pressed in picomoles per kilogram per minute (for sheep, 0.00033 pmole/kg-min; for rats, 1.36 pmole/kg-min). Even after food deprivation of only 2 hours in either lighting condition, CCK octapeptide did not affect feeding. However, food intake has been reduced in rats deprived of food for 4 hours by bolus intraventricular injection of caerulein (80, 200, and 300 pmole/kg), which has been shown to decrease food intake in rats when administered systemically (*2*); thus, rats appear to be considerably less sensitive than sheep to the CNS-mediated inhibition of feeding by CCK octapeptide or pentagastrin.

In the past decade the effects of CCK and its derivatives on feeding behavior have received much attention. Although rats have been the principal focus of these studies, a reduction of food intake following systemic administration of the hormone has been demonstrated for several other species (*9*). It has been recognized that not only do CCK and CCK octapeptide exist in the brain and CSF, but when injected there they can have specific effects. For example, caerulein injected as a bolus into the LV of rats at much larger doses than in our experiment inhibited feeding, as did microinjections of caerulein into the VMH but not the LH (*1*); CCK octapeptide injected into various areas of rat brain caused electrophysiological changes (*13*). It has been found that obese mice of the *ob/ob* genotype had significantly reduced concentrations of cerebral cortical CCK octapeptide compared to their lean littermates (*OB/-*), suggesting that brain CCK octapeptide may play a role in the genesis of obesity (*11*). Innis *et al.* (*14*) have reported that in rat brain, high concentrations of CCK octapeptide cells are located in the hypothalamus—particularly the dorsomedial area. This also supports a role of CCK octapeptide in control of food intake. Concentrations of CCK of 1 nmole per gram of tissue (wet weight) have been found in the telencephalic gray matter of human brains. This amount is at least ten times greater than those reported so far for other hormonal peptides, releasing factors, or release-inhibition factors, a finding that is consistent with the neurotransmitter role that has been suggested for CCK octapeptide (*3*).

Our use of a prolonged slow rate of injection of extremely low concentrations of these peptides is probably a more accurate test method from a physiological point of view than methods in which bolus injections of high concentrations are used. The specificity of the effect of CCK octapeptide for food intake rather than water intake and the lack of effect of systemic injections at much larger doses suggest that CCK octapeptide acts on CNS structures involved in food intake control. The fact that CCK octapeptide was more effective in sheep deprived of food for the normal intermeal interval (2 hours) than in sheep deprived of food for longer periods suggests a physiological role for this peptide in their control of food intake. The decrease in food intake produced by pentagastrin injection could be explained by the structural similarity between that portion of the gastrin molecule and the active portion of the CCK molecule. This explanation can be supported in that (i) pentagastrin was effective only at much higher concentrations (several hundred times those of CCK octapeptide) despite the fact that, peripherally, gastrin is approximately 1/20 as potent as CCK in its effect on gallbladder contraction (*15*), and (ii) these doses of pentagastrin resulted in abnormal behaviors. In this study, as in others, secretin had no effect on food intake.

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Stimulation of Abducens Nucleus Supports Classical Conditioning of the Nictitating Membrane Response

Abstract. *The acquisition and terminal performance of a classical conditioning group compared with a control group indicated that extension of the nictitating membrane elicited by direct electrical stimulation of the abducens nucleus was successfully conditioned to a previously neutral stimulus. The conditioning so obtained was associative and not due to such nonassociative factors as sensitization, pseudoconditioning, or alteration in base-rate responding.*

One of the fundamental goals of the neurosciences is to specify the neural pathways involved in learning. Following the pioneering studies of Loucks (*1*), a number of investigators (*2*) have reported that electrical stimulation of cortical motor neurons could serve as unconditioned stimuli (US's) in the classical conditioning of movements in response to a variety of conditioned stimuli (CS's). However, there are "many vagaries in the appearance of conditioned responding" (*3*) when stimulation of cortical motor centers serve as the US. Thus, even after extensive training, conditioning is not reliably obtained, and when obtained it is at a low level. In addition, conditioned responses (CR's) often occur in response systems different from that of the unconditioned response (UR). Finally, there has been a failure to demonstrate that CR's, when they do occur, are due to associative rather than nonassociative factors (*2*, *3*).

