Grill and R. Norgren, *Science* **201**, 267 (1978); *Brain Res.* **143**, 281 (1978); C. Kornblith and W. G. Hall, *J. Comp. Physiol. Psychol.*, in press].
24. Even if the young pup normally exhibited this independent type of ingestive behavior, it is unlikely that it would have survival value since any liquid food available to the pup is unlikely to be nutritionally adequate.
25. Feeding can be analyzed in pups which have had no feeding experience other than suckling. Suckling experience is not required for the normal appearance of feeding [W. G. Hall, *Science* **190**, 1313 (1975)]. This report represents one of several demonstrations of the independence of systems underlying suckling and feeding (*7, 8*).
26. I am grateful to T. Bryan for assistance; to E. M. Blass, S. Coyle, C. Kornblith, R. W. Oppenheim, J. S. Rosenblatt, and C. L. Williams for comments on an earlier version of the manuscript; and to A. E. Johnson for photography. Supported by NSF grant BNS 77 23051 and the North Carolina Division of Mental Health.

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Medial Septal Lesions Retard Classical Conditioning of the Nictitating Membrane Response in Rabbits

Abstract. *Lesions of the medial septum were produced in 7 of 14 rabbits prior to classical conditioning of the nictitating membrane response. Lesions significantly altered the hippocampal electroencephalogram, attenuated conditioned hippocampal unit responses, and slowed the behavioral rate of acquisition. The contrast of the behavioral results with those of studies of massive septal or hippocampal ablation suggests a functional subdivision of the septo-hippocampal system in learning.*

The hippocampal formation of the mammalian forebrain is thought to be involved in processes central to learning and memory (*1*). It has been suggested that disrupting the hippocampal electroencephalogram (EEG), especially the highly synchronous theta rhythm, with electrical stimulation (*2, 3*), drugs affecting central cholinergic mechanisms (*4*), or lesions of the medial septal nucleus (MSN) (*5, 6*) impairs the learning of a variety of tasks. Interpretations have implicated both acquisition and retention and have questioned whether impairment is restricted to specific tasks (for example, spatial or cue learning) (*5, 6*), or involves more general processes (for example, learning, consolidation, attention, or arousal) (*2, 4*).

We have recently reported evidence of a strong relationship among frequencies in the hippocampal EEG, learning-related changes in hippocampal neuronal activity, and differences in acquisition rate of the classically conditioned nictitating membrane (NM) response in rabbits (*7*). We have also demonstrated that subareas of the septal region show changes in activity related to different aspects of this conditioning paradigm (*8*). The MSN response can best be interpreted as one of arousal in that MSN units show brief, stimulus-evoked responses that decrease with repeated stimulus presentations (*8*). In contrast, units of the lateral septal nucleus, like those of the hippocampus, show learning-dependent plasticity—that is, marked increases in activity that model the amplitude-time course of the conditioned NM response (*9*).

In the light of this evidence, it is surprising that massive ablations of either hippocampus or septum have little effect on acquisition or retention of the NM response in rabbits (*10*). Abnormal activity may be more detrimental behaviorally than removal of the hippocampus itself (*11*), an interpretation supported by studies of NM conditioning using disruptive hippocampal stimulation (*3*). Massive septal lesions not only disrupt hippocampal activity by damaging the medial septal "pacemaker" for theta, but also interrupt major subcortical hippocampal efferents through the lateral septal nucleus (*12*). It can thus be hypothesized that lesions restricted to the MSN impair NM conditioning more than large septal or hippocampal lesions, because major pathways conveying abnormal hippocampal activity would be left intact. Also, such specific disruption of the septo-hippocampal system during NM conditioning of rabbits can address questions concerning the task specificity of septo-hippocampal processes. If septal damage disrupts nonspatial learning (such as NM conditioning), arguments can be made for a septal role in learning common to both spatial and nonspatial tasks.

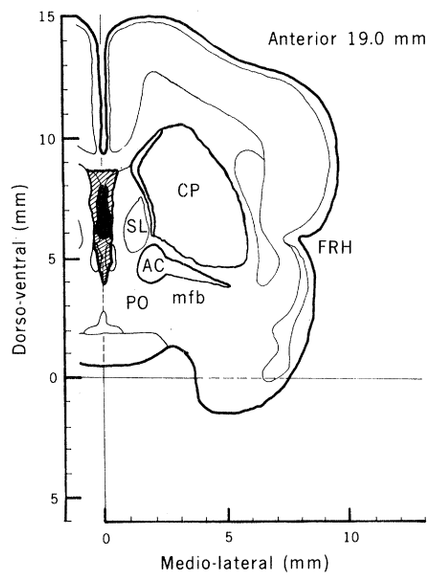

Fig. 1. Coronal section of rabbit forebrain showing the minimum (solid) and maximum (striped) extent of MSN lesions. The regions of the lateral septum and diagonal band are undamaged. Abbreviations: *AC*, anterior commissure; *CP*, caudate putamen; *FRH*, rhinal fissure; *mfb*, medial forebrain bundle; *PO*, preoptic area; and *SL*, lateral septal nucleus.

