nucleus produced what appears to be an active opening of the nictitating membrane (cf. Szentagothai & Schab, 1956). Therefore, a system antagonistic to the performance of the NMR may be available for use as a mechanism to inhibit the execution of the NMR on CS- trials during conditioned inhibition training.

Finally, Konorski's notion of a unidirectional inhibitory center could account for the observed learned inhibition of the rabbit NMR. If this mechanism controlled the manifestation of inhibition, the inhibitory influence would arise at some higher level in the CNS independent of the excitatory system and would act to reduce the neural activity of all lower

neural structures. It is possible that the inhibition may be specific to the structures related to the execution of the CR. For example, Szentagothai & Schab (1956) observed that electrical stimulation of the nucleus of Darkschewitsch produces complete inhibition of activity in the extraocular muscles of the cat, while Siegel and Wang (1974) have observed that electrical stimulation of the caudate nucleus produces a general arrest of all ongoing behaviors and if continued long enough induces sleep in the organism. The major distinction between this mechanism of inhibition and the notion of reciprocal inhibition resides in the fact that during a CS+ trial there should be little if any suppression in the activity of the neurons that make up this system. However, the notion of reciprocal inhibition requires that the neuronal activity in the center associated with not responding be suppressed during a CS+ trial.

Asratian (1972) proposes that the formation of inhibition occurs in the nerve elements controlling the conditioned response. In the NMR preparation then, the inhibition would be observed at the level of the abducens nucleus or in one of the higher CNS structures governing the conditioned NMR. This proposed mechanism of inhibition is similar to Konorski's notion of a unidirectional inhibitory process. In both cases, the physiological correlate of learned inhibition would be manifested in the system responsible for the execution of the response. However, in Konorski's proposed mechanism the

inhibition is developed from outside of the system controlling the response, while Asratian's notion requires that the inhibition somehow be generated from within the system controlling the learned response.

Although there is evidence for a general descending inhibitory process, this evidence indicates that the inhibition generated by these systems is an overriding shutdown of large portions of the nervous system. Therefore, it does not appear that the descending inhibitory systems are involved in the development of conditioned inhibition of a specific response. However, it is possible that a descending inhibitory system may be discovered which is related to the development of learned inhibition of a specific set of responses. It should be noted that the notion of reciprocal inhibition between various systems would probably involve activity occurring only in the CNS, while the notion that inhibition is the result of the activation of antagonistic systems would probably involve both the CNS and associated muscular systems.

#### The Extraocular Motor System

In addition to controlling the retractor bulbi muscle, the abducens nucleus also controls the lateral rectus muscle which is part of the extraocular motor system found in vertebrates. This suggests that an analysis of the neural system(s) which control the extraocular muscles may provide a basis from which one might delineate the components of a neural

system which modulates the activity necessary to initiate a contraction of the retractor bulbi muscle, and its associated NM sweep.

Before discussing the anatomy and physiology of the extra-ocular motor system, a precise description of the execution of the NMR may aid in understanding the development of conditioned inhibition in this preparation. The sweep of the nictitating membrane across the eyeball is the result of the retractor bulbi muscle drawing the eyeball into the socket. This inward motion of the eyeball pushes fatty tissue and the Harderian gland against the base of the nictitating membrane which then causes the membrane to sweep across the eyeball (Motaïs, 1885). Activation of the retractor bulbi muscle reflex is accomplished by tactile stimulation of the region around the eye. In terms of rate of conditioning of this reflex, Salafia, Daston, Bartosiak, Hurley, and Martino (1974) reported that the optimal stimulation area was located around the nasal canthus. Information from the tactile receptors located in this region of the face is carried to the central nervous system via the trigeminal nerve (cf., Boudreau & Tsuchitan, 1973). Although this tactile stimulation is necessary to elicit a NMR and eyeblink, the stimulation may play a small role in the actual elaboration of a conditioned response. Thompson (1974) reported that pairing a tone with direct electrical stimulation of the abducens nucleus can lead to the development of a conditioned NMR. In addition,



Black-Cleworth, Woody, and Niemann (1975) reported that direct stimulation of the branch of the facial nerve which controls the orbicularis oculi muscles in the cat could effectively be used as a UCS to establish a conditioned eye-blink response. Transection of the trigeminal nerve did not eliminate the effectiveness of the facial nerve stimulation suggesting that the UCS functions to merely guarantee the performance of the response.

As seen in Figure 1a the sweep of the triangular piece of tissue which makes up the nictitating membrane is in the nasal-temporal direction. This nasal-temporal sweep of the nictitating membrane appears to function as a device which protects the eyeball from damage while the rabbit is moving through the underbrush (Prince, 1964).

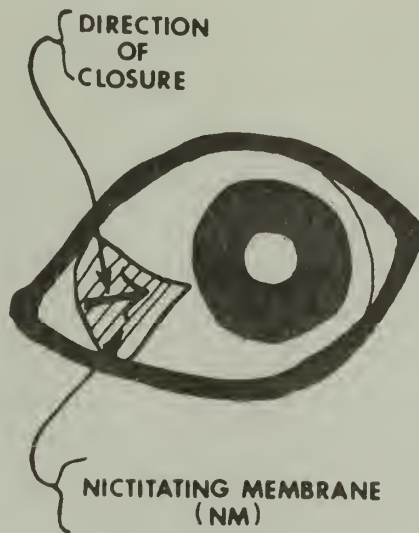
Figure 1b presents a vertical section through the eye of the rabbit and depicts two portions of the retractor bulbi muscle in relation to some of the other extraocular muscles. The four rectus muscles, two oblique muscles, and the four components of the retractor bulbi muscle which surround the optic nerve as it enters the eyeball are attached to the head in an area adjacent to the optic foramen. Although at one time there was some discussion as to which cranial nerve supplied the retractor bulbi muscle (cf. Hopkins, 1916; Prince, 1964) most researchers agree that the abducens nerve contains the efferent control system of the retractor bulbi muscle and

## FACE PAGE FOR FIGURE 1

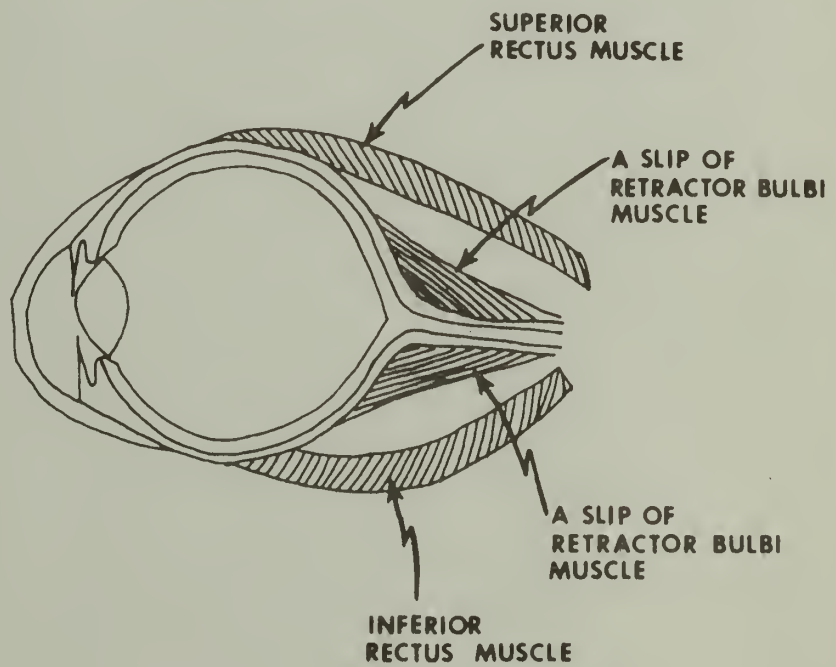
Figure 1. Panel A is a schematic drawing of the left eye of a rabbit, depicting a slightly extended nictitating membrane and its direction of closure.

Panel B, redrawn from Bensley (1946 , Figure 48, p. 89) is a schematic drawing of a vertical section through the eye of a rabbit which depicts two slips of the retractor bulbi muscle and the superior and inferior rectus muscles.

**A**



**B**



therefore the nictitating membrane closure response (Hopkins, 1916; Rosenblueth & Bard, 1932; Bach-y-Rita & Murato, 1964; Thompson, Cegavske, & Patterson, 1973).

In the cat, Bach-y-Rita and his associates (Bach-y-Rita & Ito, 1966; Alvarado, Steinaker, & Bach-y-Rita, 1967; Bach-y-Rita, Levy, & Steinaker, 1967; Lennerstrand, 1974; Bach-y-Rita & Lennerstrand, 1974) have demonstrated that the retractor bulbi muscle is physiologically, histologically, and pharmacologically different from the other six extraocular muscles. Given these findings, one might suspect that the central control of the retractor bulbi muscle is to some degree independent of the other six extraocular muscles, but to date no mention has been made in the literature indicating such independence. In addition, Bach-y-Rita (1971) indicates that in the cat the retractor bulbi muscle does not have tonic properties and is not capable of producing fine graded movements. Observations in the rabbit suggest that the retractor bulbi controlled nictitating response is also incapable of producing fine graded movements, and is limited to a basic all or none response. The absolute amplitude of the NMR may be a function of the number of motor units in the retractor bulbi muscle which are activated.

Anatomy. Although there have been no attempts to precisely locate the neurons supplying the extraocular muscles of the rabbit using histological techniques, Bensley (1946) and Hopkins (1916) using gross dissection, and Highstein

(1973a) employing electrophysiological techniques describe the efferent control of the four rectus, two oblique and retractor bulbi muscles in a manner which suggests that the innervation pattern is similar to that described in other mammals (Bienfang, 1968; Gacek, 1974; Naito, Tanimure, Taga, & Hosoya, 1974; Tarlov & Tarlov, 1971; Warwick, 1953). Specifically, the oculomotor nucleus controls the inferior (IR), medial (MR), and contralateral superior rectus (SR) muscles, and the inferior oblique (IO) muscle. The trochlear nucleus controls the contralateral superior oblique (SO) muscle, while the abducens nucleus controls the lateral rectus (LR) muscle and in the rabbit and other animals which possess a nictitating membrane, the retractor bulbi muscle.

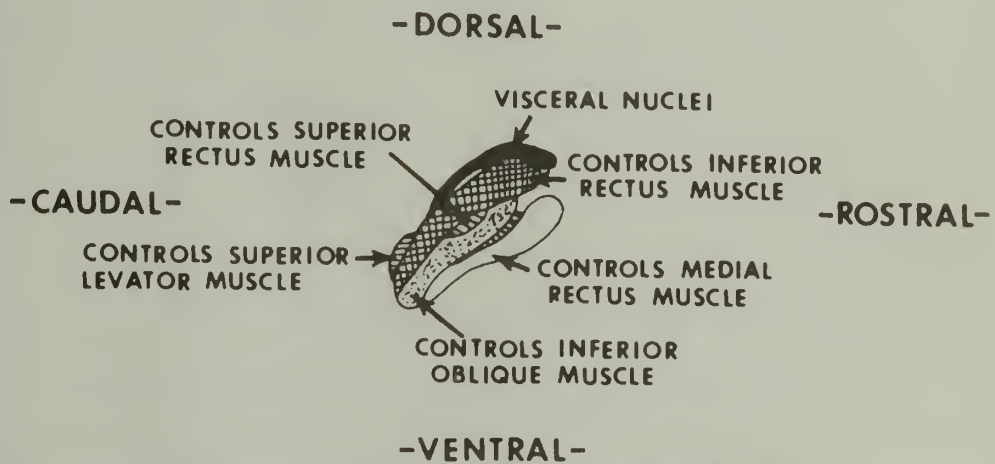
One question that immediately arises, is how the oculomotor and abducens nuclei are organized with respect to the various extraocular muscles they control. Warwick (1953) in an extensive study of the monkey oculomotor nucleus observed the following organization within the nucleus. As seen in Figure 2, the origin of the motoneurons controlling the IR muscle are located in the dorsal-lateral aspect of the nucleus and are found throughout the rostral-caudal extent of the nucleus. The motoneurons of the MR muscle are also located throughout the rostral-caudal extent of the nucleus but are located in the ventral aspect of the nucleus. The motoneurons controlling the SR muscle are found in the medial aspect of

## FACE PAGE FOR FIGURE 2

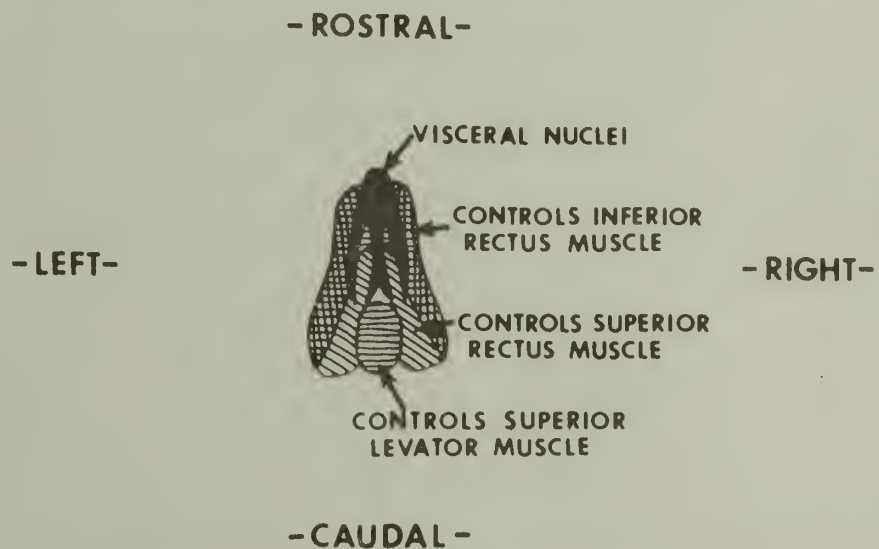
Figure 2. Panel A depicts a lateral view of the oculomotor nucleus, while Panel B depicts a dorsal view of the oculomotor nucleus in the monkey. (Redrawn from Warwick, 1953, p. 480, Figure 21.)



# A



# B



the caudal portion of the contralateral nucleus, while the motoneurons controlling the IO muscle are located in the caudal 2/3 of the nucleus and lie between the motoneurons controlling the IR and MR muscles.

A number of recent investigations of the motoneurons supplying the extraocular muscles of the cat (Tarlov & Tarlov, 1971; Gacek, 1974) have observed a slightly different organization within the oculomotor nucleus. Specifically, the motoneurons controlling the MR muscle in the cat are located in the rostral and dorsal portions of the nucleus. The motoneurons controlling the IO muscle found in the rostral aspect of the group are located in the dorsal portion of the nucleus while the units lying in the caudal end of the group are located in the ventrolateral portion of the nucleus. Lastly, the motoneurons controlling the IR muscles are located in the rostral portion of the ventral third of the nucleus. Observation of potential changes in the oculomotor nucleus following stimulation of the various branches of the oculomotor nerve tend to confirm this basic organization of the nucleus in the cat (Bienfang, 1968; Naito, et al., 1974). The basic difference in the organization of the oculomotor nucleus in the cat and monkey involves the portions of the nucleus having ipsilateral input to the extraocular muscles. More precisely, the motoneurons controlling the IR muscle have been compressed in the rostral third of the nucleus and have moved to an area below the motoneurons controlling the MR muscle. This in turn



has caused the arrangement of the motoneurons controlling the IO muscle to run in a slightly dorsal-ventral plane rather than in a horizontal plane as is found in the monkey oculomotor nucleus.

Following observations of the change in the field potentials produced by antidromic activation of the oculomotor nerve, Highstein (1973a) concluded that the organization of the oculomotor nucleus in the rabbit more closely resembled that of the monkey than the cat. However, Highstein did note that the motoneuron pools in the oculomotor nucleus of the rabbit tended to be located in specific regions in the rostral-caudal extent of the nucleus, while in the monkey, the motoneuron pools tend to run throughout the entire rostral-caudal extent to the nucleus.

Gacek (1974a, 1974b) reported that in cats in which horseradish peroxidase was injected into the LR muscle, there were a significant number of cells in the abducens nucleus which were not labeled with the peroxidase. Even in cases where the amount of peroxidase injected into the muscle should have stained all the motoneurons in the nucleus if all the motoneurons in the nucleus were only innervating the LR muscle, there were still some cells which did not take up the stain. These results suggest that there is a separation within the abducens nucleus for the control of the retractor bulbi and LR muscles, but this study does not provide any

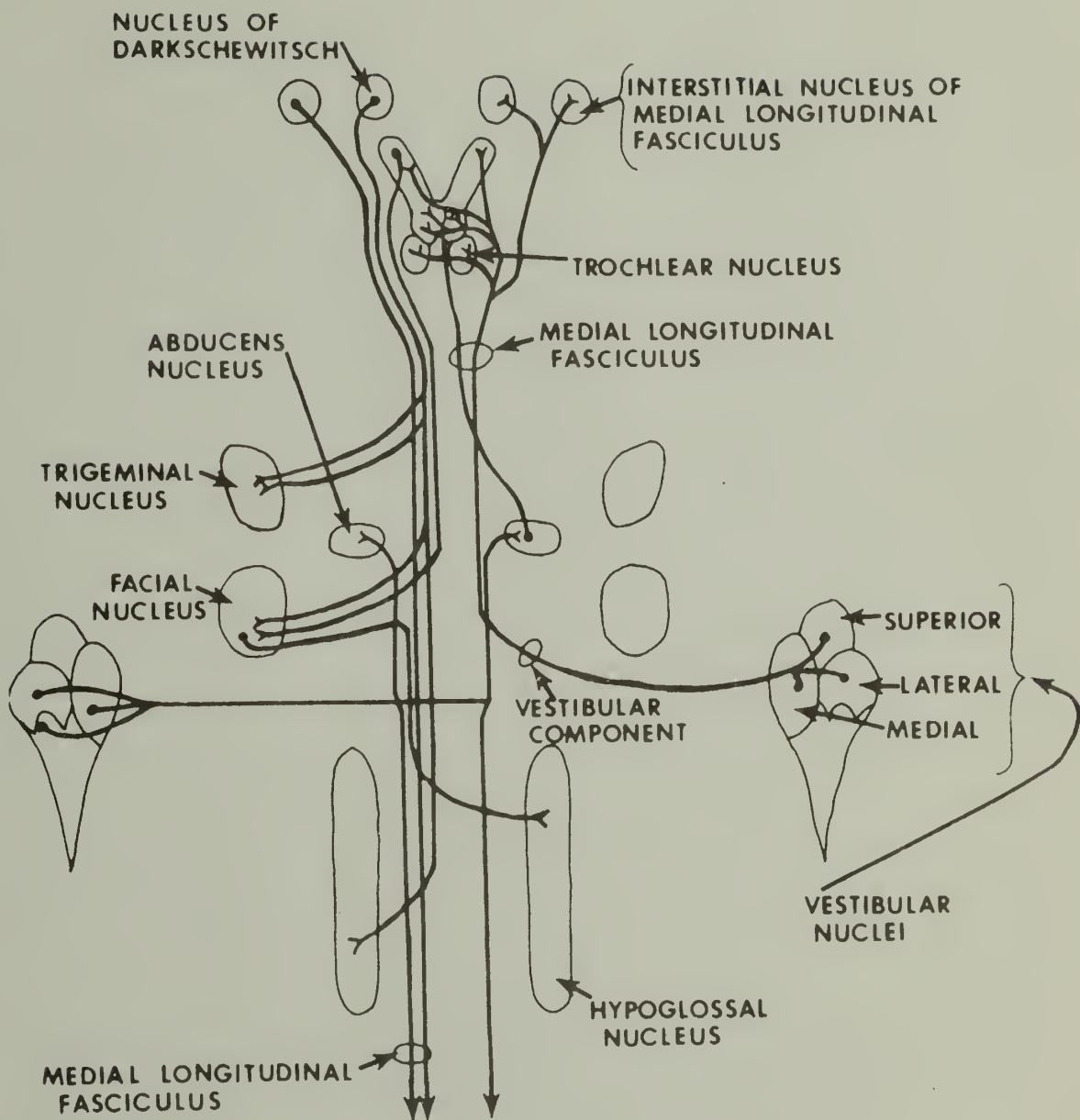
evidence of a specific organization within the nucleus for the neurons controlling these two extraocular muscles. In addition, Goldberg, Hull, and Buchwald (1974) have reported that the abducens nucleus contains a population of interneurons which are directly affected by the activity generated by the motoneurons in the nucleus. Specifically, they observed that a population of cells located in the dorsal aspect of the nucleus responded orthodromically to stimulation of the abducens nerve with latencies of from 1.4 to 10 msec and these neurons were unable to follow high frequencies of stimulation of the nerve. Goldberg, et al. postulated that this population of interneurons may be involved in relaying information concerning the activity of the abducens nucleus to other neural centers. Another interesting aspect of the abducens nucleus concerns the possibility that there may be electrotonic coupling between the motoneurons of the nucleus (Gogan et al., 1974). The presence of this type of coupling between the motoneurons in the nucleus, especially in those neurons controlling the retractor bulbi muscle, would enable the organism to activate this response with a minimum of excitatory input to the motoneurons controlling the response. However, electrotonic coupling between the motoneurons controlling the other extraocular muscles would probably interfere with the precise control of eye position necessary to track objects moving in space. In summary, it appears that

the abducens nucleus is composed of two types of motoneurons, some of which may be electrotonically coupled, and at least one group of interneurons which may serve to relay information concerning the activity of the abducens nucleus to the rest of the CNS.

