T_s on the dorsal and laterodorsal aspects of the snout was relatively low during panting (Fig. 2), which indicates that cooling of the nasal passages is transmitted to the body surface through the intervening bone and skin (the total thickness of these tissues is less than 2 mm). At a T_a of 45.5°C, T_s on the cheeks was 42° to 45°C, whereas T_s on the coolest parts of the snout was 40° to 41°C.

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5. Dry heat transfer includes transfer by conduction, convection, and radiation, but not transfer by evaporation.
6. Animal 1 was a quiet animal that was in late pregnancy at the time of study. Animal 2 was agitated and had given birth to a premature and stillborn litter 1 week before study. Animal 3 seemed to be a young animal, was not pregnant at the time of capture, and was the least affected by human activity. Body weights (measured postpartum in the cases of animals 1 and 2) were 1.4 to 1.9 kg.
7. Sheets of cloth coated with infrared-black paint (emittance .98 between 1 and 20 μm) were suspended freely in the air between the animal cage and the top and sides of the temperature cabinet, and the platform on which the cage rested was coated with the same paint.
8. We analyzed dried pinnae from two animals that were born to animal 1, grew nearly to adulthood, and later died, for spectral total hemispheric reflectance and spectral transmittance in the range of 2.8 to 20 μm with a reflectance spectrophotometer (Cary-White 90) and a transmittance spectrophotometer (Perkin-Elmer 621). Reflectance varied with wavelength between .01 and .13, and transmittance varied between .00 and .04. Integrated emittance over the range of sensitivity of the AGA Thermovision system was .89, calculated according to the methods of R. Siegel and J. R. Howell [*Thermal Radiation Heat Transfer* (McGraw-Hill, New York, 1972)]. We determined surface temperatures using the integrated emittance according to formulas in AGA Operating Manual 7202, section 8. We analyzed 72 color infrared radiographs.
9. To calculate the average surface temperature in a given radiograph, the proportion of the total pinna surface covered by each isotherm in the radiograph was determined by polar planimetry. The proportions were then multiplied by the respective average temperatures represented by the isotherms, and the products were added.
10. We calculated convective and radiative losses or gains by the methods of Wathen *et al.* (*3*), using their field values for the convection coefficient, their value of 0.8 for the radiative view factor, and 0.95 for the overall infrared emittance of the pinnae. Calculations were carried out for the average 2.3-kg animal as reported in Schmidt-Nielsen *et al.* (*1*). Total pinna surface area was thus taken to be 332 cm^2, and comparisons of heat exchange across the pinnae were made to metabolic data and overall heat exchange data reported in Schmidt-Nielson *et al.* (*1*).
11. We thank D. M. Lay for procuring the jackrabbits and L. Blaine for making available the spectrophotometers of the Goddard Space Flight Center, Greenbelt, Md. We are grateful for use of the facilities of the 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base.

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The Amnesia Gradient: Inadequate as Evidence for a Memory Consolidation Process

Abstract. *Rats were conditioned to fear a tone paired with shock to the feet. Retention tests 4 days later showed that consolidation had occurred. Other animals were not tested for retention at 4 days, but the tone was presented in order to reactivate their memories of the conditioning. An amnesia gradient was generated by low-intensity electrical stimulation of the amygdaloid complex at different intervals after the tone, but stimulation was without effect either when given to rats not previously conditioned or when given to conditioned rats without preceding memory reactivation. Thus, stimulation of the amygdaloid complex can affect memory retrieval. Moreover, the data call into question the assumption that an amnesia gradient indicates that the memory consolidation process has been modified.*

A variety of treatments, including specific, low-intensity stimulation of some regions of the brain, produce retrograde amnesia (*1*), which is shown experimentally when different groups of animals are treated at various intervals after a training trial. In the resulting "gradient of amnesia" for the training, the degree of amnesia varies inversely with the interval between training and treatment until the treatment no longer has an effect. The presence of such an amnesia gradient has long been considered to be strong evidence that the amnesic treatment disrupts memory consolidation (*1, 2*).

However, we have found that low-intensity stimulation of the amygdaloid complex (AC) given at different intervals after the reactivation of an old, well-consolidated memory can effect an amnesia gradient. The generation of an amnesia gradient can thus no longer serve as undisputed evidence of a modified consolidation process.

Male Long-Evans rats between 90 and 120 days of age had bipolar electrodes stereotaxically implanted unilaterally into the AC (*3*). After a 5- to 7-day recovery period, the rats adapted for several days to various aspects of the experimental procedure, for example, by drinking water while in the training-testing apparatus and by having the stimulation cable attached. Some rats were then conditioned in the training-testing apparatus by a single pairing of a tone (15 seconds, 12,000 hertz, 90 db) with shock to the feet (1.6 ma) administered through the grid floor of the apparatus during the last 3 seconds of the tone. Other rats received noncontingent shock to the feet in an apparatus distinctly different from the training chamber to control for any systemic effects of shock per se. Four days after conditioning or noncontingent shock some animals from both groups were tested for retention.