With these questions in mind, we assessed the effects of small, theta-disrupting MSN lesions on NM conditioning, hippocampal EEG, and multiple-unit activity of the CA1 area of the dorsal hippocampus. Fourteen New Zealand White rabbits (*Oryctolagus cuniculus*) had stainless steel insect pins, insulated except for 50 to 70 μm at the tip, implanted in the dorsal hippocampus for recording EEG and unit activity. All surgery was performed under halothane anesthesia, and electrodes were localized by a combination of stereotaxis and physiological recording during implantation. Skull screws and dental acrylic secured the recording electrodes to the skull. One skull screw served as a reference for recordings. Seven of the rabbits received midline septal lesions as follows. An insect pin insulated except for 200 μm at the tip, was lowered along the midline of the forebrain until the characteristic bursting pattern of MSN cells was observed. The electrode was lowered further until this bursting began to fade, at which point a d-c electrolytic lesion was made. Two additional lesions were made, 0.5 and 1.0 mm dorsal to the original lesion. Current parameters were 0.8 to 1.0 mA for 8 to 10 seconds at each placement.

Animals were given 8 to 10 days to recover from surgery and were then conditioned according to a standard paradigm (*7–9*). They were trained to a criterion of eight of nine consecutive conditioned responses (CR's) (*13*) or for a total of 4 days. Each daily session consisted of 13 blocks of trials [eight paired CS-UCS trials, and one test CS-alone trial per block (*14*)]. The CS was a 350-msec, 1-kHz, 85-dB tone and the UCS was a 100-msec corneal air puff at 210 g/cm^2, which began 250 msec after CS onset and terminated with the CS. Prior to the first conditioning trial, 2-minute EEG samples were recorded to assess the amount of theta in the spontaneous EEG. During training, multiple-unit activity from the

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pyramidal cell layer of the dorsal hippocampus was recorded for standard score analysis.

Multiple-unit activity from the recordings was filtered (0.5 to 5 kHz) and analyzed by computer (PDP-12) (*8*, *9*). Pulse height discriminators were set to pass only larger spikes, and poststimulus histograms were accumulated for each block of paired trials. Unit analysis consisted of computing standard scores (*15*) based on changes in amount of unit activity in the CS and UCS periods. Records from the EEG were put through a low-pass filter (0 to 25 Hz) (*7*, *16*). After behavioral training, animals were anesthetized with pentobarbital (Nembutal), a small d-c lesion was made to mark the recording electrode site, and tissue was fixed by intracardial perfusion with 0.9 percent saline followed by 10 percent formalin. Recording placements were located and lesions in the septal region were reconstructed with wet-tissue photography and Nissl-stained sections.

For EEG analysis, only animals with recording electrodes in the dorsal hippocampus were used (MSN = 6, control = 7); for unit analyses the recording electrodes were in or near the pyramidal cell layer (MSN = 5, control = 7). Figure 1 shows a summary of lesion extent in the MSN lesion group (*17*). Lesions were purposefully small, sparing portions of the MSN to avoid damage to the lateral septal nucleus or fornix. Typically, only 35 to 50 percent of the MSN was damaged bilaterally. Even these small lesions significantly reduced the percentage of theta (2 to 8 Hz) in the EEG [t (11) = 2.85, $P < .02$]. In addition, the ratio of high to low frequencies, previously shown to be highly correlated (negatively) with acquisition rate in this task (*7*), was also raised in MSN-damaged rabbits [t (11) = 2.42, $P < .05$].

An analysis of variance on multiple-unit standard scores for the first day of training indicated less responsiveness during the CS period [F (1, 9) = 10.26, $P < .05$]; responses to the UCS by the MSN group were no lower [F (1, 9) = 3.64, $.05 < P < .10$]. By the last day of training, the differences in activity during the UCS period were significant [F (1, 9) = 5.43, $P < .05$]. In addition, during the last half of the CS period (when CR's normally occur), animals with MSN lesions were still less responsive to the CS [F (1, 9) = 13.1, $P < .01$]. Thus, the effects of MSN lesions were reflected in the responsiveness of hippocampal unit activity to the conditioning paradigm, even over several days of training.