Given this general organization of the motoneuron pools controlling the extraocular muscles, an examination of the interconnections among these nuclei, and the primary and secondary inputs to them might provide the first approximation of a system which could potentially account for the development and modulation of the conditioned NMR. In trying to specify the interconnections among the nuclei controlling the extraocular muscles and the CNS structures which modify their activity, one comes to realize that the notion that all of the structures which make up the brain are connected to one another in some fashion is a reasonable first approximation to describe the makeup of the CNS. For example, Figure 3 depicts the nuclei controlling the extraocular muscles, and the interconnections among each of the nuclei and a number of structures which communicate with these structures via the medial longitudinal fasciculus (MLF). It should be noted that this figure does not differentiate the connections according to whether they inhibit or excite the neurons upon which they synapse, and the figure does not specify direct and indirect inputs to the system which arise at other levels in the CNS.

## FACE PAGE FOR FIGURE 3

Figure 3. An illustration of the major pathways and components of the extraocular motor system. This figure was redrawn from Crosby, Humphrey & Lauer (1962, Figure 192, p. 255) to illustrate the elements and connections of the extraocular motor system as they are known to exist in the rabbit.



The complexity of inputs to the oculomotor nucleus is best exemplified in a recent paper by Graybiel and Hartwieg (1974). Following injection of horseradish peroxidase into the oculomotor nucleus and some surrounding structures, Graybiel and Hartwieg observed retrograde marking related specifically to the oculomotor nucleus in the following structures: interstitial nucleus of Cajal, nucleus of the posterior commissure, the rostral pole of the trigeminal complex, the abducens nucleus, the superior, lateral, medial and descending portions of the vestibular nucleus, nucleus prepositus hypoglossi, and the dorsal medial portion of the reticular formation. The most interesting findings of this study include the confirmation of a direct link between the abducens nucleus and the oculomotor nucleus, and the description of a pathway connecting the nucleus prepositus hypoglossi to the oculomotor nucleus. The connection between the nucleus prepositus hypoglossi and the oculomotor nucleus may serve to provide the third nucleus with efference copy of some cerebellar inputs.

The densest marking observed in this study was located in the medial and superior portions of the vestibular nucleus. Given the findings of Tarlov (1970) and Gacek (1971) regarding the number and complexity of connections between these two structures, one would expect to observe a large degree of retrograde marking in these two portions of the vestibular complex. For example, Tarlov observed that the superior portion



of the vestibular nucleus sends projections to the trochlear nucleus, the various components of the oculomotor nucleus, the interstitial nucleus of Cajal, and the nucleus of Dark-schewitsch via the ipsilateral MLF. In addition, Tarlov suggests that the superior portion of the vestibular nucleus may send projections to the ipsilateral abducens nucleus, and the contralateral interstitial nucleus of Cajal and nucleus of Darkschewitsch. According to Tarlov, the medial aspect of the vestibular nucleus sends projections to the ipsilateral abducens nucleus, then crosses the midline and may send projections to the contralateral abducens nucleus. At the level of the abducens nucleus, these fibers enter the MLF and via the MLF send off collaterals to synapse on the trochlear nucleus, the various components of the oculomotor nucleus, the interstitial nucleus of Cajal, and the nucleus of Darkschewitsch. Tarlov also suggests that there may be fibers in this projection which recross the midline rostral to the oculomotor nucleus to innervate the ipsilateral interstitial nucleus of Cajal and the nucleus of Darkschewitsch.

In addition to confirming some of the findings of Tarlov, Gacek (1971) was able to specify the precise location of some of the projections to the extraocular motor system which arise within the vestibular nucleus. For example, Gacek observed that there was an ipsilateral projection to the portion of the oculomotor nucleus which controls the inferior rectus muscle that travels lateral to the MLF and arises in the

ventral portion of the lateral aspect of the vestibular nucleus (cf. Szentagothai & Schab, 1956). Gacek also failed to observe an ipsilateral projection from the superior aspect of the vestibular nucleus to the abducens nucleus.

In addition to the massive vestibular input to the oculomotor, trochlear and abducens nuclei, the extraocular motor system receives many direct and indirect inputs from other structures within the CNS. For example, Shimo-oku (1970) observed that severing the trigeminal nerve eliminated some of the inhibitory input to the oculomotor nucleus. Also a number of investigations have reported that the pontine reticular formation ventral to the abducens nucleus is involved in the initiation of complex eye movements. Cohen and Komatsuzaki (1972) reported that electrical stimulation in the pontine reticular formation produced distinct eye movements similar to the slow phases of nystagmus and pursuit movements, as well as some other specific eye movements. Cohen and Komatsuzaki postulated that this area of the reticular formation may serve to integrate the vestibular or visual ocular reflexes. Similarly, Keller (1974) recording and stimulating single units in the same general region confirmed the involvement of this area in the elaboration of complex eye movements. One particular observation made by Keller indicated that microstimulation of one of the subpopulations of neurons in this area could produce an abrupt inhibition of all saccadic and quick-phase eye movements. Highstein, Cohen



and Matsunami (1974) have also reported that electrical stimulation of the reticular formation can produce inhibitory potentials in the extraocular motor system. Specifically, Highstein et al. observed that stimulation in a slightly more anterior portion of the reticular formation produced IPSPs and EPSPs in the oculomotor nucleus. The latency of the observed potential changes was brief enough to suggest a monosynaptic connection between the RF and the OMN.

The accessory oculomotor nuclei (Carpenter & Peter, 1970) have been shown to receive inputs from many of the structures which innervate the extraocular motor system (Tarlov, 1970) and also may send projections to this system. For example, Schwindt, Precht, and Richter (1974) have demonstrated that stimulation of these structures produces EPSPs and IPSPs in trochlear nucleus motoneurons, and EPSPs in neurons just ventral to the abducens nucleus (cf., Graybiel & Hartweg, 1974). In addition, Szentagothai and Schab (1956) have observed an indirect inhibitory connection between the nucleus of Darkschewitsch and the portion of the oculomotor nucleus controlling the inferior rectus muscle. Using physiological levels of stimulation, Markham, Precht, and Shimazu (1966) were able to demonstrate a reciprocal connection between the interstitial nucleus of Cajal and the vestibular nucleus. These findings suggest that the accessory oculomotor complex is intimately related to the regulation of the extraocular motor system and a number of the system's

major inputs. In addition, it appears that this region may be a relay-integrative center for the visual-ocular reflex. For example, Tarlov and Moore (1966) observed that lesions created in the inferior and superior colliculus did not produce degeneration in any of the components of the extraocular motor system, but did produce substantial degeneration in the interstitial nucleus of Cajal. In addition, Kawamura, Brodal, and Hodderik (1974) have observed a large degree of degeneration in the reticular formation surrounding the oculomotor nucleus following lesions in the superior colliculus. Precht, Schwindt, and Magherini (1974) and Shimo-Oku (1974) have observed that visual system stimulation, (actually stimulation of the optic nerve or tectum), produced modifications in motoneuron activity within the oculomotor nucleus. The latency of the observed modifications suggested a polysynaptic input which may pass through the accessory oculomotor nucleus (Tarlov & Moore, 1966) or a reticular formation (Kawamura et al., 1974). In addition, Wright, Hilsz, and Locke (1974) have found that the medial occipital cortex has direct projections to the nucleus of Darkschewitsch, while Leonard (1969) observed direct projections from the medial frontal cortex to the midbrain area containing the accessory oculomotor nuclei. Carpenter and Strominger (1964) have shown that the dentate nucleus of the cerebellum sends projections to the nucleus of Darkschewitsch.

In addition to this complex array of major inputs to the

extraocular motor system, a number of other smaller inputs have been suggested. Rasmussen (1946) suggests that there may be a direct projection from the superior olivary complex to the abducens nucleus. Also, Carpenter and Strominger (1964) have reported direct cerebellar projections to specific portions of the oculomotor nucleus. For example, they observed that the entire dentate nucleus sends projections to the area of the oculomotor nucleus controlling the inferior rectus muscle, while the ventral aspect of the dentate nucleus sends projections to the contralateral oculomotor nucleus controlling the superior rectus muscle.

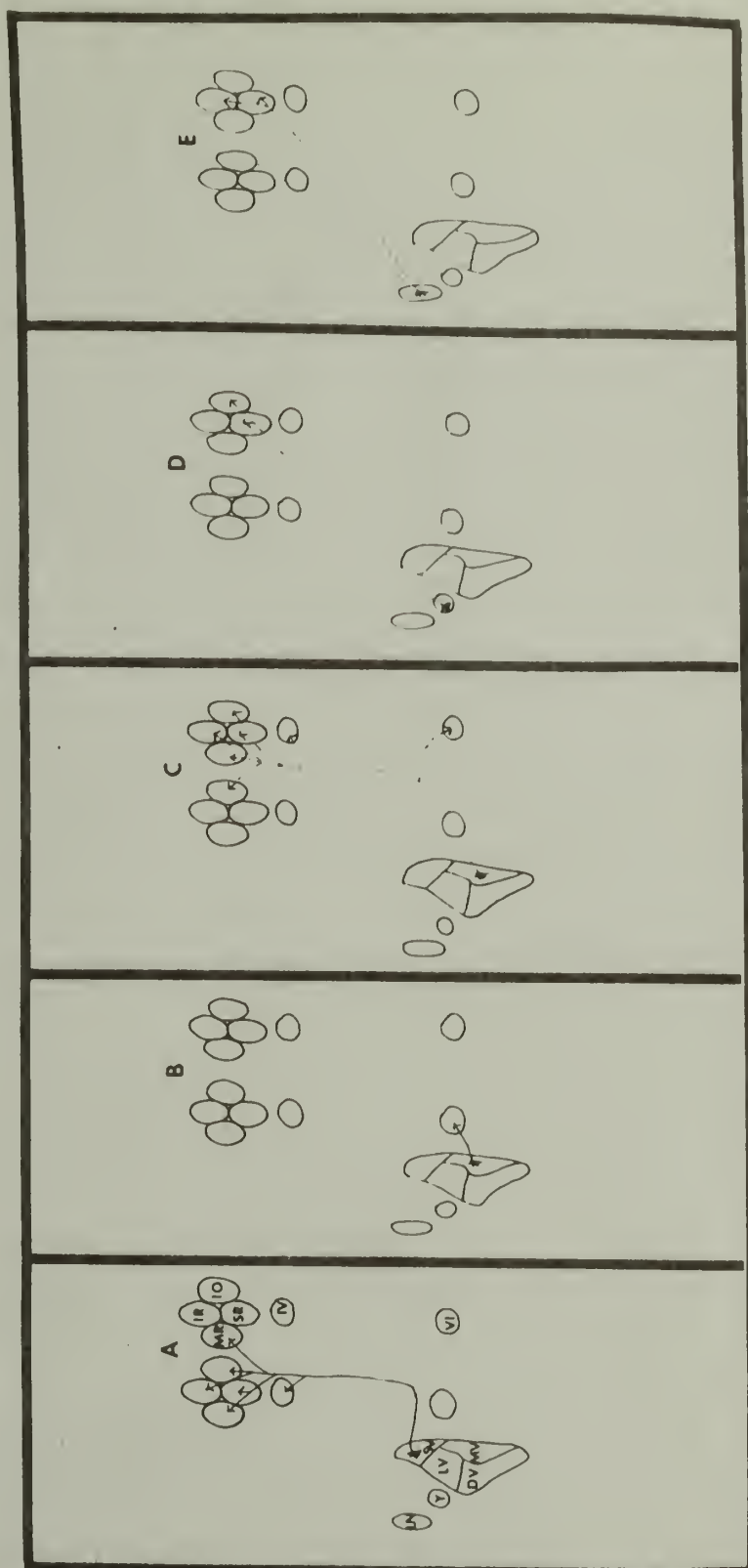
Physiology. The number and variety of inputs to the extraocular motor system suggests that some relatively complicated functional relationships exist between the various components of the system. As with the anatomical analysis of the system, the major thrust of the investigations attempting to delineate the physiological relationship between the components of the system has been in analyzing how information arising in the vestibular system modifies activity in the motoneurons controlling the extraocular muscles. The relationship between activity in the vestibular system and its modifying effect on the motoneurons of the extraocular muscle system in the rabbit has been extensively investigated by Ito and his associates using field potential and intracellular recordings (Ito, 1974; Ito, Highstein & Fukuda, 1970; Ito, Highstein & Tsuchiya, 1970; Fukuda, Highstein, & Ito, 1972; Highstein &

Ito, 1971; Highstein, Ito, & Tsuchiya, 1971; Highstein, 1971, 1973a,b). The origin and termination of the vestibular inputs to the system are summarized in Figure 4. As seen in the figure, inhibitory inputs to the oculomotor nucleus and trochlear nucleus arise in the ipsilateral superior vestibular nucleus. This portion of the vestibular nucleus also has an inhibitory effect on the motoneurons in the contralateral portion of the oculomotor nucleus controlling the MR muscles (Ito et al., 1970b; Highstein & Ito, 1971; Highstein et al., 1971; Highstein, 1973a). The inhibitory input to the abducens nucleus arises in the rostral half of the ipsilateral medial vestibular nucleus (Highstein, 1973b). Systemic injection of a number of pharmacological agents (bicuculline, esserine, strychnine, and picrotoxin) has suggested that the inhibitory transmitter in this system is a gamma-amino-butyric acid or a related substance (Highstein, 1973b; Ito et al., 1970a,b). As seen in the figure, the excitatory inputs to the system arise primarily in the contralateral medial vestibular nucleus. Stimulation in the rostral portions of the MVN produces EPSPs throughout the oculomotor nucleus, the trochlear nucleus and the contralateral abducens nucleus. In addition, this structure also provides excitatory input to the ipsilateral portion of the OMN controlling the MR muscle. The Y group of the vestibular nucleus provides excitatory input to the portions of the contralateral oculomotor nucleus controlling the IO

## FACE PAGE FOR FIGURE 4

Figure 4. A schematic representation of a horizontal section through the IIIrd, IVth, and VIth nuclei and the projections to them from the superior and medial aspects of the vestibular nucleus, the Y-group of the vestibular nucleus, and the lateral nucleus of the cerebellum. The solid lines indicate inhibitory processes while the dashed lines indicate excitatory processes. The figure was redrawn from Highstein (1973a, Figure 10, p. 297; 1973b).







and SR muscles while the lateral nucleus of the cerebellum provides excitatory inputs to the portions of the contralateral OMN controlling the IR and SR muscles (Highstein, 1973b; Fukuda et al., 1972; Ito et al., 1970a; Ito, 1974).

In addition to this direct monosynaptic effect of vestibular activity on the motoneurons of the extraocular system, these investigations have demonstrated that cerebellar activity significantly modifies the character of the vestibulo-ocular reflex. Electrical stimulation of the flocculus produces a depression in the components of the system producing IPSPs in the motoneurons of the extraocular muscle system. This includes the superior vestibular nucleus which produces inhibition in the ipsilateral oculomotor and trochlear nuclei and the medial vestibular nucleus which produces IPSPs in the ipsilateral abducens nucleus. On the other hand, stimulation of the flocculus did not disrupt EPSPs in the contralateral abducens, oculomotor, and trochlear nuclei which were generated by activity in the medial vestibular nucleus. However, stimulation of the flocculus did produce an inhibition of the EPSPs which were generated in either the Y group of the VN or the LN of the cerebellum.

Examination of the vestibulo-ocular reflex system in the cat suggests that the physiological mechanisms controlling the reflex system are similar to those in the rabbit (Baker, Mano & Shimazu, 1969; Baker & Precht, 1972; Baker, Precht, &

Llinas, 1972; Berthoz, Baker, & Precht, 1973; Precht & Baker, 1972; Precht, Baker, & Okada, 1973; Richter & Precht, 1968; Schwindt, Precht, & Richter, 1974). For example, Baker et al., (1969) have reported that stimulation of the ipsilateral vestibular nerve produces IPSPs in the abducens nucleus while stimulation of the contralateral vestibular nerve produces EPSPs in the abducens nucleus. Also, Precht and Baker (1972) found that stimulation of the ipsilateral superior vestibular nucleus produced IPSPs in the trochlear nucleus while stimulation of the contralateral medial vestibular nucleus produced EPSPs in the nucleus (cf., Figure 4).

In addition to the modification in the activity of the motoneurons produced by vestibular and cerebellar stimulation, a number of other brain stem areas have been implicated in the regulation of extraocular motor systems. For example, Baker and Berthoz (1975) have reported that the stimulation in the vestibular complex produces modifications in the neurons of the nucleus prepositus hypoglossi which are similar to those produced in the motoneurons of the extraocular muscle system. In addition, they have observed that one of the projections of this nucleus synapses on the oculomotor nucleus (cf. Graybiel & Hartweg, 1974) either directly or indirectly via the interstitial nucleus of Cajal. They have postulated that this area may function to modify the vestibulo-ocular reflex or form part of a system for the regulation of tracking movements. Two other studies (Cohen & Komatsuzaki, 1972; Keller,

1974) have reported that the reticular formation immediately anterior and ventral to the nucleus prepositus hypoglossi may be involved in the coordination of eye movements. For example, Cohen and Kamatsuzaki reported that electrical stimulation of this area produced saccades, the various phases of nystagmus or pursuit movements which were followed by a fixation period similar to those arising naturally. On the other hand, Keller (1974), recording from single units in the pontine reticular formation, observed three classes of units whose discharge properties were directly related to the amplitude of a gazeshift, eye position, and velocity of the eye movement. In addition, he observed two classes of units whose activity decreased during movements. Microstimulation of these pause units produced an inhibition of all "voluntary" saccadic eye movements.

The pontine reticular formation has also been implicated in the modulation of the motoneurons in the third nucleus (Highstein, Cohen and Matsunami, 1974). In the majority of third nucleus motoneurons examined, the activation of the PRF produced EPSPs, although in a few cases IPSP and EP-IPSP sequences were noted. It was also demonstrated that these potentials affected the motoneurons of the oculomotor nucleus independent of the activity of the vestibular nucleus. Specifically, they reported that paired stimulation to both areas resulted in a summation of postsynaptic potentials rather than

an occlusion of the two potentials as would be the case if they were arising within the same system.

Besides these reticular formation produced modifications in the extraocular muscle system, other lower centers have been shown to modify the activity of neurons in this system. Shimo-Oku (1970) has shown that repeated trigeminal stimulation produces a reduction of the complex evoked potential recorded from the oculomotor nucleus when the inferior oblique nerve is stimulated. In addition, Hikosaka and Maeda (1973) have found that stimulation of the contralateral dorsal root at the level of C2 or C3 induced IPSPs in abducens motoneurons or facilitated vestibular induced IPSPs in the abducens nucleus. On the other hand, stimulation of the ipsilateral dorsal root at the same levels produced EPSPs in the abducens nucleus or facilitated vestibular nucleus induced EPSPs in the nucleus. Given the latencies of the EPSPs and IPSPs induced in the abducens nucleus, it appears that these potential changes were the result of activation of vestibular neurons rather than direct activation of the abducens motoneurons.