Recently, a number of investigators (*4*-*7*) have come to recognize the unique advantages of the classically conditioned rabbit nictitating membrane response (NMR) for investigating the neural processes involved in learning. The conditioning parameters have been well specified, and there is essentially no contribution of nonassociative factors to the CR (*8*). In addition, both conditioned and unconditioned NMR's—retraction of the eyeball, via the retractor bulbi muscle, and passive extension of the membrane over the globe—result from activation of the abducens motor neurons (*4*, *6*). Thus, electrical stimulation of the abducens nu-

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cleus directly activates the final neural pathway (cranial nerve VI) for eliciting membrane extension (*4*, *5*). Moreover, in addition to the inputs from peripheral US's such as paraorbital shock or corneal air puff (*4*, *5*), the abducens nucleus also receives inputs from the auditory and visual systems (*9*). Not only does the abducens nucleus receive inputs from stimuli that can serve as CS's and US's during conditioning, but these stimuli also interact with each other. For example, prior to conditioning, a tone CS presented alone does not elicit an unconditioned NMR (*5*, *8*), but the tone does increase the excitability of the unconditioned reflex as measured by an increased amplitude of the unconditioned NMR to the subsequent application of a peripheral US (*5*). Furthermore, Young *et al.* (*5*), using direct stimulation of the abducens nucleus, not only demonstrated that a tone increased the excitability of abducens motor neurons, but also observed a small number of CR's, which suggested the possible occurrence of conditioning. We now report the results of systematic investigations that revealed substantial classical conditioning of the rabbit NMR when tone CS's are paired with direct electrical stimulation of the abducens nucleus as the US (*10*).

In experiment 1, we implanted five experimentally naive rabbits (New Zealand, albino, 100 days old, and weighing 2.2 kg) with monopolar stimulating electrodes (00 insect pins insulated with Epoxylite except for 50 μm at the tip) aimed at the right abducens nucleus (*4*). Placement of the electrode was fixed when an NMR occurred to electrical stimulation at a pulse amplitude no greater than 80 μA (*5*). Electrical stimulation was generated by a solid-state stimulator and delivered through constant-current isolation units.

One week after surgery, the threshold for eliciting a 0.5-mm extension of the membrane (criterion response) to electrical brain stimulation of 1000 Hz, 0.2-msec pulse duration, and 40-msec train duration was determined. Beginning 2 days later, animals were given one adaptation session followed by 16 daily conditioning sessions. The conditioning apparatus, method of restraint, and response transduction have been described elsewhere (*8*). Each conditioning session consisted of 60 paired presentations of a 200-msec tone CS (1000 Hz, 82 dB) whose offset coincided with the onset of a US consisting of a 40-msec train of brain stimulation (1000 Hz, 0.2-msec pulse duration) at a pulse amplitude set three times above each animal's threshold. The pulse amplitude for the five animals ranged from 100 to 380 μA, and evoked UR's of 2.4- to 4.1-mm membrane extension. The intertrial intervals (ITI's) of 50, 60, and 70 seconds were randomized. During the adaptation session, membrane movement was recorded during the intervals when CS's were to be presented in subsequent sessions in order to obtain a measure of base-rate responding.

Electrical stimulation of the brainstem as the US produced an orderly acquisition of CR's to a tone CS over 16 days of training; by the last 2-day block, CR's were being made on a mean [$\pm$ standard error of the mean (SEM)] of 79 $\pm$ 6 percent of the trials (range 64 to 98 percent). In addition, the latencies and shapes of the observed CR's were indistinguishable from those obtained with peripheral US's (*8*).

Given the systematic acquisition function obtained in experiment 1, experiment 2 was conducted to determine whether variations in the pulse amplitude of brain stimulation would produce variations in CR acquisition. Thirty-nine rabbits were prepared and tested for threshold. The mean threshold was 80 μA (range 40 to 150 μA). After 2 days, animals were given one 60-minute adaptation session followed by 20 daily training sessions. Three experimental groups of rabbits (N = 9 per group) received 30 paired presentations of a tone CS and brain stimulation US each day, as described above except that the pulse amplitude of brain stimulation for each group was set at 240, 320, or 400 μA. The ITI's (110, 120, and 130 seconds) were randomized. In addition, three explicitly unpaired control groups (N = 4 per group) received 30 presentations of tone CS alone and brain stimulation US alone, each day, at a pulse amplitude of 240, 320, or 400 μA. These control groups were used to assess the possible contributions of nonassociative processes to CR acquisition. For these control groups, stimulus presentations were arranged so that there were no more than three consecutive presentations of either CS or US, with randomized ITI's of 50, 60, and 70 seconds. For all groups, responses occurring during the 200 msec of tone presentation were scored as CR's. For the control groups, the responses occurring during the 200 msec prior to US onset were also recorded to derive a