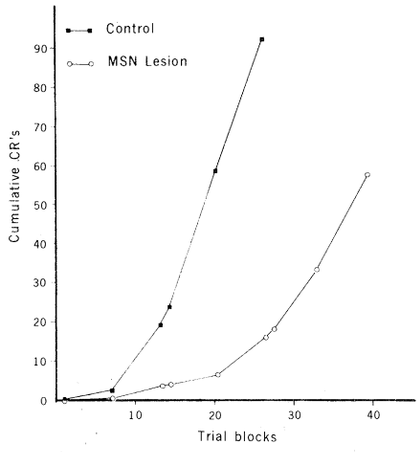

Fig. 2. Mean cumulative CR's across training blocks. The first CR's in the MSN group are relatively delayed.

The MSN group took longer to reach criterion than did controls [t (12) = 5.52, $P < .001$] (Fig. 2). The treatment seems to have postponed the onset of conditioning, rather than prevent it completely. Although three of the MSN animals failed to reach criterion, each member of this group increased CR's across training. This particular form of impairment has been described as following other disruptions of hippocampal function (*3*) and has been characterized as a delay in the onset of conditioning, followed by a relatively normal increase to near normal levels of performance. Although testing was terminated before the performance of the MSN group reached asymptote, our data support this interpretation of the lesion deficit. The MSN animals required more trials to reach their third CR than did controls [t (12) = 2.34, $P < .05$]. Such an effect on learning could be described as a lengthening of phase 1 in the two-phase model of classical conditioning proposed by Prokasy (*18*).

The concomitant impairment in hippocampal theta and rate of learning following small MSN lesions provides support for our earlier observation (*7*) of a predictive relationship between hippocampal EEG and rate of learning. A recent report (*6*) that spatial memory functions of rats are impaired after subtotal MSN lesions, which disrupted hippocampal theta, is consistent with our observations. However, our results indicate that septo-hippocampal involvement in learning processes is not restricted to spatial tasks. The relationships between the rate of NM conditioning and (i) changes in single- and multiple-unit activity in both septum and hippocampus (*8*, *9*), (ii) pretraining spontaneous hippocampal EEG and learning-related changes in EEG frequencies (*7*), and (iii) disruption of normal patterns of hippocampal activity (as shown here) suggest a strong involvement of the septo-hippocampal system in the acquisition processes of learning (*19*).

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13. A conditioned response (CR) was defined as NM movement of greater than 0.5 mm before UCS (*14*) onset or within 500 msec of CS onset on test trials. Learning criterion was defined as the occurrence of eight CR's on any nine consecutive trials.
14. Abbreviations: CS, conditioned stimulus; UCS, unconditioned stimulus.
15. Standard scores were computed as follows: (PreCS = 250 msec before CS onset; CS period = CS-UCS interval; UCS period = 250 msec after UCS onset; S.E., standard error)

CS period score:
$$(\bar{X}_{\text{CS period}} - \bar{X}_{\text{PreCS period}})/\text{S.E.}_{\text{PreCS period}}$$
UCS period score:
$$(\bar{X}_{\text{UCS period}} - \bar{X}_{\text{PreCS period}})/\text{S.E.}_{\text{PreCS period}}$$

16. The EEG analysis consisted of measuring the period between successive positive-going zero crossings, converting period to frequency, and accumulating the number of waves into frequency categories. Categories were (in hertz): 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 18, and 18 to 22.
17. Coronal section modified from I. Urban and P. Richard, *A Stereotaxic Atlas of the New Zealand Rabbit's Brain* (Thomas, Springfield, Ill., 1972).
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Inhibitory, GABAergic Nerve Terminals Decrease at Sites of Focal Epilepsy

Abstract. *Using an immunocytochemical method for the localization of the γ-aminobutyric acid (GABA) synthesizing enzyme, glutamic acid decarboxylase (GAD), we have observed GABAergic nerve terminals distributed throughout all layers of normal monkey sensorimotor cortex. These terminals displayed ultrastructural characteristics that suggested that they arose from aspinous and sparsely spinous stellate neurons. In monkeys (*Macaca mulatta *and* M. fascicularis*) made epileptic by cortical application of alumina gel, a highly significant numerical decrease of GAD-positive nerve terminals occurred at sites of seizure foci indicating a functional loss of GABAergic inhibitory synapses. A loss of such inhibition at seizure foci could lead to epileptic activity of cortical pyramidal neurons.*

Epilepsy is a neurological disorder characterized by intermittent, generalized seizures involving motor and sensory systems. Most epilepsies in humans are caused by tumors or by trauma to a brain region as a result of cranial injuries, including those produced by birth canal obstructions (*1*). Human epilepsy has not been studied in as much detail as disorders in experimental animals produced by the application of various agents that initiate seizure activity; for example, alumina gel, cobalt, and penicillin (*2*). Since these experimentally produced epileptic foci develop reproducibly they have provided models for the study of human focal epilepsy.