In addition to the potential changes produced in the motoneurons of the extraocular muscle system by brainstem nuclei, a number of investigations have reported that stimulation in the accessory oculomotor nuclei produces potential changes in this system and some of its related structures. For example, Markham, Precht and Shimazu (1966) reported that

stimulation of the interstitial nucleus of Cajal produced an inhibition of activity in the vestibular nucleus. Stimulation in areas immediately adjacent to the interstitial nucleus of Cajal as well as in the reticular formation, posterior commissure, and cerebral peduncles produced much less of an effect on the neurons in the vestibular nucleus. Schwindt, Precht and Richter (1974) reported that stimulation of the internuclear area, consisting of the interstitial nucleus of Cajal and nucleus of Darkschewitsch, produced an EPSP-IPSP sequence in the motor neurons of the trochlear nucleus. Given the latency of the potential changes and the results of control experiments, it does not appear that the observed effects were the result of activating collaterals of the ascending vestibular pathway. Stimulation in the medial aspects of the internuclear region failed to effect abducens motoneurons but did produce EPSPs and IPSPs in reticular formation neurons just ventral to the nucleus (cf. Cohen & Komatsuzaki, 1972). However, stimulation just lateral to the effective region for trochlear motoneurons did produce short latency EPSPs in abducens motoneurons. In addition, Szentagothai and Schab (1956) reported that vestibular induced excitation in the oculomotor nucleus is completely eliminated during stimulation of the nucleus of Darkschewitsch. However, they reported that lesioning of the nucleus of Darkschewitsch did not produce degeneration in the oculomotor nucleus, suggesting



that the inhibitory effect of the stimulation was relayed to the oculomotor nucleus via the disruption of one of the excitatory pathways to the nucleus.

Stimulation of the visual system has also been shown to modify the activity of the motoneurons of the extraocular muscle system. Precht, Schmidt and Magherine (1971) observed that tectal stimulation produced substantial inhibition in abducens motoneurons ipsilateral to the stimulation site while producing excitation in contralateral motoneurons. Precht et al. also reported that neurons adjacent to the VI nucleus but not antidromically activated by VI nerve stimulation were also affected by tectal stimulation. The response characteristics of these interneurons were similar to those produced in the abducens motoneurons except that ipsilateral tectal stimulation produced only EPSPs in these neurons and not IPSPs as was observed in the abducens motoneurons. In addition, they observed that stimulation of the tectum produced little effect on the motoneurons of the trochlear nucleus. Shimo-Oku (1974) reported that optic nerve stimulation produces a facilitation of antidromically induced activity in the oculomotor nucleus. In both investigations, the latency of the observed effects were usually greater than 1 msec and sometimes as high as 6 to 10 msec, suggesting that induced activity was mediated by at least a disynaptic pathway.



Summary. The extraocular motor system has three major inputs. They consist of (a) the internuclear area which is made up of the various accessory oculomotor nuclei and may serve to integrate information from higher CNS levels necessary for the regulation of the extraocular motor system, (b) the vestibular system consisting principally of the superior and medial portions of this nucleus, and (c) the reticular formation--nucleus prepositus hypoglossi area. The vestibular system input appears to be a simple reflexive network which regulates eye position as a function of head orientation. Therefore, this system would probably not be involved in development or modulation of a learned response related to the extraocular motor system even though Miles and Fuller (1974) have presented evidence suggesting that there may be some adaptive plasticity in this system. A number of factors suggest that the other two systems, either separately or together function as the system responsible for the major types of adaptive plasticity which can develop within the components of the extraocular motor system. First these structures receive and integrate a variety of sensory information while the vestibular system's primary function is to integrate information related to position of the head in space. Secondly, the modulating role of these structures in relation to eye movements is rather complex in comparison to the movements produced via the vestibular system. For

example, stimulation in portions of the reticular formation produces rather complex ocular movements while activation of the vestibular system produces either excitation or inhibition in specific regions of the extraocular motor system. Thirdly, Groves and Lynch (1972) have suggested that the anatomical makeup of the reticular core makes it an ideal location for the fundamental neural changes which take place during the habituation process, and Groves, Miller and Parker (1972) have observed habituation and sensitization to repetitive sensory stimuli in neurons located within the reticular formation. Given these findings, it may be possible that the reticular core is responsible for the plastic changes which govern the acquisition of all basic responses. For example, Markel and Adams (1969) have demonstrated that encephalo-isolé rats can acquire a conditioned heart rate response and preliminary observations in our laboratory suggest that encephalo-isolé rabbits can manifest a conditioned NMR. Except for the cerebellum, the predominant neural tissue left functioning in these encephalo-isolé animals is diffuse reticular type tissue. It would seem that this reticular tissue located in the midbrain and brain stem is at least capable of developing plastic changes in the nervous system which determine how the organism will respond to a particular set of sensory information. If this area is not the primary locus of the plastic changes occurring during the acquisition

of a particular response, its minimal function must be to modulate the activity related to the manifestation of the response.

### General Overview

The anatomical and physiological investigations of the extraocular motor system have localized the major regulatory inputs for the system within the midbrain and brain stem. Although few of these investigations have focused on the retractor bulbi response, the intimate relationship of this defensive reflex with the other components of the extraocular motor system suggests that some of the elements of this basic system may also play a role in modulating the retractor bulbi response (NMR). The following experiments were designed in an effort to specify which elements of the midbrain and brain stem are part of the neural system which modulates the input to the abducens nucleus necessary for the execution or inhibition of a conditioned NMR. The purpose of Experiment 1 was to determine which portions of the midbrain and brain stem facilitate or inhibit the unconditioned NMR while Experiment 2 was designed to extend this analysis by delineating the elements of the midbrain and brain stem which disrupt the manifestation of a conditioned NMR. Experiment 3 examined the effects lesions placed in the midbrain and brain stem had on the development of learned inhibition in Pavlov's conditioned inhibition paradigm. The results of these

experiments suggest that areas of the CNS which were once thought to play little if any role in the development of adaptive responses are intimately involved in the development and modulation of at least one of these learned responses, the rabbit NMR.

### EXPERIMENT 1

The aim of Experiment 1 was to electrically stimulate various regions of the midbrain and brain stem of the rabbit in an effort to determine if other neural structures, in addition to the abducens nucleus, are involved in the manifestation (modulation) of the NMR. For this purpose, trains of brain stimulation preceded and overlapped a peripherally applied stimulus which would unconditionally elicit a reflexive NM closure. The primary dependent variable of interest was the effect of brain stimulation to a particular midbrain site on the amplitude of the elicited NMR.

### Method

#### Animals

Twenty-seven male and female albino rabbits weighing between 2.6 and 3.6 kgs were used in this experiment.

#### Apparatus

Two Grass model S-88 stimulators in conjunction with stimulus isolation and constant current units were used to present the ESB and unconditioned stimulus (US). A rotary

minitorque potentiometer coupled to a suture in the right nictitating membrane of the rabbit served to convert any lateral movement of the NM to a dc signal that was recorded on a Grass model 5D polygraph. One channel of the polygraph was also used to record the onset and offset of the ESB and US.

### Surgery and Procedure

One hour prior to the start of surgery, each rabbit was injected with approximately 12 mg/kg of Thorazine (IM) to potentiate the effects of the Nembutal anesthetic (IV, 20-25 mg/kg, diluted with physiological saline). Maintenance of a sustained analgesic level throughout the experiment was accomplished through the use of intraperitoneal injections of Demerol (33 mg/kg/hr).

After the rabbit's head was fixed in the head holder of a large Kopf stereotaxic instrument equipped with a rabbit adapter, the effective unconditioned stimulus (US) intensity necessary to elicit a reflexive nictitating membrane closure (unconditioned response, or UR) was determined. Because the rabbits were given large doses of Demerol to maintain the analgesic level, the US found to elicit a reflexive NM closure comparable in amplitude to those observed in conditioning studies was a 4 mA square wave pulse of 50 msec duration. The US was presented across two wound clips, one of which was placed approximately 1/2 cm below the inferior right eyelid,



while the other was placed 1/2 cm posterior to the temporal canthus. In the first few rabbits the shock electrode locus was varied to determine the most effective locus but in most cases the previously described placement elicited the most robust NMR.

Following this test for UR amplitude, a 4 to 5 cm midline incision was made extending caudally from between the eyes. The skull was exposed, cleaned and dried. Next a craniotomy was performed to remove the skull beginning 6 mm posterior to bregma and extending to a point approximately 18 to 19 mm behind bregma. Care was taken to leave lambda intact. The lateral extent of the craniotomy was approximately 5 mm on both sides of the midline. At the completion of the craniotomy, the dura surface was covered with mineral oil to prevent drying of the dura and underlying cortical tissue.

The skull was then aligned with the stereotaxic instrument such that lambda was 1.5 mm below bregma. Following this, the effect that midbrain and brain stem stimulation had in modifying the UR was determined. Between 12 and 24 electrode approaches were made in each animal. For the electrodes aimed in the vicinity of the oculomotor nucleus, testing for the physiological effects of the ESB and the effects of the stimulation on the UR were first assessed when the electrode tip was approximately 11 mm below the dura surface, and continued through approximately another 4 to 5 mm of neural



tissue. This corresponded to an area about 1 mm above the oculomotor nucleus and extended to a point about 1 mm above the base of the brain. When the electrodes were aimed in the vicinity of the abducens nucleus, the physiological effects of the stimulation were first assessed when the electrode was approximately 13 mm below the dura surface and continued through approximately another 7 mm of neural tissue. In this area, the testing began when the electrode was located deep in the cerebellum and continued to a point below the abducens nucleus into the reticular formation. During the course of the experiment more than 150 electrode approaches were made and between 3 and 6 neural levels were tested during each approach.

The parameters of the ESB were varied from a minimum of 500 pps, .01 msec pulse duration, and 50  $\mu$ A pulse amplitude to a maximum of 1000 pps, .25 msec pulse duration, and 300  $\mu$ A pulse amplitude. The usual train duration was 500 msec, although in a few instances it was decreased to as low as 100 msec. The level of the ESB used to assess the effects of stimulation at a particular loci on the UR was usually set at the highest total stimulus energy (TSE) level (pulse frequency X pulse duration X pulse amplitude) which did not produce any gross body movements. The temporal relationship between the ESB and the peripheral shock which elicited the UR was arranged so that the eyeshock overlapped the last 50 msec of the brain stimulation.

The general procedure employed when examining a particular locus was to first determine if the ESB elicited some physiological response. Then a four trial sequence consisting of a test trial, two control trials, followed by another test trial was presented. On the test trials both ESB and eye shock were presented while on control trials only eye shock was presented. This double alternation sequence was presented from a minimum of 1.5 times to a maximum of 8 times at any given locus. The ITI was usually 15 seconds, although during a few experiments a very short ITI was employed to determine if there was some habituation to the ESB. Using this stimulus presentation sequence, the probability that the ESB modified the amplitude of the UR in the same direction on 5 test trials was .031. This was the criterion for classifying a neural locus as an effective site for modifying the reflexive NMR.

Following completion of the experiment, the rabbits were given an overdose of Nembutal and then perfused with isotonic saline followed by 10% formalin. The brains were then removed, stored in formalin, embedded in low viscosity nitrocellulose, and then cut at 40  $\mu$ . Every fourth section through the area investigated was then stained either with cresyl violet or with a modification of the Kluver-Barrera method for staining cells and fibers. The location of the electrode tips was determined with the aid of the Gerhard (1968) and Meessen and Olszewski (1949) atlases.

## Results and Discussion

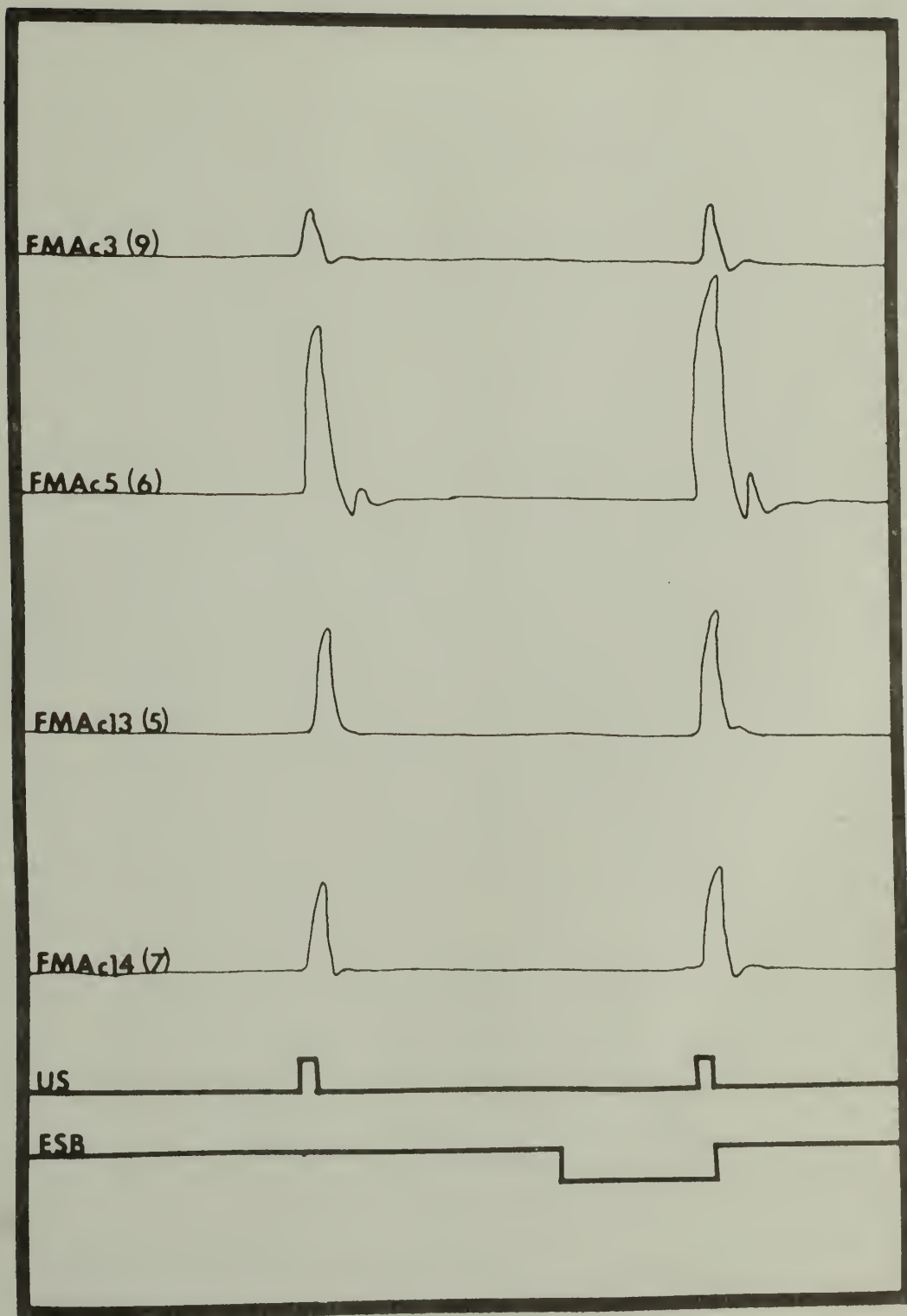
Given complications which arose during the course of the experiment, the data from only 9 of the animals was subjected to analysis.

Figure 5 depicts tracings of polygraph records which demonstrate the relative augmentation of the reflexive NMR produced by the brain stimulation. As seen in the figure, the augmentation was relatively small, usually on the order of 1 to 4 mm, and in only Electrode 6 for rabbit FMA-5 was the augmentation greater than 5 mm. In general, the ESB increased the NMR amplitude by an additional 5 to 20% of the control NMR amplitude. The parameters of brain stimulation employed during test situations from which Figure 5 was derived were 1000 pps, pulse duration varied from .03 to .1 msec, pulse amplitude varied from 200 to 300  $\mu$ A and train duration varied from 300 to 500 msec.

Figure 6 shows tracings of polygraph records which demonstrate the relative diminution in the reflexive NMR produced by the brain stimulation. As in the situations where ESB produced an augmentation of the NMR, the ESB reduced the NMR amplitude by between 1 and 4 mm which corresponded to relative decreases in NMR amplitude of between 5 and 20%. The parameters of ESB employed during these testing situations corresponded to those used during tests where the ESB produced an augmentation of the NMR.

## FACE PAGE FOR FIGURE 5

Figure 5. Tracings of polygraph records which depict the augmentation in the unconditioned NMR produced by brain stimulation. The responses depicted in column one were taken from trials during which no brain stimulation was presented, while the responses depicted in column two were redrawn from trials during which brain stimulation preceded the US. The number in parentheses refers to the electrode locus (cf. Figure 7) which produced the observed augmentation.



## FACE PAGE FOR FIGURE 6

Figure 6. Tracings of polygraph records which depict the diminution in the unconditioned NMR produced by brain stimulation. The responses depicted in column one were redrawn from trials during which no brain stimulation was presented while the responses depicted in column two were redrawn from trials during which brain stimulation preceded the US. The number in parentheses refers to the electrode locus (cf. Figure 7) which produced the observed diminution.



FMA 3 (12)

FMAc 5 (8)

FMAc 6 (9)

FMAc 14 (10)

US

ESB

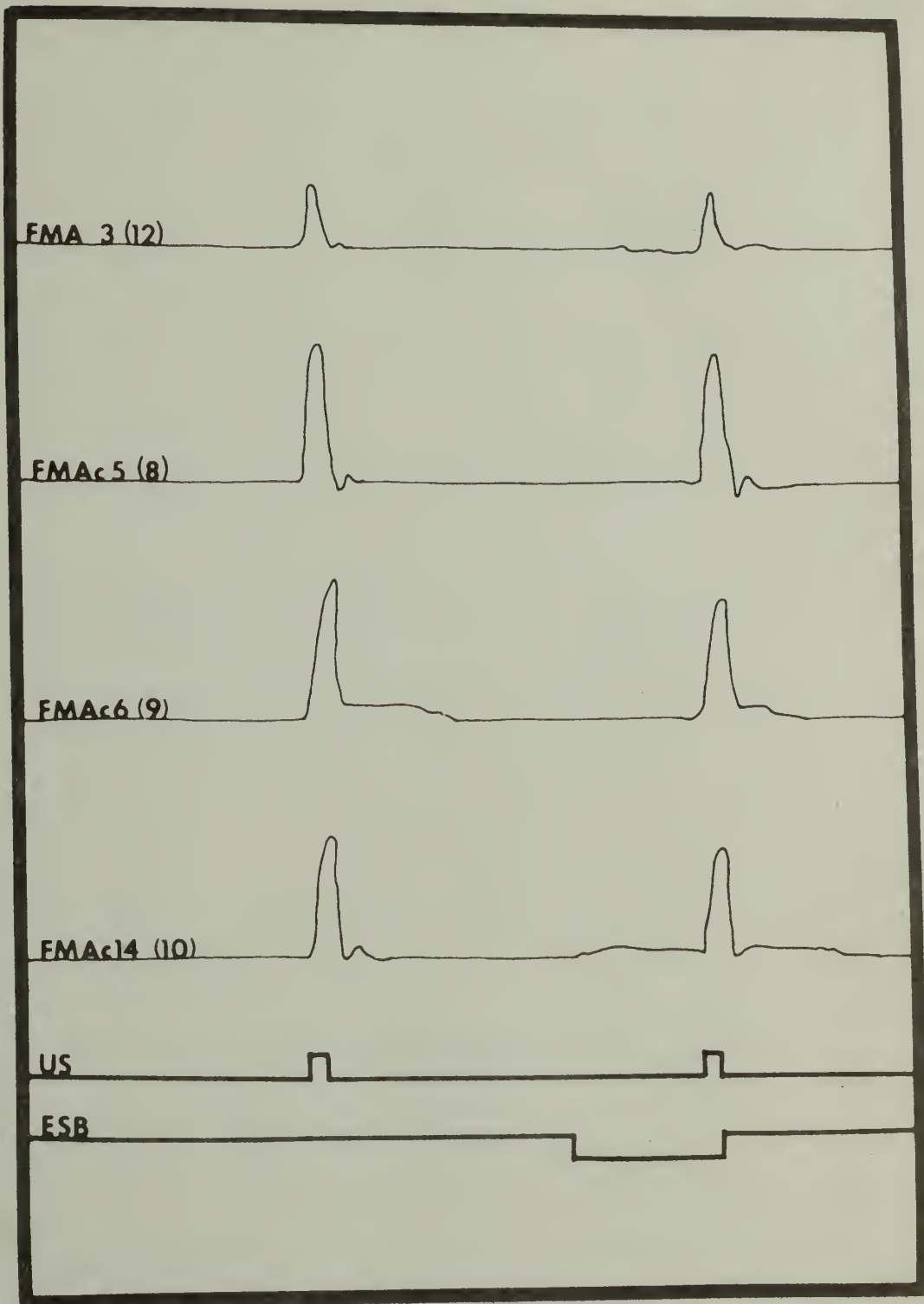


Figure 7 depicts the electrode tips or approximate location in the electrode tract where ESB produced its effect for all the approaches made during this experiment. As seen in the figure, the majority of the loci which were effective in modifying the UR were located within a few millimeters of the midline, and were usually located below the level of the third or fourth ventricle, or the Aquaduct of Silvius. Specifically, stimulation of the red nucleus, locus coeruleus, portions of the reticular formation and the small-celled nuclei surrounding the abducens nucleus tended to produce a diminution in the amplitude of the UR. On the other hand, stimulation in the interstitial nucleus of Cajal, the central gray and portions of the reticular formation tended to produce an augmentation in the amplitude of the UR. Unfortunately, the results do not unequivocally suggest a specific arrangement of the augmentation or diminutive areas within the midbrain and brain stem of the rabbit. However, it appears that stimulation in the more anterior portions of the midbrain tended to produce a facilitation of the response.