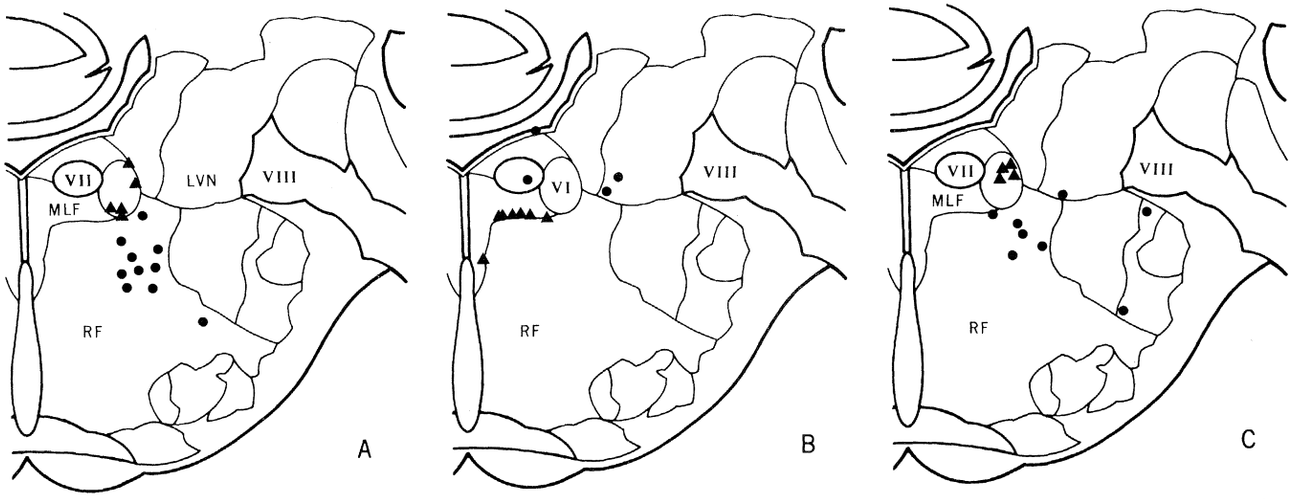

Fig. 1. Anatomical locus of electrode tips for 38 rabbits in experiment 2 (histological verification for one animal was unavailable). In the paired CS-US condition, five electrode tips were in the abducens nucleus (*VI*) (A, ▲), ten in the pontine reticular formation (*RF*) (A, ●), seven in the medial longitudinal fasciculus (*MLF*) bordering the pontine reticular formation (B, ▲), and four in other structures (B, ●). In the unpaired CS, US condition, four electrodes were in the abducens nucleus (C, ▲), and eight were in surrounding structures (C, ●). Abbreviations: *LVN*, lateral vestibular nucleus; *VII*, genu of the facial nerve; and *VIII*, auditory nerve.

measure of the possible synergistic action of CS's and US's on base-rate responding. As in experiment 1, a measure of base-rate responding was obtained during the adaptation session.

There was a significant acquisition of CR's over days in the paired CS-US condition [$F(4,96) = 22.9$; $P < .001$] which reached terminal levels (mean ± SEM) of 45 ± 7 percent ($N = 27$) by the last 2-day block of training. There was no evidence of CR acquisition in the unpaired CS,US condition, in that responding to the tone averaged 1.7 percent throughout training. Moreover, base-rate responding was not elevated, since it (1 percent) was essentially equivalent to that observed during adaptation (2 percent) when no stimuli were presented. Therefore, as with peripheral US's (*8*), the acquisition of conditioned NMR's produced by the pairing of a tone CS with a brain stimulation US was due to associative factors and not to such nonassociative factors as sensitization, pseudoconditioning, or an alteration in base-rate responding.