Several factors, including glial hypertrophy, ischemia, and increased concentrations of acetylcholine, have been suggested as being involved in the onset of seizures (*3*). Another such factor, and one that is the concern of this report, involves the inhibitory neurotransmitter γ-aminobutyric acid (GABA). It has been suggested that a reduction of this inhibitory substance could be responsible for seizure activity because results from biochemical studies have shown decreases in both GABA (*4*) and its synthesizing enzyme, glutamic acid decarboxylase (GAD) (*5*), at seizure foci.

In a recent immunocytochemical study, GAD has been localized within the axon terminals of aspinous and sparsely spinous stellate neurons in the rat cerebral cortex (*6*). Since GAD has been found in a number of other brain regions within neurons that have been identified as GABAergic (*7*), it is probable that the presence of GAD is indicative of neurons that use GABA as a neurotransmitter. Therefore, the localization of GAD within aspinous and sparsely spinous stellate neurons, together with evidence from physiological and pharmacological studies (*8*), strongly suggests that these neurons are responsible for GABA-mediated inhibition in the cerebral cortex. Since these neurons are found in every cortical layer and project numerous axon terminals to pyramidal cell somata, they could exert a powerful inhibitory effect on cortical projection neurons. Furthermore, a decrease in the number of these inhibitory axon terminals could lead to seizure activity of pyramidal neurons. To test this possibility, we compared the

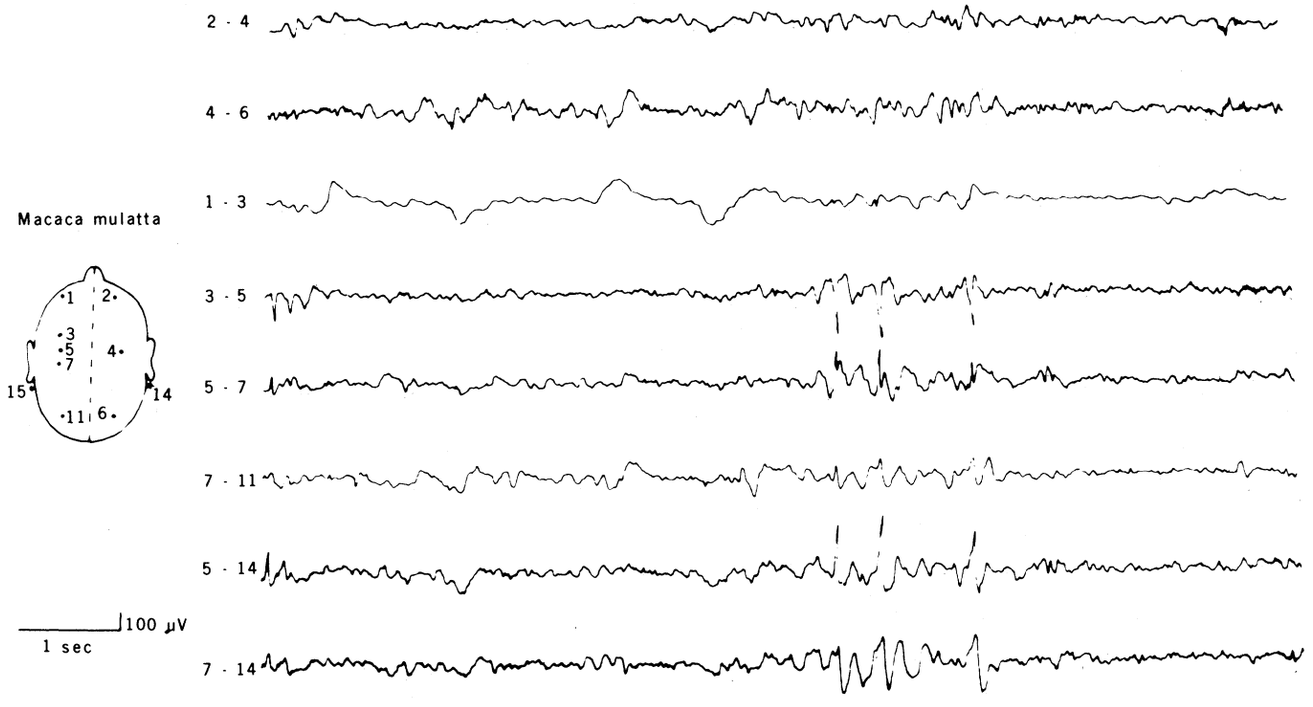

Fig. 1. An ECG recording that was made just prior to fixation of the brain by intravascular perfusion shows an alumina-induced epileptic focus in *M. mulatta* located around lead 5 as indicated by spike phase reversals in traces 3 to 5 and 5 to 7.

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