In general, this midbrain-brain stem facilitory-inhibitory arrangement is similar to that described by Magoun and Rhines (1946) and Rhines and Magoun (1946) for the cat and monkey. However, examination of the kymograph records of the effect brain stem stimulation had on the blink reflex evoked in the Magoun and Rhines (1946) investigation indicates that their

## FACE PAGE FOR FIGURE 7

Figure 7. The location of the electrode tips for all the rabbits used in this experiment. An open square depicts the approximate location of an electrode in which ESB applied via that electrode produced an augmentation in the amplitude of the UR. A solid square represents the approximate location of an electrode in which ESB applied via that electrode produced a diminution in the amplitude of the UR. A solid circle is used to depict the tip of an electrode tract which did not alter the amplitude of the UR. All outlines are drawn from the Gerhard, 1968 atlas, plates 31, 32, 34, 35, and 38, and the Meessen and Olszewski, 1949 atlas, plates 10, 12, and 14. See Table 1 for a list of abbreviations used in this figure.



Table 1

Explanation of Abbreviations used in Figure 7.

Aqu	Aqueduct of Sylvius
BIC	Brachium of the Inferior Colliculus
CG	Central Gray
Coch	Cochlear Nucleus
Coe	Locus Coeruleus
D	Nucleus of Darkschewitsch
DBC	Decussation of the Brachium Conjectivium
EW	Edinger Westphal Nucleus
IC	Inferior Colliculus
INC	Interstitial Nucleus of Cajal
IP	Interpeduncular Nucleus
LLe	Lateral Lemniscus
LVNd	Lateral Vestibular Nucleus, dorsal portion
LVNv	Lateral Vestibular Nucleus, ventral portion
MGN	Medial Geniculate Nucleus
MLe	Medial Lemniscus
MLF	Medial Longitudinal Fasciculus
MVN	Medial Vestibular Nucleus
NP	Pontine Nuclei
PR	Pontine Reticular Formation
Prt	Pretectal Nucleus
PTH	Posterior Thalamus
Pyr	Pyramidal tract



Table 1 (continued)

R	Red Nucleus
Rm	Red Nucleus, magnocellular portion
Ra	Raphe
RB	Restiform Body
SC	Superior Colliculus
SCN	Superior Central Nucleus
SN	Substantia Nigra
SO	Superior Olive
SVN	Superior Vestibular Nucleus
TgR	Tegmental Reticular formation
Trap	Trapezoid Body
Y	Y group of the Vestibular Nucleus
III	Oculomotor Nucleus
IV	Trochlear Nucleus
V	Trigeminal Nerve
Vm	Trigeminal Nucleus, motor portion
Vs	Trigeminal Nucleus, sensory portion
VI	Abducens Nucleus
VII	Genu of the Facial Nerve
VIII	Auditory Nerve

observed effects were much more pronounced than those observed in the present investigation. In particular, one of the kymograph records (Figure 1A, Magoun and Rhines, 1946) indicated that the stimulation completely inhibited the blink reflex. Complete inhibition of the blink reflex was never observed in the present investigation. Given the many differences between these studies, it can only be suggested that the differences between the studies appears to be related to the nature of the conditioning stimulus employed. Specifically, Magoun and Rhines (1946) and Rhines and Magoun (1946) used relatively long brain stimulation durations and repeatedly elicited an UR during this stimulation period, while in the present experiment, a discrete 100 to 500 msec train of brain stimulation was presented prior to eliciting the response. The discrete nature of the stimulation presented in this experiment would probably have eliminated most of the modulatory effects of the ESB which were the result of a summation of inhibitory or facilitory activity over the long stimulus durations employed in these earlier studies.

## EXPERIMENT 2

The results of Experiment 1 suggest that specific regions located in the midbrain and brain stem of the rabbit, which are not directly responsible for the execution of the unconditioned NMR, may play a role in modulating the precise character of the UR. For example, electrical stimulation

applied to an area surrounding the abducens nucleus tended to reduce the amplitude of the unconditioned NMR elicited in the anesthetized rabbit. Although stimulation of these structures modified the character of the unconditioned NMR, it was not clear that stimulation of this system could also modulate the topography of the conditioned NMR. Attempts to establish a conditioned NMR in an anesthetized rabbit using a variety of CSs proved unsuccessful. Therefore, in order to determine if these structures also modulated the character of the CR, electrodes were chronically implanted in the mid-brain and brain stem of the rabbit. Following recovery from surgery and after establishing a conditioned NMR, the effects that ESB had on the topography of the CR were investigated.

There have been a number of reports suggesting that subcortical brain stimulation can produce a disruption of both learned and unlearned behaviors (Plumer, Siegel, & Cicala, 1973; Lineberry & Siegel, 1971). Most of this research has focused on the disruptive effects produced by stimulation of the caudate nucleus. Analysis of this system has shown that low frequency stimulation of this structure produces an inhibition of an ongoing operant response (Wilburn & Kesner, 1974; Deadwyler et al., 1974), the manifestation of a classically conditioned response (Plumer, et al., 1973), hypothalamically induced attack behavior (Plumer & Siegel, 1973) and eating behavior (Lineberry & Siegel, 1971). The disruption of these

behaviors has been correlated with a general inhibition of single unit activity in various portions of the mesencephalic reticular formation, the hypothalamus, the thalamus, and the cortex (Siegel & Lineberry, 1968; Siegel & Wang, 1974). The powerful inhibitory properties of caudate nucleus stimulation suggests that this structure is not explicitly involved in the elaboration of a particular response, but it may function to produce an overall inhibition of the normal activity of the CNS.

Some casual observations made during the course of Experiment 1 indicated that slightly higher levels of stimulation applied to a number of the loci which modulated the character of the UR produced distinct movements in the extraocular motor system. Still higher levels of stimulation increased the intensity of these extraocular motor system responses and as the stimulation was increased it sometimes induced more general body movements. Given that the initial motor response elicited by the ESB involved the extraocular muscles, it appears that the electrodes were located within structures (i.e., the nucleus of Cajal and the nucleus of Darkschewitsch) whose function was more closely related to the regulation of the extraocular motor system than some other motor system. The results of the present experiment in conjunction with the findings of Experiment 1 should provide a more precise analysis of which midbrain and brain stem nuclei

are specifically related to the modulation of both the learned and unlearned retractor bulbi response of the rabbit.

### Method

#### Animals

The animals were 12 experimentally naive male and female albino rabbits approximately 100 days old when brought to surgery and weighing between 2.9 and 3.35 kgs. At all times the animals were maintained in individual cages on ad lib food and water.

#### Apparatus

A detailed description of the apparatus and technique for recording from the nictitating membrane (NM) is available elsewhere (Gormezano, 1966). Two rabbits were run concurrently in the upper two drawers of a four drawer fire-proofed file cabinet that was ventilated and illuminated. A panel in front of the subjects supported two house lights (28 V dc. behind translucent white plastic) and two impedance matched speakers which were used to present the tonal CS. The unconditioned stimulus was administered via stainless steel wound clips attached approximately 1/2 cm below and posterior to the right eye, and consisted of a 2 mA shock of 50 msec duration.

Each rabbit was restrained within a Plexiglas box similar to those described by Gormezano. A rotary minitorque potentiometer coupled to a suture in the right nictitating membrane



of the rabbit served to convert any lateral movement of the right nictitating membrane to a dc signal that was recorded on a two channel Beckman RP Dynograph. A conditioned response (CR) was defined as a 1 mm positive deflection of the recording pen and was equal to less than a 1 mm lateral movement of the NM.

The ESB was delivered via a Grass Model S-88 stimulator in conjunction with stimulus isolation and constant current units. The parameters of brain stimulation were varied from 100 to 1000 pps, pulse duration varied from .001 msec to .25 msec, and the pulse amplitude varied from 30  $\mu$ A to 400  $\mu$ A.

#### Surgery and Histology

Each rabbit was implanted with 5 monopolar electrodes, aimed at various portions of the extraocular motor system. Stereotaxic placement of the electrodes was based on coordinates from Sawyer, Evert, and Green (1954). The electrodes were constructed of 00 Clay-Adams insect pins (shaft diameter 0.25 mm; tip diameter 0.03 mm), insulated with insul-X except for an area 0.5 mm from the tip.

Approximately one hour prior to surgery, each rabbit was injected with 12 mg/kg of Thorazine (IM) to potentiate the effects of Nembutal anesthetic (IV, 20-25 mg/kg diluted with physiological saline). Prior to placing the rabbit in a large Kopf stereotaxic frame equipped with a rabbit adapter, the animal was injected with Xylocaine near the zygomatic

arches and then a subcutaneous application of Xylocaine with epinephrine to the scalp.

The procedure for implanting electrodes in the rabbit is the same as for the rat (see Miller, Coons, Lewis & Jensen, 1961). Briefly, a 4 to 5 cm midline incision was made extending caudally from between the eyes. The skull was then exposed, cleaned and dried. Three stainless steel jeweler's screws .061 inches in diameter were then inserted into the skull to anchor the dental cement. One was located approximately 9 mm anterior to bregma and 5 mm lateral to the midline and served as the reference electrode, while the others were located approximately 6-12 mm posterior to bregma and 7 mm lateral to the midline.

The skull was then aligned with the stereotaxic instrument such that lambda was 1.5 mm below bregma. Then the placements for the electrodes were marked on the skull with the stereotaxic instrument, and small holes drilled into the bone. The electrodes aimed in the vicinity of the abducens nucleus were implanted first. A small amount of dental cement (William Getz Corp.) was placed around the electrode and one of the posterior anchor screws, and then the liquid fastener was applied to the cement. After the cement had hardened, the stereotaxic arm was removed from the electrode. After all the electrodes had been implanted and the protruding end of each electrode clipped off, the leads from the electrodes

were connected to an Amphenol socket and the entire assembly secured to the skull with dental cement. In addition, three steel machine screws were cemented to each subject's acrylic plug to provide a means for rigid support of the head during the testing procedure. Each rabbit was given a minimum of 10 days to recover from surgery prior to being run in the experiment.

Following training and testing, the animals were given an overdose of Nembutal and perfused with isotonic saline followed by 10% formalin. The brains were then removed, stored in formalin, and then embedded in low viscosity nitro-cellulose. Sections of the brain were cut at 40  $\mu$  and every fourth section was then stained with a modification of the Kluver-Barrera method for cells and fibers. The location of the electrode tips was determined with the aid of the Meessen and Olszewski (1949) and Gerhard (1968) atlases.

### Procedure

A summary of the testing sequence employed in this experiment is presented in Table 2. On the day prior to the first conditioning session, a small loop was placed in the right nictitating membrane and the rabbits were habituated to the apparatus by being placed in the restraining box and remaining in the experimental enclosure for at least 45 minutes.

Training began on the next day and consisted of 100

Table 2

## Summary of Procedures Employed in Experiment 2\*

<u>Phase 1</u>	<u>Phase 2</u>	<u>Phase 3</u>	<u>Phase 4</u>
Sutured and habituated to	Acquisition of CR	Testing of each electrode for disruptive properties	Retesting of electrodes which appear to disrupt the CR
<u>Phase 5</u>	<u>Phase 6</u>	<u>Phase 7</u>	<u>Phase 8</u>
Videotape the muscular responses produced by the ESB at each electrode loci	Test for effects of ESB on UR	Transect extraocular muscles in 5 of the rabbits	Retest each electrode for disruptive properties

\* It should be noted that only some of the rabbits underwent Phases 6,7, and 8.

presentations of a 550 msec tone (1200 Hz, 85 dB SPL) with the US overlapping the last 50 msec of the CS. The inter-trial interval was 30 seconds. This phase of the experiment continued until the CS elicited better than 90% conditioned responses (CRs) on any one day. Usually 2 days of acquisition training was sufficient to reach this criterion.

On the following day testing was begun to determine what effect ESB had on the CR. Testing consisted of 100 reinforced conditioning trials. On half of the trials ESB was presented to one of the electrode loci. The testing sequence consisted of a double alternation procedure in which two control trials (trials during which no ESB was presented) were followed by two test trials. During the first 60 to 80 trials of the session, on those trials when ESB was presented (test trials), the ESB preceded the CS by 250 msec. The train duration during this testing sequence was 750 msec. The general procedure employed for determining if the parameters of the ESB should be altered was: (a) if the ESB elicited vocalizations or sustained kicking from the rabbit the total stimulus energy (TSE) of the ESB was substantially reduced; (b) if the ESB reduced the amplitude of the CR after 5 to 10 test trials, one parameter of the ESB was reduced by a factor of two; and (c) if the ESB produced no alterations in the character of the CR or only a slight modification of the topography of the CR, one parameter of the ESB was increased by



a factor of two. During the last 20 to 40 trials of the session, the ESB-CS relationship was reversed such that CS onset occurred 250 msec prior to the onset of the ESB. The CS duration during this testing sequence was 750 msec, while the ESB train duration was 500 msec. Usually during this testing sequence, the parameters of the ESB were not varied. After testing all five electrode loci, those electrodes which appeared to alter the manifestation of the CR were retested to confirm the effects observed during the first testing of each electrode locus.

Following the completion of this testing sequence, a videotape record of any muscular responses elicited by the ESB to the various electrode loci was made. This record was made in order to determine whether the observed effects of ESB on the CR were the result of specific changes in the position of the facial muscles or the extraocular muscles.

After the completion of the videotape recording, six rabbits were given an additional test session during which the effects of ESB on the unconditioned NMR was assessed. During this testing sequence, the ESB preceded the onset of the US by 500 msec and remained on for an additional 250 msec (train duration was 750 msec). The US was a 2 mA AC shock of 50 msec duration. Ten test trials and their associated control trials were presented at each electrode locus. The parameters of the ESB employed in this testing sequence were

identical to those used during the last sequence to test the effects of ESB on the CR.

A few weeks after this phase of the experiment, five of the rabbits were reanesthetized and the four recti and two oblique muscles were sectioned. Ten days after this procedure, the rabbits were retested to determine the effects of ESB on the conditioned response. The procedures and parameters employed to test the effects of ESB on the CR were similar to that employed earlier.

A sign test was used to determine if the diminution of the CR observed during a given testing sequence was consistent. An .05 significance level was chosen as the criterion for specifying that ESB applied to a given locus produced a consistent reduction in the amplitude of the CR.

### Results

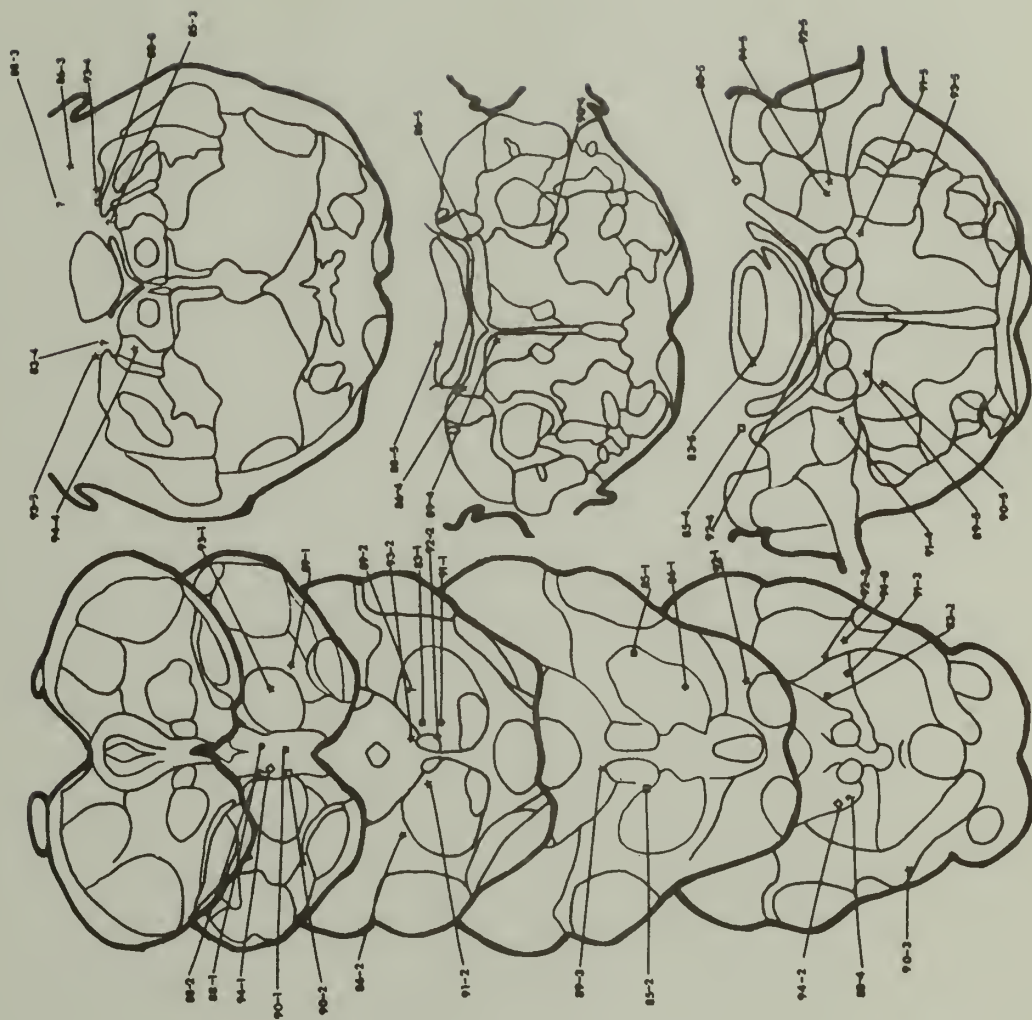
During the testing sequence, two rabbits dislodged the electrode cap and were sacrificed prior to completing the experiment. The data from these rabbits was not included in the analysis of this experiment.