Examination of the data for the three groups of animals in the paired CS-US condition revealed no significant differences in acquisition or terminal CR performance as a function of US intensity. Consistent with this finding, there was also no significant difference in the mean UR amplitudes of the three control groups. The locus of electrical stimulation was, however, critical in determining terminal CR performance. The five animals with electrode tips located within the abducens nucleus (Fig. 1A) acquired CR's and reached the highest level of asymptotic performance, 74 ± 9 percent (Fig. 2). Stimulation at sites outside of the abducens nucleus, though eliciting UR's, resulted in more variable acquisition and overall lower terminal levels of performance. Specifically, ten animals with electrode tips located in the pontine reticular formation (Fig. 1A) demonstrated asymptotic performance of only 55 ± 13 percent (Fig. 2) with three animals responding near base rate, 3.6 ± 2.2 percent. The lowest level of asymptotic performance, 16 ± 8 percent (Fig. 2) occurred in seven animals with electrodes located in the medial longitudinal fasciculus (Fig. 1B), with five animals responding near base rate, 3.8 ± 1.2 percent. Four additional animals had electrodes located outside these regions (Fig. 1B) and their overall CR performance in the last block of training was 36 ± 20 percent. The differences in terminal CR production for the paired CS-US groups (Fig. 2) were significant [$F(2,19) = 5.27$; $P < .05$]. In addition, relating terminal CR performance to linear distance from the abducens nucleus for the rabbits with electrode tips depicted in Fig. 1A indicates that the closer the electrode tip was to the abducens nucleus, the higher the overall CR performance. For the rabbits with electrode tips located in the reticular formation (Fig. 1A), increased distance from the abducens nucleus, in general, appears to have decreased the efficacy of the US. Taken together, these results indicate that optimum conditioning is obtained by direct stimulation of the abducens motor neurons as the US. Moreover, the observed conditioning was associative, since stimulation of the abducens nucleus, or other brainstem structures, in the unpaired CS,US condition (Fig. 1C) failed to provide any evidence of CR acquisition (Fig. 2).

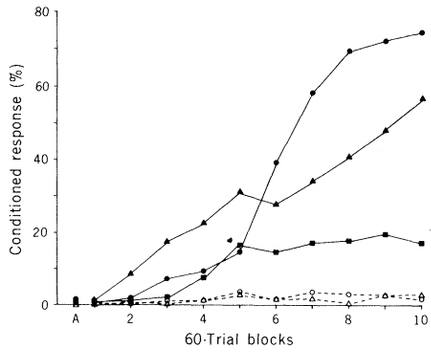

Fig. 2. Mean percent conditioned responses as a function of blocks of 60 trials. For the paired CS-US condition, separate acquisition functions are presented for animals with electrodes in: the abducens nucleus (●, $N = 5$), pontine reticular formation (▲, $N = 10$), and medial longitudinal fasciculus (■, $N = 7$). For the unpaired CS,US condition, data are given for animals with electrodes in the abducens nucleus (○, $N = 4$) and in other structures (△, $N = 8$).

The results of these experiments provide, to our knowledge, the first unambiguous evidence that electrical stimulation of a motor nucleus can effectively serve as the US in classical conditioning. Moreover, these results indicate that the rabbit NMR procedure appears to be ideally suited for determining the neural pathways involved in learning.

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Gamma Rays: Further Evidence for Lack of a Threshold Dose for Lethality to Human Cells

Abstract. *In experiments designed to measure human cell survival with ± 2 percent accuracy it was found that low doses (21 to 87 rad) of γ-rays inactivated the colony-forming ability of cultured human cells with a probability of 0.00226 ± 0.00012 per rad. There appears to be no threshold for the lethality of radiation to human cells in vitro.*

Three important effects of ionizing radiations on human cells are mutagenesis, carcinogenesis, and cell reproductive death. These effects are thought by some to occur through a common molecular mechanism (*1*). Whether or not this is the case, it would be useful to know with certainty whether any of these effects has a dose threshold. The alternative to the existence of a dose threshold is the "linear hypothesis," according to which there is no dose too small to produce an effect, so that dose response curves should be linear at very low doses. We tested the validity of the linear hypothesis for radiation lethality to human cells at low doses and obtained data that strongly support it.

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