### Histology

Figure 8 depicts the location of the electrode tips for all the rabbits used in this experiment. In general, a solid symbol indicates that ESB applied via that electrode produced a substantial reduction in the amplitude of the conditioned NMR, while an open symbol indicates that ESB applied via that

## FACE PAGE FOR FIGURE 8

Figure 8. The location of the electrode tips for all the rabbits used in this experiment. A solid square indicates that application of ESB to this locus produced a substantial reduction in CR amplitude both when the extraocular muscles were intact and following transection of the muscles. An open square indicates that a small but consistent reduction in CR amplitude was observed both when the extraocular muscles were intact and following transection of the muscles. A solid and open diamond were used to depict substantial and small but consistent reductions, respectively, in rabbits which were only tested with the extraocular muscles intact. A solid circle was used to depict the location of electrode tips which produced a substantial reduction in CR amplitude only after transection of the extraocular muscles. A question mark was used to indicate the location of electrode tips which, following transection of the extraocular muscles, produced a somewhat interesting modification in the topography of the CR. A star was used to depict the location of electrode tips which did not produce a diminution in the amplitude of the CR. Outlines are drawn from the Gerhard, 1968 atlas, Plates 31, 32, 34, 35, and 38, and the Meessen and Olszewski, 1949 atlas, Plates 10, 13, and 14.



electrode produced a small but consistent reduction in the amplitude of the conditioned NMR. A substantial reduction in the amplitude of the CR was defined as a mean decrease of at least 40% relative to the amplitude of a CR elicited during a trial during which no ESB was presented. A small reduction in the amplitude of a CR was defined as a mean decrease of at least 10% but not more than 40% relative to the amplitude of a CR elicited during a trial during which no ESB was presented. In addition, if the reduction in CR amplitude exhibited signs of habituating during the course of a test session, the observed reduction in CR amplitude was attributed to the distracting properties of the ESB rather than to the ability of the ESB to modulate the activity of the neural system controlling the CR. A star was used to indicate the location of electrode tips which did not modify the amplitude of the CR during testing.

Examination of Figure 8 suggests that a system for disrupting the conditioned NMR may originate in the internuclear area consisting of the interstitial nucleus of Cajal and nucleus of Darkschewitsch. At the level of the oculomotor nucleus the path of this system appears to move laterally through the small-celled portion of the red nucleus. As the system passes the trochlear nucleus, the structures involved in modulating the CR were the ventral portions of the central gray and the tegmental reticular formation.



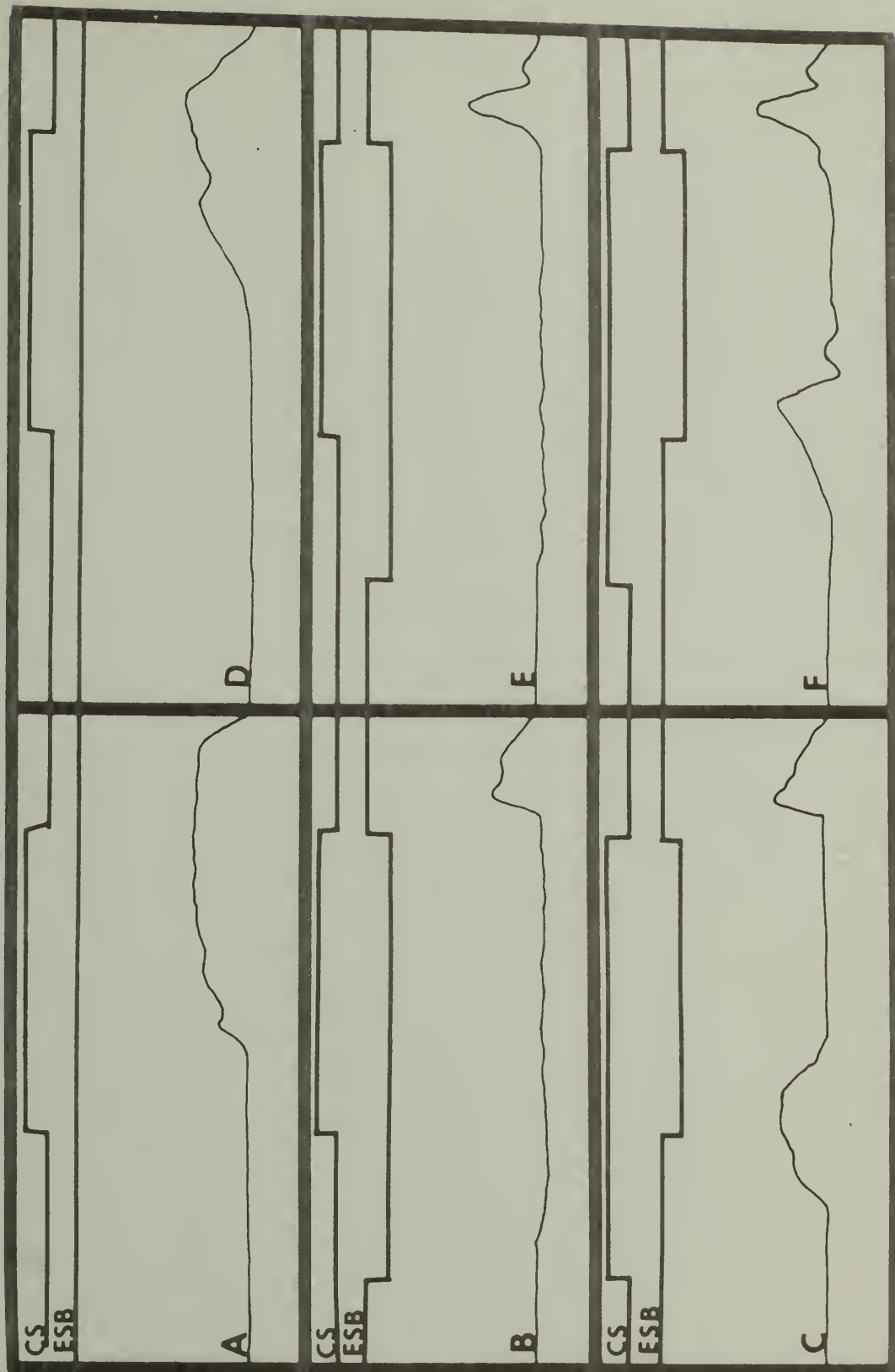
### Effects of ESB on the CR

ESB-CS trials. Figures 9b and 10b depict the topography of two CRs for rabbits FM85 and FM90, respectively, which were judged to be substantially reduced when ESB was applied to Electrode 1 for these rabbits. Comparing panels A and B of the two figures, one observes a completed inhibition of the CR for rabbit FM85, while in rabbit FM90 the ESB produced a NM closure equal to approximately one half the amplitude of the normal CR (panel A) and prevented any manifestation of the CR. Electrode 1 for rabbit FM85 was located on the right side, in the lateral aspects of the small-celled portions of the red nucleus, while in rabbit FM90 Electrode 1 was located on the right side near the midline in the internuclear area.

Figures 11b and 12b depict the topography of two CRs which were judged to be partially inhibited by the application of ESB. As seen in Figure 12b the application of ESB to Electrode 2 for rabbit FM94 basically increased the latency of the CR without producing much diminution in the amplitude of the response (cf., Figure 12a). When ESB was applied to Electrode 2 for rabbit FM90, (cf., Figure 11a & b) the disruption of the CR was manifested by both an increase in the rise time of the CR and a slight diminution in the amplitude of the CR. Electrode 2 for rabbit FM94 was located on the left side in the central gray, while in rabbit FM90 Electrode 2 was located on the left side in the internuclear area approximately 1 mm away from Electrode 1 for this rabbit.

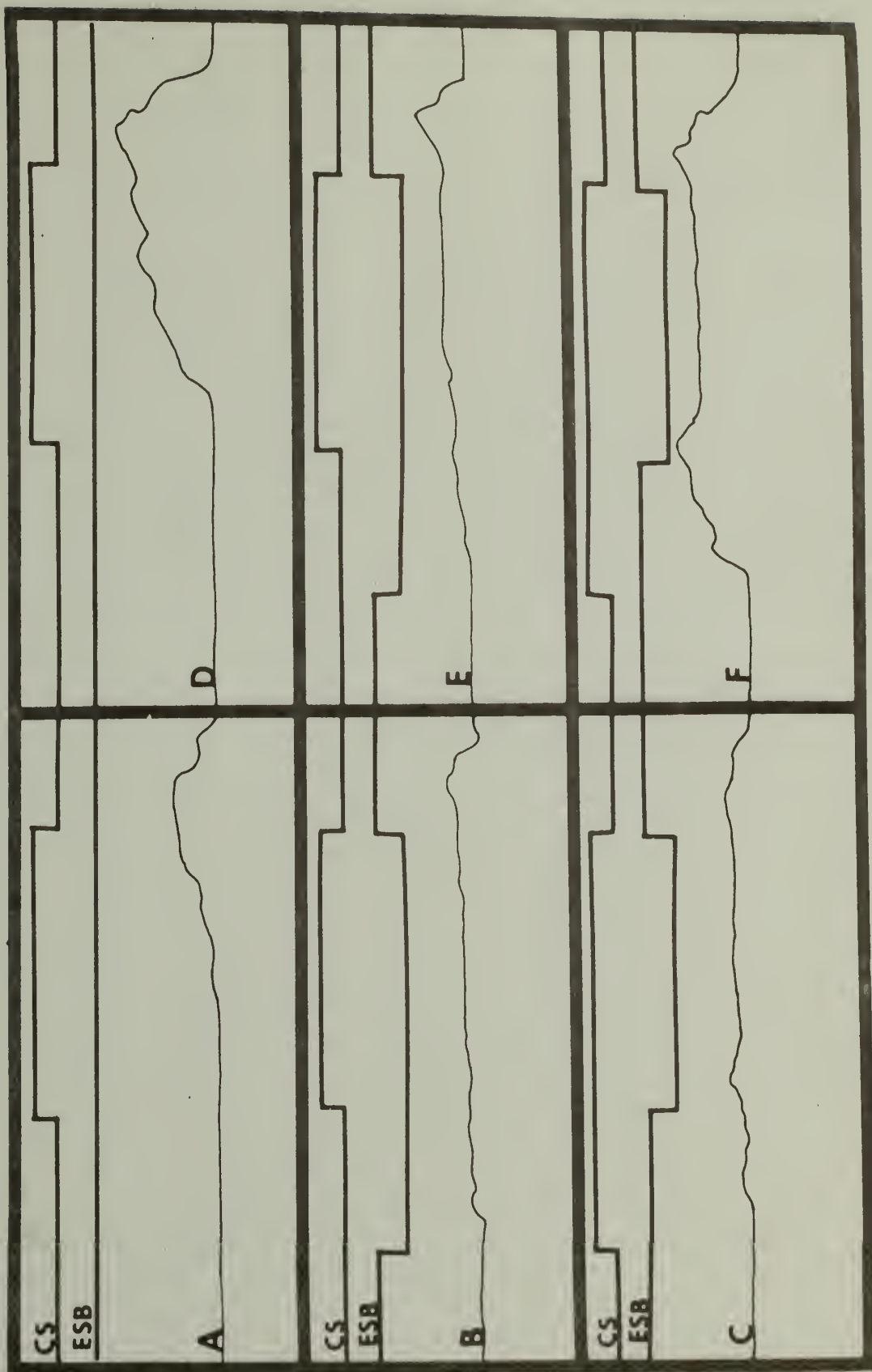
## FACE PAGE FOR FIGURE 9

Figure 9. Tracings of polygraph records which depict the substantial reduction in the CR amplitude produced by stimulation of Electrode 1 for rabbit FM85. Panel A depicts the normal CR topography when the extraocular muscles were intact while Panel D depicts the CR topography following transection of the extraocular muscles. Panels B and D depict the changes in the CR topography observed when brain stimulation precedes the onset of the CS while Panels C and F depict the changes in the CR topography observed when the onset of the CS precedes the onset of the ESB. The parameters of the ESB employed during these tasks were 1000 pps, .05 msec pulse duration, and 50  $\mu$ A pulse amplitude.



## FACE PAGE FOR FIGURE 10

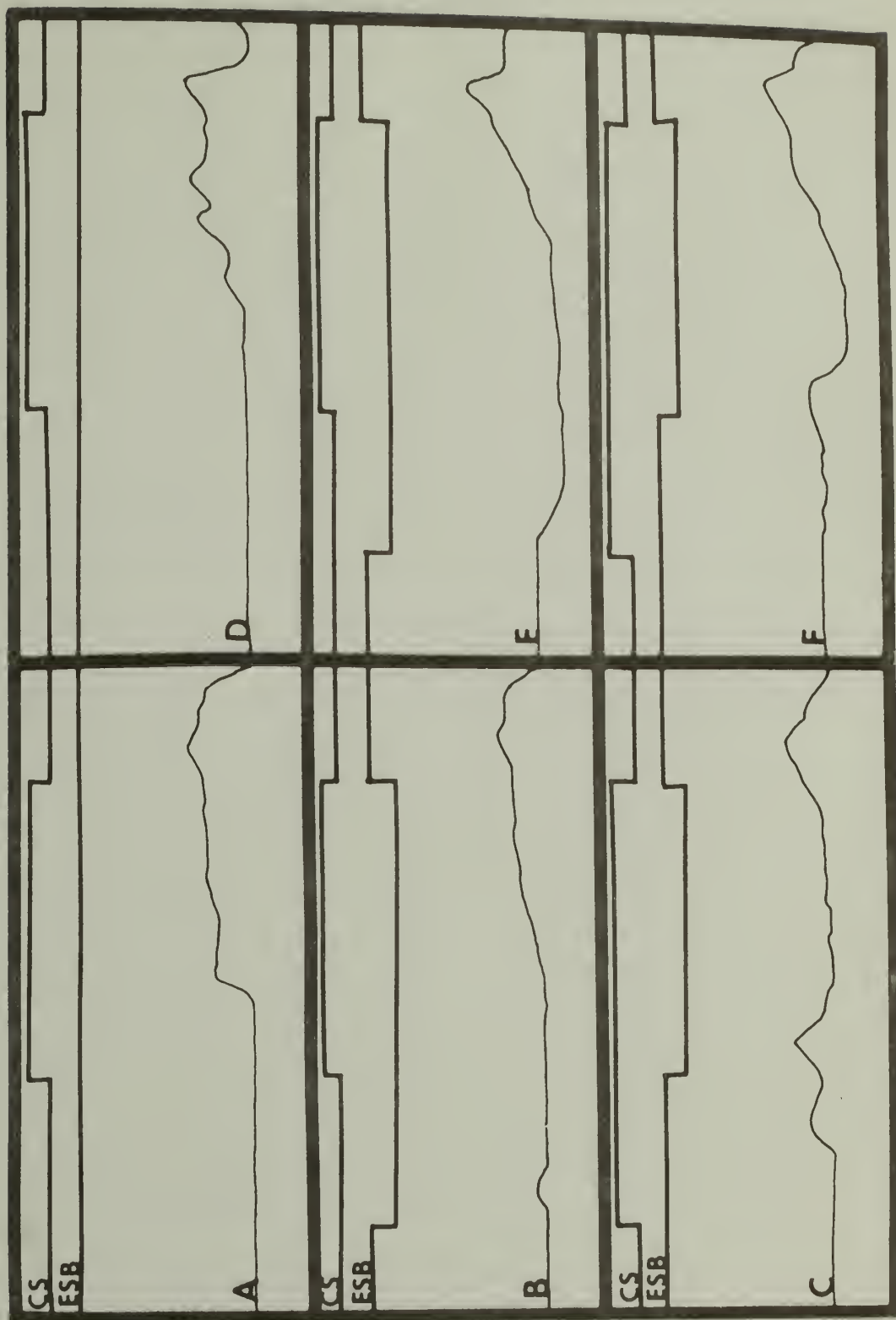
Figure 10. Tracings of polygraph records which depict the reduction in the CR amplitude produced by stimulation of Electrode 1 for rabbit FM90. The panels are arranged in the same manner as in Figure 9. The parameters of ESB employed during these tasks were 500 pps, .001 msec pulse duration, and 50  $\mu$ A pulse amplitude.





## FACE PAGE FOR FIGURE 11

Figure 11. Tracings of polygraph records which depict the small but consistent reduction in the CR amplitude produced by stimulation of Electrode 2 in rabbit FM90. The panels are arranged in the same manner as in Figure 9. The parameters of ESB employed during these tasks were 500 pps, .001 msec pulse duration, and 30  $\mu$ A pulse amplitude.



## FACE PAGE FOR FIGURE 12

Figure 12. Tracings of the polygraph records which depict the small but consistent reduction in the CR amplitude produced by stimulation of Electrode 2 in rabbit FM94. Panels A and C depict the normal CR topography while Panel B depicts the change in CR topography observed when the onset of the brain stimulation preceded the onset of the CS. Panel D depicts the change in CR topography observed when the onset of the CS preceded the onset of the ESB. The parameters of ESB employed during these tasks were 1000 pps, .2 msec pulse duration, and 150  $\mu$ A pulse amplitude.



The magnitude of the observed effects do not appear to be a function of the total stimulus energy applied to a given electrode. Rather, most of the observed disruptions in the topography of the CR were produced when the parameters of the ESB were set at the lowest levels employed in this experiment. For example, the parameters of the ESB which were used during the test of Electrode 1 for rabbit FM85 (cf., Figure 9) were 1000 pps, .05 msec pulse duration, and 50  $\mu$ A pulse amplitude.

CS-ESB Trials. Examining Figures 9, 10, 11 and 12 once again, one observes that if a CR begins to develop and then ESB is applied to an electrode, the amplitude of the CR can be reduced. For rabbit FM85 (Figure 9c) the reduction was to baseline and the CR never recovered while in rabbit FM90 (Figure 10c) the reduction was to a point approximately one half of the control amplitude. In those electrode loci categorized as producing a small but consistent disruption in the topography of the CR, application of ESB also reduced the amplitude of the CR (Figure 11c and Figure 12d). However, over the course of the CS interval the CR began to recover and in some cases the peak CR amplitude was as great as that observed during a control trial.

In general the disruption in the topography of the CR during test trials ESB-CS and CS-ESB as depicted in Figures 9, 10, 11 and 12 accurately represents the disruptive effects observed during the course of this experiment. The electrode



loci which were judged to produce no disruption of the CR did to some degree alter the topography of the CR. In most cases the stimulation initially produced a slight inhibition of the CR which dissipated during the testing sequence. In a few of the loci, application of ESB appeared to acquire the properties of a CS during the course of testing while in a few cases very low levels of ESB elicited a NM closure or eyeblink which precluded any analysis of the ability of the ESB to produce a diminution in the amplitude of the CR.

#### Videotape Records

Examination of the videotape records indicated that the ESB usually produced either a tensing of the facial muscles anterior to the nasal canthus or a distinct movement of the eyeball. For those electrode loci categorized as producing a substantial reduction in the amplitude of the CR, the ESB usually produced a tensing of the facial muscles which was not necessarily accompanied by any specific extraocular muscle responses. However, for Electrode 1 in rabbit FM85, (cf., Figure 9) the ESB produced no noticeable changes either in facial muscles or in the position of the eyeball.

For the electrodes located in areas of the brain stem adjacent to the abducens nucleus, the ESB usually produced a NM sweep and an eyeblink. Stimulation of electrodes located in the posterior two thirds of the midbrain and in the anterior portions of the brain stem produced a distinct rotation of

the eyeball. However, when the electrodes located in the cerebellum were stimulated, no changes in eyeball position or facial muscle tension were observed.

#### Effects of ESB on the UR

The effect ESB had on the unconditioned NMR was assessed in six of the rabbits. In 28 of the 30 electrode loci tested, the ESB elicited what appeared to be an unconditioned eyeblink response (20 loci) or conditioned responses (8 loci). Sixteen of the loci tested produced a reduction in UR amplitude, 2 loci produced increases in UR amplitude while the other 12 loci did not appear to produce any consistent changes in UR amplitude.

In comparing the effects ESB had on the CR with those observed during this testing phase, 9 of the 14 loci which produced a diminution in CR amplitude also reduced UR amplitude. The usual reduction in UR amplitude observed at these loci was on the order of 10 to 20%, although stimulation in a few of the loci reduced the amplitude of the UR by as much as 50%. For the other 5 loci in which ESB reduced the amplitude of the CR, the ESB did not produce any consistent reduction in UR amplitude.

#### Transection of Extraocular Muscles

In general, transection of the four recti and two oblique muscles rarely diminished any of the disruptive properties of the ESB. Examination of panels b with e and c with f in

Figures 9, 10, and 11 suggests that if anything, transecting the extraocular muscles may have enhanced the inhibitory properties of the ESB. Examination of Figure 13 further supports this hypothesis. During the initial testing of Electrode 1 for rabbit FM88, the ESB elicited what was recorded on the polygraph as an NMR or eyeblink. This response was relatively large and masked the CR. Following transection of the extraocular muscles, the ESB no longer produced the response, but it completely inhibited the manifestation of the CR on both types of test trials.

Following transection of the extraocular muscles, a few interesting modifications in the disruptive properties of the ESB were observed. For example, in rabbits FM83, FM88, and FM89, application of ESB via electrodes 4, 4, and 2, respectively produced a substantial reduction in CR amplitude, but only on the trials during which the ESB was presented after CS onset. On the test trials during which the ESB was applied prior to CS onset, the CR amplitude was not consistently altered. In rabbit FM88 a reduction in CR amplitude on the control trial following an ESB trial was observed during the testing of electrodes 2 and 3. An example of this unusual effect is depicted in Figure 14. As seen in this figure (Panels A and D), application of ESB to Electrode 3 reduced the latency of the CR and consequently the magnitude of the CR was increased. However, on the first trial after the ESB

## FACE PAGE FOR FIGURE 13

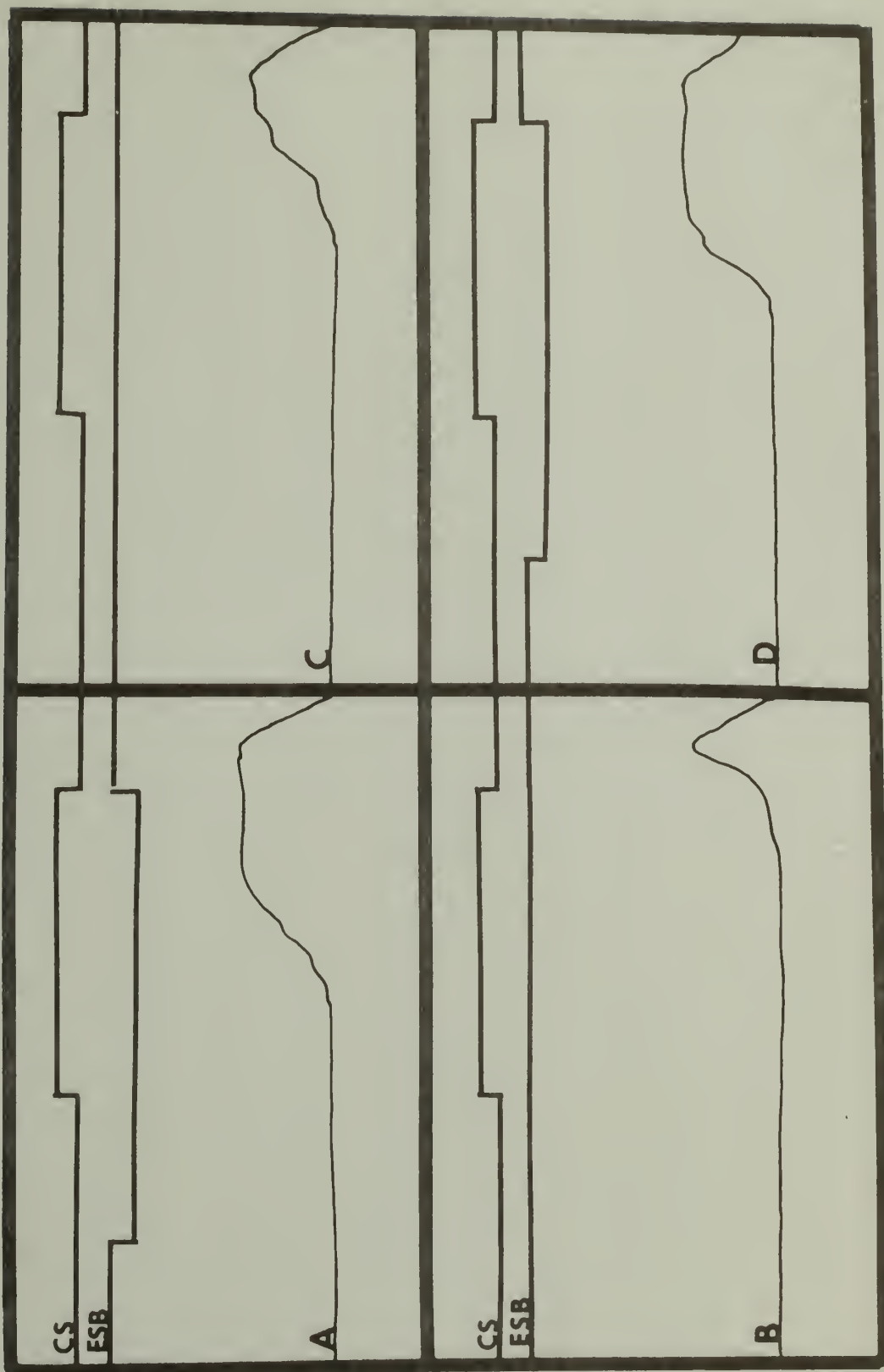
Figure 13. Tracings of polygraph records which depict the substantial reduction in the CR amplitude produced by stimulation of electrode 1 in rabbit FM88 following transection of the extraocular muscles. The panels are arranged in the same manner as in Figure 12. The parameters of ESB employed during these tasks were 1000 pps, .25 msec pulse duration, and 300  $\mu$ A pulse amplitude.





## FACE PAGE FOR FIGURE 14

Figure 14. Tracings of polygraph records which depict the changes in the CR topography produced by stimulation of Electrode 3 in rabbit FM88 following transection of the extraocular muscles. Panels A and D depict the topography of the CR observed when ESB precedes the onset of the CS while Panels B and C depict the topography of the CR on trials during which no ESB was presented. The figure depicts a four trial testing sequence consisting of an ESB trial (Panel A) followed by two control trials (Panels B and C), followed by another ESB trial (Panel D). The parameters of ESB employed during these tasks were 1000 pps, .05 msec pulse duration, and 150  $\mu$ A pulse amplitude.



was presented, (see Figure 11b) the latency of the CR was relatively long and consequently the amplitude and magnitude of the CR were substantially reduced. On the next control trial (see Figure 10c) the topography of the CR appeared to return to its normal state. When the CS preceded the onset of the ESB, there appeared to be few noticeable differences in the topography of the CR as a function of whether or not the ESB was presented on the previous trial or on that particular trial.

### Discussion

The major finding of this experiment is the delineation of a number of neural loci which inhibit the manifestation of the conditioned NMR. Specifically, it appears that the interstitial nucleus of Cajal and the nucleus of Darkschewitsch are the primary loci of this inhibitory process. The red nucleus and portions of the tegmental reticular formation also appear to be involved in this inhibition of the conditioned response. These structures may comprise part of a midbrain-brain stem circuit for inhibiting the extraocular motor system and, in particular, the neural system controlling the retractor bulbi response.

One of the requirements for specifying that application of ESB to a particular electrode produced a consistent diminution in the CR amplitude was that the CR amplitude had to be reduced on both ESB-CS and CS-ESB test trials. This

requirement and the other control procedures employed in this experiment suggest that the observed disruption in the topography of the CR was the result of a disruption in the neural system generating the CR. It does not appear that the observed disruption of the CR was caused by a disintegration of the information conveyed by the CS or was the result of a disruption in the memory retrieval processes necessary to elicit a CR. If either of these processes were responsible for the disruption in the topography of the CR, application of ESB after the initiation of the CR should have little effect on the topography of the CR (cf., Kesner & Wilburn, 1974). As observed in this experiment, application of ESB to a set of electrode loci either prior to CS onset or after CS (CR) onset produced a diminution in the amplitude of the CR.

In this experiment, transecting the extraocular muscles did not eliminate the observed reduction in CR amplitude suggesting that the diminution in the CR amplitude was the result of a central inhibitory or disruptive process. On the other hand, if transecting the extraocular muscles did eliminate the diminutive properties of the ESB, it would appear that some peripheral antagonistic system was responsible for the reduction in the amplitude of the CR (cf., Konorski, 1972).

The results of this experiment also suggest that this midbrain-brain stem circuit plays a role in modulating the amplitude of the UR. Except for the observed modifications

in UR amplitude produced by internuclear area stimulation, the results of Experiments 1 and 2 appear relatively consistent. In the anesthetized rabbit, stimulation in the internuclear area usually produced an augmentation in the amplitude of the UR. However, in the unanesthetized rabbit, stimulation of the internuclear area reduced the amplitude of both the UR and the CR. In conjunction with this UR and CR reduction, examination of the polygraph records suggests that internuclear stimulation produced a partial NMR or eyeblink response. If the portion of the internuclear area which initiated the eyeblink response had a lower threshold than the inhibitory mechanism, the addition of CNS depressants (Nembutal and Demerol) would raise the threshold for activating each mechanism, but the one with the lowest activation threshold would be most likely to manifest itself. In the anesthetized rabbit, the system associated with the eyelid closure response appears to be most easily activated by the ESB and therefore would tend to increase the amplitude of the UR. Removal of the general CNS depression, as in the chronic unanesthetized preparation employed in Experiment 2 would eliminate any bias in favor of this response facilitation and allow the inhibitory process to reduce the amplitude of the CR and the UR.

Previous research concerned with delineating the connections between the internuclear area and the extraocular motor

system indicates that there are two distinct pathways connecting these areas. Specifically, Gacek (1971) and Szentagothai and Schab (1956) employing cats as the experimental animal have reported that lesions placed in the vestibular nucleus produced degeneration in two distinct central pathways. One pathway was the tightly grouped medial longitudinal fasciculus (MLF) which connects the medial and superior portions of the vestibular nucleus with the motor nuclei controlling the extraocular muscles and some related midbrain and brain stem nuclei (cf., Tarlov, 1970). The second pathway runs lateral to the MLF and is rather diffuse. According to Gacek, this pathway sends projections to the oculomotor nucleus, while Szentagothai and Schab observed that the pathway projected to the oculomotor nucleus, the interstitial nucleus of Cajal, and the nucleus of Darkschewitsch. Szentagothai and Schab also observed that lesions placed in the nucleus of Darkschewitsch produced degeneration in the pathway running in the direction of the vestibular nucleus. In addition, Szentagothai and Schab reported that electrical stimulation, when applied to the region of the midbrain containing the nucleus of Darkschewitsch and the interstitial nucleus of Cajal, produced a relaxation of the vestibular-induced contraction in the extraocular muscles. Transecting the MLF eliminated the tonic excitation in the extraocular muscles produced by vestibular stimulation but did not eliminate the relaxing



effect that midbrain stimulation had on the extraocular muscles. Szentagothai and Schab concluded that this lateral pathway which arose in the internuclear area is responsible for inhibiting the motoneurons of the extraocular motor system, while the MLF is associated with the coordinated activation of the system.

Although Highstein (1973b) reported that ipsilateral stimulation of the vestibular nerve, and in particular the medial vestibular nucleus, produced IPSPs in the abducens nucleus, stimulation of the vestibular nucleus in this experiment failed to disrupt the CR. The vestibular-induced activity in the extraocular motor system is probably associated only with the maintenance of eye position. Therefore, this input to the abducens nucleus would not necessarily be involved in the modulation of the NMR.

The present experiment confirms that the internuclear area and a number of other midbrain nuclei play a role in inhibiting at least one of the extraocular muscles (retractor bulbi) and suggests that the inhibitory properties of the midbrain-brain stem circuit are somewhat complex. It appears that this "circuit" is not only involved in the inhibition of reflex induced activity in the extraocular muscles (Szentagothai & Schab, 1956), but also disrupts learned responses involving the extraocular muscles. The slight disruption of the CR produced by stimulating contralateral portions

of the pathway would seem to be the result of an antidromic activation of the neurons in this midbrain-brain stem circuit. The neurons which make up the system appear to have collaterals which facilitate a bilateral initiation of this inhibitory process. Stimulation of only the contralateral pathway produced minimal inhibition of the NMR in this experiment because very few neurons which modulate the motoneurons controlling the retractor bulbi on the recorded side were activated.

In conclusion, the extraocular motor system and its related structures appear to be under the control of two distinct systems. At one level there are the direct connections between the vestibular nucleus and the various nuclei controlling the extraocular muscles. This system appears to be involved merely in the regulation of eye position as a function of head and body position. A second system involves the internuclear area. This neural region receives a number of different modalities of sensory information and influences the extraocular motor system via at least two distinct pathways. One pathway involves the MLF and is most likely related to the control of eye position as it relates to visual system input. The second pathway and the structures it passes through appears to be related to a more general regulation of the extraocular muscles and the accessory protective system for the eye (eyelids and NMR) of the rabbit. In

particular this system appears to inhibit to some degree the protective devices associated with the eye, as well as the four recti and two oblique muscles. The results of the present experiment indicate that activation of the midbrain-brain stem circuit produces an unconditional inhibition of both a conditioned and unconditioned NMR. However, from the results of this experiment, it is not clear that elimination of portions of this system will prevent the acquisition of learned inhibition of the NMR in the rabbit.

### Experiment 3

The findings of Experiment 2 indicated that electrical stimulation of a midbrain-brain stem circuit which begins in the internuclear area consisting of the interstitial nucleus of Cajal and nucleus of Darkschewitsch produced an inhibition of an ongoing conditioned NMR in the rabbit. The present experiment sought to determine if this neural system was intimately involved in the development of learned inhibition in Pavlov's conditioned inhibition paradigm. Specifically, radio frequency lesions were placed in and around the internuclear area, and the various components of this "circuit". If this midbrain-brain stem circuit were involved in the development of conditioned inhibition of the rabbit NMR, lesions placed in this system should disrupt its development and prevent any manifestation of inhibition. On the other hand, lesions placed in the midbrain and brain stem but

outside of this system should not prevent the development or manifestation of conditioned inhibition of the rabbit NMR.

### Method

#### Animals

The animals were 37 experimentally naive male and female albino rabbits approximately 100 days old when brought to surgery (or at the start of the experiment for unoperated controls) and weighed between 2.6 and 3.4 kgs. At all times the rabbits were maintained in individual cages on ad lib food and water.

#### Apparatus

The basic apparatus used in this experiment was similar to that employed in Experiment 2. Three or four rabbits were run concurrently in a four drawer fire proofed file cabinet that was ventilated. Because of some observed fluctuations in the quality (tone and white noise intensity) of the stimuli presented in one of the drawers of the file cabinet (the second drawer from the bottom), rabbits were not trained or tested in this drawer after the first few replications of the experiment. A panel in front of the rabbits supported two house lights (6 V dc behind translucent plastic) which were used as a CS in this experiment, along with two impedance matched speakers. One of the speakers was used to present the tonal CS (1200 Hz, 85 dB SPL) which served as the conditioned inhibitor in this experiment, while the other

speaker presented continuous white noise (76 dB SPL). A Variac transformer was used to generate a 10 volt AC shock which was presented across two safety pin electrodes inserted through the skin on the back of the rabbits. This served as a third CS in a portion of the experiment. The US was a 2 mA shock of 50 msec duration applied to two wound clips attached approximately 1/2 cm below and posterior to the right eye. A Grass model 5D polygraph was used to record movements of the NM. The criterion used to define a CR was the same as that in Experiment 2.

### Surgery and Histology

In general the same surgical and histological procedures employed in Experiment 2 were used in this experiment. The lesions were created by passing current generated by a Grass model LM4 radio frequency lesion maker. The voltage was gradually increased from 0 to between 80 and 100 volts during the 15 seconds current was allowed to pass through the electrode. The electrode was a size 00 Clay Adams insect pin insulated with Epoxylite except for about .75 mm at the tip.

In addition to those rabbits given radio frequency lesions, 4 additional rabbits were anesthetized and then the extraocular muscles were transected. In one of these rabbits the tissue which connects the upper portion of the NM to the wall of the orbit was also transected.



## Procedure

A summary of the general training and testing sequence employed in this experiment is presented in Table 3. On the day prior to the first conditioning session, the right NM was sutured, and the rabbits were habituated to the apparatus by being placed in a restraining box and remaining in the experimental enclosure for at least 45 minutes.

Training began on the next day and consisted of 100 reinforced presentations of a 550 msec CS per day. The US overlapped the last 50 msec of the CS. For the rabbits trained using Sequence A of Table 3, the CSs were either the onset of the house lights or the backshock. Each stimulus was presented 50 times during each session. This phase of the experiment usually lasted 3 days. For the rabbits trained and tested using Sequence B of Table 3, the CS was the onset of the house lights. There were 100 presentations of this CS during each session and a maximum of 5 sessions were given during this phase of the experiment.

During the next phase of this experiment, all the rabbits were given conditioned inhibition training. This phase of the experiment involved differential conditioning during which presentation of the light CS was reinforced while presentation of the compound consisting of light and tone was not reinforced. For most of the rabbits this training continued for 14½ days. In addition, four rabbits were given another day of conditioned inhibition training.



Table 3

	<u>Training Sequence A</u> ,	<u>Training Sequence B</u>
	(17 rabbits: FML32, L33,L34,L35,L36,L37, L38,L39,L41,L42,L43, L45,L46,N1,N2,EOM2, EOM3)	(13 rabbits: FML48, L50,L51,L52,L54, L55,L56,L57,L58, L59,N3,N8,EOM5)
Phase 1:	Acquisition to light and backshock. (50 trials of each CS per day) <u>3 days</u>	Acquisition to light (100 trials per day) <u>4-5 days</u>
Phase 2:	CI training. Light reinforced; light & tone not reinforced. (50 trials of each CS per day) <u>14½ days</u>	CI training. Light reinforced; light & tone not reinforced. (50 trials of each CS per day) <u>14½ days</u>
Phase 3:	Summation test. Tone and backshock not rein- forced. (50 trials of each CS per day) <u>2½ days</u>	-----      -----
Phase 4:	Retardation test. Acquisition to tone. (100 trials per day) <u>3 days</u>	Retardation test. Acquisition to tone. (100 trials per day) <u>2½ days</u>

The rabbits run under Sequence A then received a summation test (Phase 3) which involved nonreinforced presentations of the backshock CS and the compound consisting of the backshock CS and tonal CS. The first day of testing followed the last half day of discrimination training. There were 25 presentations of the compound stimulus and the backshock CS during this first testing session. On the second and third days of testing, 50 trials of each type were presented. Prior to the start of this phase of the experiment, the rabbits were given a total of 30 reinforced trials with the backshock stimulus in order to insure that this stimulus still possessed excitatory properties.

The last phase of the experiment consisted of retardation testing during which the conditioned inhibitor (tonal CS) was now paired with the US. For the rabbits run under Sequence A, this phase of the experiment lasted for at least 3 days, and consisted of a minimum of 300 reinforced presentations of the tonal CS. For the rabbits run under Sequence B, this phase of the experiment began after the last  $\frac{1}{2}$  day of discrimination training and continued for at least another 2 days. If a rabbit did not acquire a conditioned NMR to the tonal CS within these time limits, additional training sessions were given until the rabbit gave at least 85% CRs to the CS on a given day. Data analysis for this phase of the experiment focused on the first 300 (Sequence A) or 250 (Sequence B) conditioning trials.

## Results

Of the 37 rabbits which began this experiment, one died during the postoperative recovery period and 6 rabbits were dropped from the study because of apparatus problems. This group included 2 unoperated controls, one rabbit which had its extraocular muscles cut and 3 rabbits with lesions.

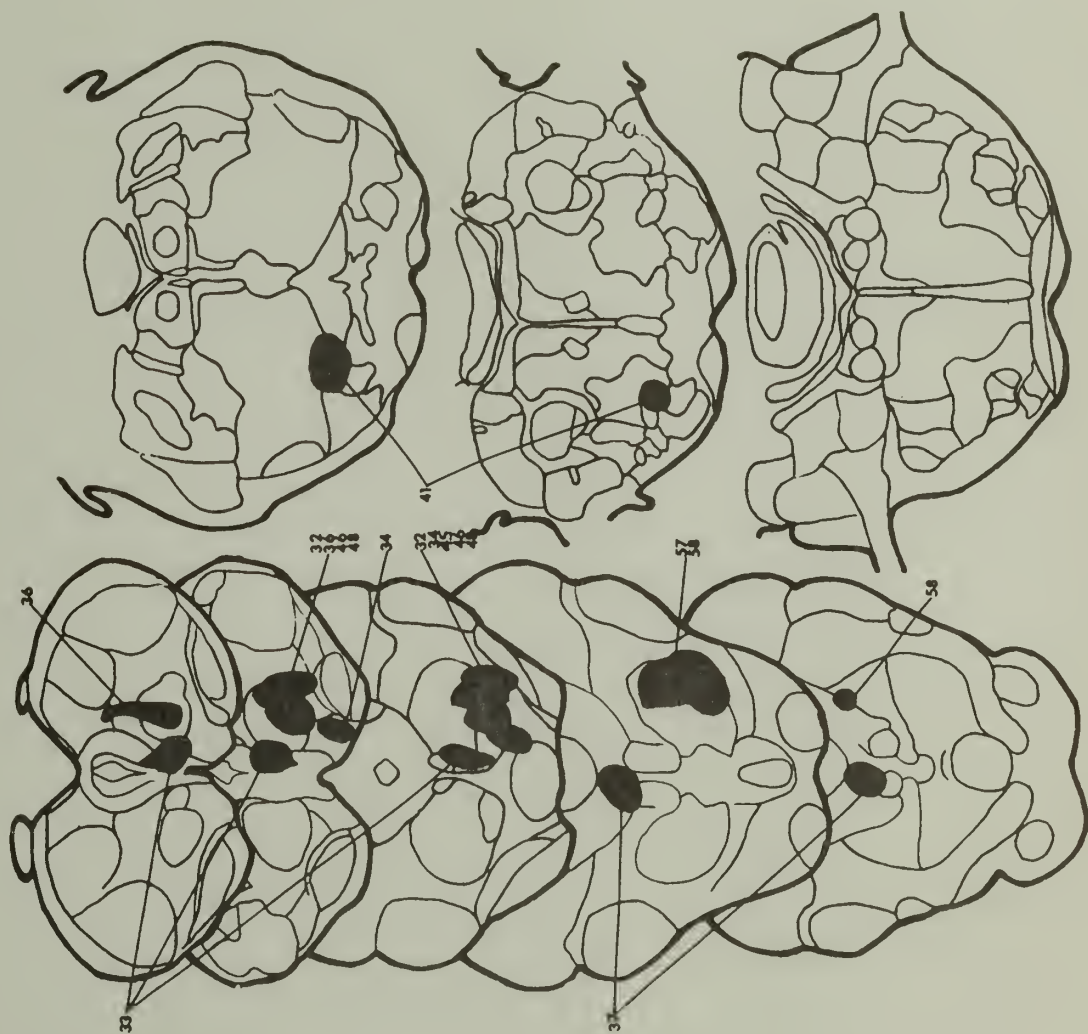
Of the remaining rabbits, a total of 23 rabbits which had lesions placed in the midbrain and/or brain stem were given conditioned inhibition training. Of these 23 rabbits, 11 failed to discriminate between the light (L; CS+) and the compound consisting of light and tone (LT; CS-), while 8 rabbits were able to form this discrimination and in this group of 8 rabbits the tone manifested the properties of a conditioned inhibitor. The data from the other four rabbits with midbrain or brain stem lesions did not fit nicely into either of these categories and therefore their data will be presented separately.

Seven additional rabbits were given conditioned inhibition training. These included 4 unoperated control rabbits and 3 rabbits in which the extraocular muscles were cut.

Figure 15 depicts the extent of the lesions for all the rabbits which did not discriminate between the light (CS+) and the light plus tone compound (CS-). The lesions for these rabbits were located in the internuclear area, the anterior portions of the red nucleus, the central gray,

## FACE PAGE FOR FIGURE 15

Figure 15. The location of the lesions for all the rabbits which failed to develop conditioned inhibition in this experiment. Outlines were redrawn from the Gerhard, 1968 atlas, Plates 31, 32, 34, 35, and 38 and the Meessen and Olszewski, 1949 atlas, Plates 10, 13, and 14.



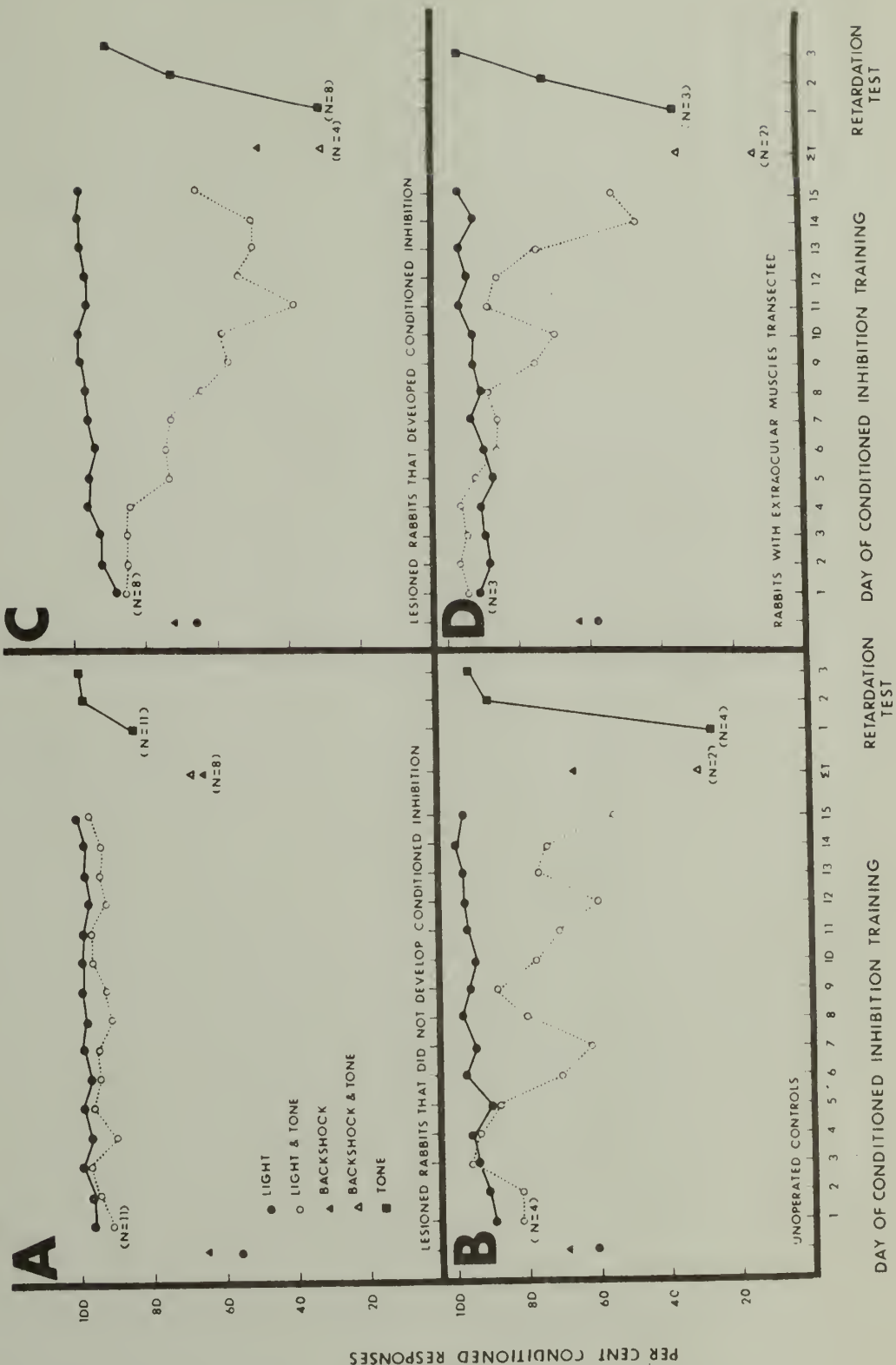
tegmental reticular formation, and in one rabbit the lesion was located in the ventral pontine reticular formation.

During the differential conditioning phase of the experiment the overall ratio of CRs to the CS- compound versus the CS+ for the 11 nondiscriminators was .963. Examination of Figure 16 emphasizes the total inability of the rabbits in this group to differentiate between the L+ and LT-. As seen in the figure, over the 15 days of discrimination training the greatest reduction in responding to the CS- was on the order of 10%. Examination of the last few days of discrimination training does not suggest that these rabbits were beginning to differentiate between the CS+ and CS-. Eight of these rabbits were also given a summation test. The mean suppression ratio (CRs to the compound consisting of tone (T) and backshock (BS)/CRs to BS + CRs to T&BS) was .5104. A ratio of .50 indicates that the test stimulus possesses neither excitatory nor inhibitory properties while a ratio greater than .50 suggests that the test stimulus may possess excitatory properties. The results of the retardation test also suggest that the tonal CS may have possessed excitatory properties for the rabbits in this group. During the retardation test (cf., Figure 16), 7 of the 11 rabbits in this group began to give CRs to the tonal CS within the first 10 paired presentations of the tone. The overall level of responding to the tone in this phase of the experiment for the rabbits in this group was .94.



## FACE PAGE FOR FIGURE 16

Figure 16. Mean percent CRs during the initial acquisition, conditioned inhibition training, summation testing, and retardation testing phases of this experiment. Panel A depicts the mean percent responses for the lesioned rabbits which did not develop conditioned inhibition while Panel C depicts the mean percent responses for the lesioned rabbits which did develop conditioned inhibition. Panel B depicts the mean percent CRs for the unoperated controls while Panel D depicts the mean percent CRs for the rabbits with their extraocular muscles transected.



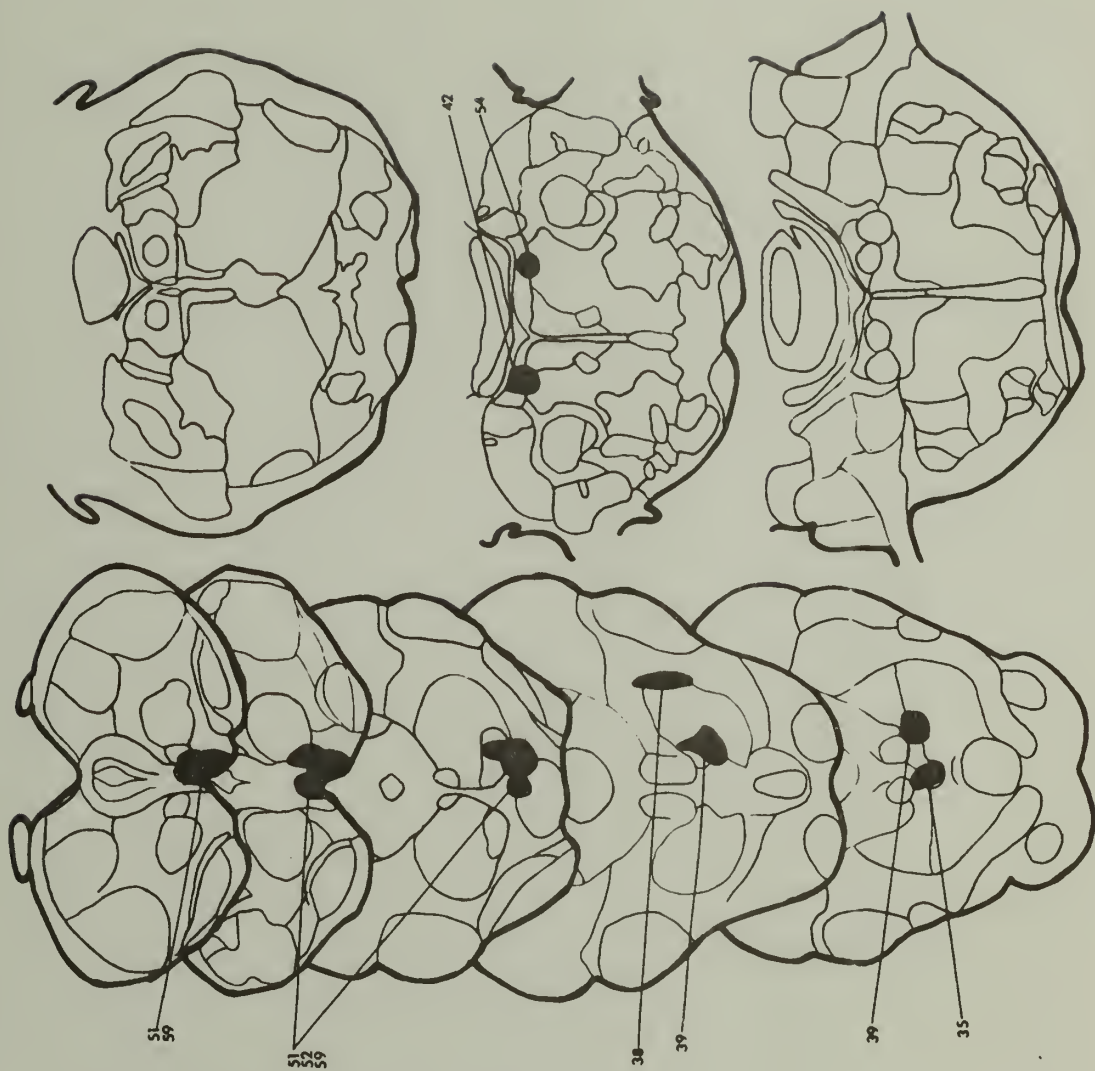
For the rabbits which were judged to have formed the discrimination between L+ and the LT- compound, the following pattern of responding was observed during the various phases of the experiment. Initially, the rabbits were unable to differentially respond to the two CSs, and during the first few days of differential conditioning some of the rabbits responded a bit more to the nonreinforced compound (LT) than to the CS+ (L). As seen in Figure 16 (Panels B,C, and D), by the last few days of differential conditioning, these rabbits were able to suppress their responses to the CS-. The ratio of responding to the CS- compound versus the CS+ during the last day and a half of training for the lesioned rabbits which discriminated was .57, the ratio for the unoperated controls was .68, and the ratio for the rabbits with their extraocular muscles cut was .52. In addition, the rabbits in these groups exhibited inhibitory summation. The suppression ratios for the 8 rabbits given the summation test were generally below .40, which suggests that the tone possessed inhibitory properties. As seen in Figure 16, during the first day of retardation testing the rabbits in these groups responded very little to the now reinforced tone. In a few of the rabbits the tone did not begin to reliably elicit a CR until the third day of retardation testing. By contrast, naive rabbits usually acquire a CR to the tonal CS employed in this experiment during the first conditioning session (Marchant & Moore, 1974).

Figure 17 depicts the extent of the lesions for those rabbits which were able to form the discrimination between L+ and LT-, and in which the tone exhibited inhibitory properties. As seen in the figure, in three rabbits the lesion was located just below the internuclear area and destroyed the rootlets of the oculomotor nerve. In these rabbits, examination of the oculomotor nucleus revealed that few motoneurons were left intact on the side ipsilateral to the lesion. In another three rabbits the lesions invaded the red nucleus. However, in these rabbits destruction of the red nucleus was very limited or began in the more posterior regions of the nucleus. In addition, small lesions of the tegmental reticular formation, the anterior aspects of the pontine reticular formation, or the most posterior portions of the tegmental nuclei did not disrupt the development of conditioned inhibition.

Four additional rabbits with lesions in the midbrain or brain stem were given conditioned inhibition training. In two of these rabbits (FML50 and FML56) the various CSs employed in this experiment during training and testing did not consistently elicit a CR. The size of the lesions for these two rabbits (Figure 18) is not unusually large or in a location (e.g., abducens nucleus) which should prevent the acquisition of the CR. The other two rabbits (FML43 and FML55) did not differentiate between the L+ and the LT-.

## FACE PAGE FOR FIGURE 17

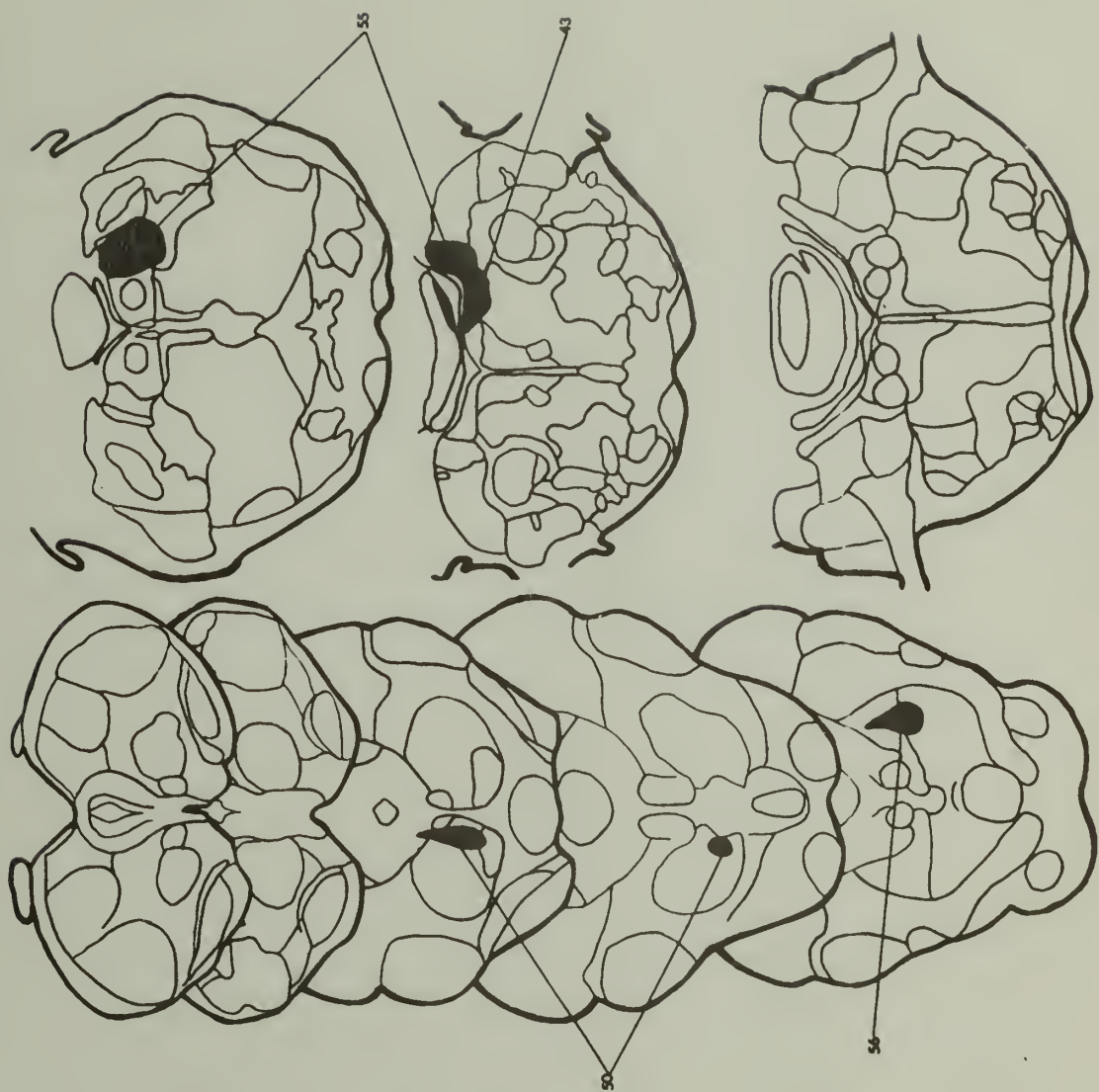
Figure 17. The location of the lesions for all the rabbits which developed conditioned inhibition in this experiment. Outlines were redrawn from the Gerhard, 1968 atlas, Plates 31, 32, 34, 35, and 38 and the Meessen and Olszewski, 1949 atlas, Plates 10, 13, and 14.





## FACE PAGE FOR FIGURE 18

Figure 18. The location of the lesions for the two rabbits which failed to develop a robust conditioned response during this experiment and the two rabbits which failed to discriminate between the L+ and LT- but in which the tone appears to manifest the properties of a conditioned inhibitor. Outlines were redrawn from the Gerhard, 1968 atlas, Plates 31, 32, 34, 35, and 38 and the Meessen and Olszewski 1949 atlas, Plates 10, 13, and 14.



However, in both rabbits the tone appeared to possess the properties of a conditioned inhibitor. In rabbit FML43 the tone suppressed responding to the backshock CS (suppression ratio = .40), and during the retardation test the tonal CS did not reliably elicit a CR until after about 50 conditioning trials had been given. Rabbit FML55 also did not form the discrimination (discrimination ratio = .994) but during the retardation test, the tonal CS did not reliably elicit a CR until the second day of testing. For both rabbits the lesion was located in the anterior portions of the dorsal tegmental nucleus, in portions of the locus coeruleus, and in rabbit FML43 the lesion destroyed part of the cerebellum.

#### Discussion

In general, the findings of this experiment support the hypothesis that a midbrain-brain stem circuit which appears to originate in the internuclear area consisting of the interstitial nucleus of Cajal and the nucleus of Darkschewitsch plays a role in the development of conditioned inhibition of the rabbit's NMR. The results of this investigation also provide further evidence that the path of the circuit runs lateral to the midline through the small-celled portions of the red nucleus and then through the central gray and the tegmental reticular formation. In the brain stem the path of the circuit may pass in the vicinity of the dorsal tegmental nuclei and the locus coeruleus.

A number of investigations have implicated midbrain and brain stem structures in the development or retention of a variety of learned responses. For example, Smith (1970a, b) observed that lesions of the red nucleus disrupted the acquisition of conditioned responses in both cats and rats. In cats, unilateral lesions placed in the red nucleus impaired the performance of a conditioned flexion response using the contralateral forelimb (Smith, 1970b). However, conditioned flexion responses could be initiated in the forelimb ipsilateral to the lesion. In the present investigation, lesions placed in the anterior portions of the red nucleus did not impair the acquisition of a conditioned NMR ipsilateral to the lesion but did disrupt the development of conditioned inhibition. On the other hand, a lesion in the contralateral red nucleus did impair the acquisition of a conditioned NMR in rabbit FML50 (cf., Figure 17). At the completion of the experiment an attempt was made to establish a conditioned NMR on the left side in 8 rabbits. Although 6 of these rabbits had lesions which invaded the red nucleus on the right side, these rabbits had little difficulty acquiring the new conditioned response. These findings suggest that the disruption in CR performance observed by Smith (1970a,b) may not hold for rabbits or for responses which involve the extraocular motor system. In particular, his findings do not appear to account for the impairment in the acquisition of a CR in rabbit FML50.

In addition, a number of investigations have attempted to delineate the role of the reticular formation and other deep midbrain and brain stem structures in the formation and retention of various discrimination problems. Thompson and his associates (Craddock & Thompson, 1971; Spiliotis & Thompson, 1973; Thompson & Thorne, 1973) have reported that lesions in the reticular formation, red nucleus, tegmental nucleus and other subcortical structures interfere with the retention of a variety of complex discrimination problems. The many differences between these investigations and the present series of experiments prevents any precise comparison. It should be noted, however, that the retention deficits observed by Thompson and his associates were produced by massive lesions to structures in the midbrain and brain stem. In the present investigation, the disruption in the development of conditioned inhibition was produced by placing relatively small unilateral lesions in a midbrain-brain stem circuit which appears to modulate the neural activity necessary to initiate a retractor bulbi response.

The rabbit NMR preparation has previously been employed in a number of investigations concerned with determining the relative importance of a number of cortical and subcortical structures in the acquisition and modulation of a conditioned NMR. This research has indicated that complete or partial decortication (Oakley & Russell, 1972; Eichenbaum, Potter,



Papsdorf, & Butter, 1974), dorsal hippocampal lesions (Solomon, 1975; Solomon & Moore, in press), septal lesions (Lockhart & Moore, 1975) and amygdaloid lesions (Kembel, Alkin, & Leonard, 1972) do not interfere with the acquisition of a conditioned NMR. In addition, complete decortication (Oakley & Russell, 1974) did not disrupt differential conditioning when the CS+ and CS- were from different modalities, and Solomon, (1975) has reported that dorsal hippocampal lesions did not disrupt the development of conditioned inhibition. On the other hand, frontal lesions (Eichenbaum, et al., 1974) and septal lesions (Lockhart & Moore, 1975) interfered with the formation of a discrimination when CS+ and CS- were from the same modality. The deficits observed following frontal or septal lesions appear to be related to the rabbit's inability to withhold responses during nonreinforced trials. The disruption in the development of conditioned inhibition in the present experiment can also be attributed to a failure in the rabbit's ability to withhold responses to the nonreinforced CS. In the investigations involving frontal and septal lesions, when the rabbits were given sufficient training they were able to form the appropriate discrimination. In the present experiment, even after 14 to 15 days of conditioned inhibition training, some rabbits continued to respond on almost every CS- trial. While the deficit in discrimination learning following septal lesions does not appear to be related to



the midbrain-brain stem circuit described in the present series of experiments, a number of studies have provided evidence which indicates that the frontal cortex has projections to the midbrain portion of this circuit. For example, Leonard (1969) has observed terminal degeneration in the central gray just below the posterior commissure following medial frontal lesions in rats. Her description of this area corresponds to the internuclear area of the rabbit containing the nucleus of Darkschewitsch and the interstitial nucleus of Cajal. The elimination of one of the inputs to this inhibitory midbrain-brain stem circuit as a result of frontal cortical lesions might interfere with the functioning of the circuit and produce a deficit in the relearning of a discrimination as observed by Eichenbaum et al. (1974).

Although the performance of most of the rabbits in this experiment support the hypothesis that a specific midbrain-brain stem circuit is involved in the development of conditioned inhibition, the performance of a few rabbits questions the specificity of this circuit. For example, in rabbit FML41 a lesion placed in the pontine reticular formation completely disrupted the development of conditioned inhibition. How this portion of the pontine reticular formation might relate to the proposed midbrain-brain stem circuit is unclear. However, given that only one rabbit had a lesion placed in this region, the possibility exists that this structure is not

actually involved in the development of conditioned inhibition but is related to some other process which prevented the formation of the discrimination. Also the lesion in rabbit FML34 was slightly smaller and in approximately the same location as the lesions in rabbits FML51 and FML59. During the experiment rabbit FML34 failed to form the discrimination and the tone did not manifest the properties of a conditioned inhibitor, while rabbits FML51 and FML59 did discriminate and the tone did manifest the properties of a conditioned inhibitor. The slight differences in the size and location of the lesion in rabbit FML34 do not appear to account for the differences in performance between this rabbit and rabbits FML51 and FML59 observed in this experiment.

Two other rabbits also failed to discriminate between the L+ and the LT- but during the testing of these rabbits, the tone appeared to possess the properties of a conditioned inhibitor. The lesion in both of these rabbits was located in the dorsal tegmental nucleus and invaded the locus coeruleus. If these lesions destroyed a small component of the inhibitory circuit, during discrimination training the inhibitory input to the abducens nucleus may not have been sufficient to override the excitatory input generated by the light CS. During the summation test, which was carried out in extinction, the excitatory properties of the back shock CS may have been relatively less intense than those of the

light which continued to be reinforced during the discrimination phase of the experiment. This relative reduction in the excitatory input to the abducens nucleus during the summation test appears to have been sufficient to allow the inhibitory properties of the tone to manifest themselves. The small inhibitory input to the abducens nucleus generated by the tone would have to be eliminated or suppressed before the tone could acquire excitatory properties and elicit a CR. The time (number of conditioning trials) it should take for suppression of the inhibitory action of the tone to develop could account for the impairment in the acquisition of a CR to the tonal CS observed during the retardation test for these rabbits.

#### GENERAL DISCUSSION

The major findings of this series of experiments is the delineation of a midbrain-brain stem circuit which is responsible for the development of conditioned inhibition of the rabbit's NMR. In addition to describing a neural system related to the development of conditioned inhibition, the results of Experiments 2 and 3 also suggest that a peripheral antagonistic inhibitory system (cf., Konorski, 1972) was not responsible for the modulation of the CR observed in these experiments. In particular, transection of the extraocular muscles did not eliminate the diminution in the amplitude of the CR produced by electrical stimulation of the circuit

in Experiment 2, and did not prevent the development of conditioned inhibition in Experiment 3. Furthermore, those rabbits in Experiment 3 in which the oculomotor nerve was lesioned also developed conditioned inhibition. Therefore, the development of conditioned inhibition is either (a) the result of a reciprocal inhibition between the motoneurons in the abducens nucleus and one or more components of this midbrain-brain stem circuit or (b) the result of a descending inhibitory process which may arise in this circuit and is related specifically to the retractor bulbi response and possibly to the other components of the extraocular motor system (cf., Szentagothai & Schab, 1956).

One way to determine whether a reciprocal or descending inhibitory process is responsible for the development of conditioned inhibition of the NMR would be to record multiple unit activity in one or more components of the midbrain-brain stem circuit and the abducens nucleus during conditioned inhibition training. The recording of multiple unit activity during conditioned inhibition training should not only aid in determining which process is involved in the development of inhibition but may also help to localize the origin of the inhibitory process.

Preliminary observations from a few rabbits suggest that the level of activity in the abducens nucleus during CS-trials is correlated with the amplitude of the CR on that

trial. Specifically, on CS- trials when the rabbits emitted a CR comparable in amplitude to one elicited on a CS+ trial, the activity in the abducens nucleus appeared to be identical. As the amplitude of the CR on the CS- trials decreased during the course of conditioned inhibition training, the multiple unit activity associated with these smaller CRs also decreased. On the CS- trials in which no CR was elicited, the multiple unit activity in the abducens nucleus was either equal to or less than the pre-CS activity. Unfortunately, an analysis of the changes in the multiple unit activity in the midbrain-brain stem circuit accompanying the development of conditioned inhibition has not been completed.

These observations further suggest that the inhibitory process does not involve the activation of an antagonistic muscle system which prevents the execution of the CR but instead acts directly on the abducens nucleus to either suppress the motor unit activity or eliminate the excitatory input necessary to manifest the CR. In addition, the results of Experiment 3 indicate that a midbrain-brain stem circuit is intimately involved in the development of conditioned inhibition. Therefore, during the presentation of a CS- the multiple unit activity in the midbrain-brain stem circuit should increase relative to the pre-CS period, while the activity in the abducens nucleus should either decrease or remain the same. Observation of this set of changes on a CS-



trial coupled with no change in the multiple unit activity in the midbrain-brain stem circuit during a CS+ trial would suggest that a descending inhibitory process was responsible for the development of conditioned inhibition. These results would tend to support the hypothesis that the integrative action necessary to initiate the inhibitory process was located in the midbrain-brain stem circuit.

Observation of an increase in the multiple unit activity of the midbrain-brain stem circuit and a decrease in the multiple unit activity in the abducens nucleus during a CS-trial, coupled with a decrease in the multiple unit activity of the midbrain-brain stem circuit and an increase in the multiple unit activity in the abducens nucleus on a CS+ trial, would provide direct support for the hypothesis that a reciprocally inhibitory process was responsible for the development of conditioned inhibition of the rabbit's NMR.

Although the observation of an increase in the multiple unit activity of the midbrain-brain stem circuit on a CS-trial would suggest that the circuit integrated the sensory information necessary to suppress the manifestation of a conditioned NMR, this system may just amplify or relay the inhibitory action initiated by some other neural system. The origin of this higher level inhibitory process could be the frontal cortex. For example, Leonard (1969) has observed terminal degeneration in the midbrain area of the rat containing the nucleus of Darkschewitsch and the interstitial nucleus



of Cajal. In addition, Eichenbaum, et al. (1974) have reported that frontal cortical lesions in the rabbit disrupted the retention of an intensity discrimination, and frontal cortical lesions have been shown to impair the acquisition of a go-no go discrimination task in a number of species (cf., Zielinski & Czarkowska, 1973, 1974). Although frontal cortex lesions impair the formation of a discrimination, the deficit produced by these lesions does not appear to be as severe as that observed following lesions to the midbrain-brain stem circuit. In addition, Oakley and Russell (1974) have reported that complete decortication does not disrupt the rabbit's ability to discriminate between tone and light CSs; and Norman, Villablanca, Brown, Schwafel, and Buchwald (1974) reported that hemispherectomized cats could differentiate between tones of different frequencies. These results suggest that the cortex plays a minor role in the inhibitory process governing the modulation in the conditioned NMR observed in this series of experiments. In addition, although the hippocampus has often been referred to as the structure responsible for the development of learned inhibition (Douglas, 1972), Solomon (1975) has reported that hippocamp-ectomized rabbits were able to develop conditioned inhibition in Pavlov's conditioned inhibition paradigm. Therefore, even if these higher level systems initiated the inhibitory process, the results of Experiment 3 suggest that the midbrain-brain stem circuit must at least amplify this process to produce the

complete suppression of the conditioned NMR observed during a CS- trial in Pavlov's conditioned inhibition paradigm. However, if completely decorticate or encephalo isolé rabbits were able to develop conditioned inhibition, then it would appear that this midbrain-brain stem circuit was initiating the inhibitory process. In addition, a thorough analysis of the electrophysiological changes which accompany the development of conditioned inhibition should specify the inhibitory mechanism responsible for the manifestation of conditioned inhibition.

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### Appendix A

A breakdown of the number of inhibitory and facilitory loci observed in each rabbit in Experiment 1.

Table 1

Subject	Sex	Weight	Duration of Testing Procedure	Number of Facilitatory Loci	Number of Inhibitory Loci	Number of Neutral Loci
FMAc2	M	2.95 kgs	4 hrs 30 min	1	1	10
FMAc3	F	2.75 kgs	6 hrs	2	2	15
FMAc5	F	2.95 kgs	6 hrs	4	7	13
FMAc6	M	2.6 kgs	3 hrs 40 min	2	4	6
FMAc8	F	3.0 kgs	5 hrs 10 min	5	2	11
FMAc10	F	2.6 kgs	6 hrs 20 min	4	6	12
FMAc13	F	2.8 kgs	4 hrs 30 min	10	2	1
FMAc14	F	2.8 kgs	5 hrs	2	4	10
FMAc16	M	3.0 kgs	3 hrs 40 min	2	1	11

## Appendix B

Median reduction (in millimeters) of the amplitude of the conditioned and unconditioned responses for the rabbits in Experiment 2.

Table 1

CR Reduction				UR Reduction	
Subject	Electrode	Test 1	Test 2	Test Following Transection of Extraocular Muscles	Test 1
FM83	1	8mm	8mm	6mm	8mm
	2	11mm	6mm	10mm	8mm
	3	2mm	--	4mm	4mm
	4	2mm	4mm	*	--
	5	--	--	--	2mm
% Conditioned Responses on last day of acquisition - 98%					
FM85	1	6mm	8mm	8mm	2mm
	2	3mm	4mm	2mm	2mm
	3	2mm	2mm	--	--
	4	4mm	2mm	4mm	--
	5	2mm	6mm	--	2mm
% Conditioned Responses on last day of acquisition - 100%					
FM86	1	2mm	7mm	Not tested	--
	2	--	--	Not tested	Increased UR amplitude 3mm
	3	2mm	--	Not tested	Increased UR amplitude 6mm
	4	--	--	Not tested	--
	5	--	--	Not tested	2mm
% Conditioned Responses on last day of acquisition - 99%					

Table 1 (continued)

CR Reduction			UR Reduction	
Subject	Electrode	Test 1	Test 2	Test 1
Test Following Transection of Extraocular Muscles				
FM88	1	--	--	3mm
	2	4mm	6mm	3mm
	3	6mm	4mm	3mm
	4	2mm	2mm	1mm
	5	--	--	1mm
10mm				
**				
**				
*				
--				
% Conditioned Responses on last day of acquisition - 100%				
FM89	1	--	--	4mm
	2	6mm	8mm	7mm
	3	--	--	--
	4	--	--	--
	5	--	--	4mm
* --				
% Conditioned Responses on last day of acquisition - 94%				
FM90	1	6mm	4mm	4mm
	2	3mm	3mm	5mm
	3	4mm	--	--
	4	--	--	--
	5	2mm	--	3mm
6mm				
3mm				
--				
--				
--				
% Conditioned Responses on last day of acquisition - 99%				

Table 1 (continued)

CR Reduction		UR Reduction		
Subject	Electrode	Test 1	Test 2	Test 1
Test Following Transection of Extraocular Muscles				
FM91	1	5mm	9mm	Not tested
	2	--	--	Not tested
	3	12mm	15mm	Not tested
	4	--	--	Not tested
	5	--	--	Not tested
% Conditioned Responses on last day of acquisition - 100%				
FM92	1	--	--	Not tested
	2	3mm	5mm	Not tested
	3	--	--	Not tested
	4	--	--	Not tested
	5	--	--	Not tested
% Conditioned Responses on last day of acquisition - 100%				
FM93	1	6mm	--	Not tested
	2	--	--	Not tested
	3	--	--	Not tested
	4	--	--	Not tested
	5	3mm	--	Not tested
% Conditioned Responses on last day of acquisition - 100%				



Table 1 (continued)

CR Reduction		UR Reduction		
Subject	Electrode	Test 1	Test 2	Test 1
FM94	1	3mm	4mm	Not tested
	2	2mm	4mm	Not tested
	3	--	--	Not tested
	4	--	--	Not tested
	5	2mm	2mm	Not tested
Test Following Transection of Extraocular Muscles				
% Conditioned Responses on last day of acquisition - 93%				Not tested
				Not tested
				Not tested
				Not tested
				Not tested

### Appendix C

Number of conditioned responses during all phases of  
Experiment 3 for all subjects.

Table 1

Number of conditioned responses during all phases of Experiment 3 for the lesioned rabbits that failed to develop conditioned inhibition.

Subject	Acquisition Phase		Differential Conditioning Phase		Summation Test		Retardation Test
	Light	Backshock	Light	Light + Tone	Backshock	Backshock + Tone	
FML32	92	120	707	707	22	20	242
FML33	66	96	723	721	107	124	297
FML34	105	139	713	682	81	115	300
FML36	54	71	708	591	14	12	290
FML37	73	103	714	719	117	119	300
FML41	96	108	705	707	88	81	278
FML45	92	83	660	598	125	119	268
FML46	62	69	707	661	102	94	297
FML48	212	---	702	686	---	---	246
FML57	237	---	722	724	---	---	249
FML58	197	---	724	724	---	---	244

Table 2

Number of conditioned responses during all phases of Experiment 3 for the lesioned rabbits that developed conditioned inhibition.

Subject	Acquisition Phase		Differential Conditioning Phase		Summation Test		Retardation Test
	Light	Backshock	Light	Light + Tone	Backshock	Backshock + Tone	
FML35	111	123	705	582	77	52	257
FML38	145	140	718	368	22	6	244
FML39	96	94	614	399	65	50	147
FML42	61	77	719	582	76	51	262
FML51	433	---	720	660	---	---	177
FML52	309	---	738	551	---	---	98
FML54	122	---	677	328	---	---	119
FML59	207	---	694	439	---	---	198

Table 3

Number of conditioned responses during all phases of Experiment 3 for the unoperated controls.

Subject	Acquisition Phase		Differential Conditioning Phase		Summation Test	Backshock + Tone	Tone
	Light	Backshock	Light	Light + Tone			
N1	96	115	690	516	78	42	225
N2	59	93	635	547	87	36	---
N3	237	---	710	489	---	---	179
N8	248	---	721	710	---	---	212

Table 4

Number of conditioned responses during all phases of Experiment 3 for the rabbits that had their extraocular muscles transected.

Subject	Acquisition Phase		Differential Conditioning Phase		Summation Test	Backshock + Tone	Tone
	Light	Backshock	Light	Light + Tone			
EOM2	81	123	716	680	11	2	261
EOM3	146	109	717	608	75	29	161
EOM5	94	---	560	509	---	---	163

Table 5

Number of conditioned responses during all phases of Experiment 3 for the lesioned rabbits whose behavioral data is somewhat anomalous.

Subject	Acquisition Phase		Differential Conditioning Phase		Summation Test		Retardation Test
	Light	Backshock	Light	Light + Tone	Backshock	Backshock + Tone	
FML43	105	112	718	721	77	56	257
FML50	11	---	114	212	---	---	41
FML55	201	---	724	720	---	---	118
FML56	1	---	112	36	---	---	2