In the retention test, the rats, deprived of water for 24 hours, were placed in the training-testing chamber and allowed access to a drinking tube. Through a drinkometer circuit and programming equipment, the conditioning tone was automatically presented after the rat had completed 50 seconds of tube contact. Both the response time (in seconds) to complete the initial 50 seconds of tube contact and the latency from the onset of the tone to the completion of an additional 5 seconds of tube contact by the rat were converted to logarithms. Long drink latencies during the presentation of the tone were considered evidence of retention, and short drink latencies, of amnesia. Details of the apparatus and the training-testing procedure have been described (*4*).

Other rats were not tested for retention but were individually placed in the training-testing chamber and presented with the conditioning tone without the shock, as a memory reactivation treatment (*5*). Rats previously given noncontingent shock but not tested were also presented with the tone as a control for the reactivation procedure. The AC was stimulated (*6*) either immediately, 30 minutes, 60 minutes, or 240 minutes after the termination of the tone. Only animals in the immediate stimulation groups had the stimulation cable attached and received stimulation in the training-testing chamber. Animals in the other stimulation conditions were returned to their home cages for the appropriate interval and were then taken to a different experimental room where the stimulation cable was attached and the AC was stimulated. One group of conditioned animals (TS-C-NS) received no stimulation of the AC but were given the memory reactivation treatment and handled identically to stimulated animals. Another group of conditioned animals (TS-NC-S) were not given memory reactivation treatment but were placed in a different box and given AC stimulation to determine the effects of the stimulation alone. The animals given memory reactivation treatment, AC stimulation, or both, 4 days after training or noncontingent shock were tested, 24 hours water deprived, the next day. The

animals were then killed and perfused with physiological saline followed by an 11 percent formalin solution. The brains were then prepared for frozen section histology by the procedure described by Pieri and Hoffmann (*7*).

The test results of animals with histologically verified electrode placements in the AC (*8*) were analyzed by two separate analyses of variance, one performed on the 50-second contact response times and the other on the drink latencies obtained in the presence of the conditioning cue, the tone. As there were no interval differences when stimulation was given to animals with noncontingent shock, the data from these subgroups were pooled to form a single control group for any nonmemorial interactions between shock, presentation of training cues, and stimulation. Similarly, since the trained animals given sham stimulation showed no effects at the various intervals, their data were pooled so that the animals formed a single control group to which the effect of stimulation in trained animals was compared.

The analysis of the 50 seconds of tube contact on day 4 showed that the trained group had longer response times than the noncontingent shock group ($t = 2.37$, d.f. = 11, $P < .05$). Statistical treatment of the data from the seven groups tested on day 5 again showed only that trained groups had longer response times than the noncontingent shock group [analysis of variance ($F = 2.81$, d.f. = 6,44, $P < .05$); subsequent pairwise Newman-Keuls statistic ($P < .01$ for all comparisons between trained and nontrained animals) (*9*)]. Response time, which was refractory to the effects of AC stimulation, is generally thought to reflect retention of nonspecific situational cues.

In the presence of the specific conditioning cue, the tone, conditioned animals showed retention 4 days after training, as their drink latencies were longer than those of the control animals that received noncontingent shock ($t = 7.85$, d.f. = 11, $P < .001$) (Fig. 1A). Thus, there was consolidation of the tone-shock contingency within the 4-day interval between training and testing.

Stimulation of the AC at different intervals after reactivation of this consolidated memory produced an amnesia gradient (Fig. 1B). There were significant differences in the drink latencies of the seven groups tested 5 days after training ($F = 24.66$, d.f. = 6,44, $P < .001$). Subsequent pairwise comparisons indicated that, compared to that of trained animals given sham stimulation, stimulation of the AC in trained animals resulted in reliably shorter drink latencies when given immediately and 30 minutes after (Newman-Keuls statistic, P's $< .01$), but not 60 or 240 minutes after memory reactivation. Moreover, stimulation immediately after memory reactivation (0 minute) resulted in shorter drink latencies than stimulation at 30 minutes ($P < .05$), 60 minutes ($P < .01$), or 240 minutes ($P < .01$). In turn, the 30-minute group had shorter drink latencies than either the 60- or 240-minute groups ($P < .01$ in both cases). With the exception of the trained group given AC stimulation immediately after memory reactivation, all groups had longer drink latencies than the noncontingent shock group given AC stimulation ($P < .01$ in all cases). Stimulation of the AC in trained animals did not affect memory in the group not given memory reactivation, as the latencies of this group were similar to those of trained animals not given AC stimulation ($P > .05$) and longer than those of the animals stimulated either immediately or after 30 minutes ($P < .01$). Thus, it was the interaction between the memory reactivation treatment and the time until AC stimulation, and not memory reactivation or AC stimulation alone, that effected retrograde amnesia.

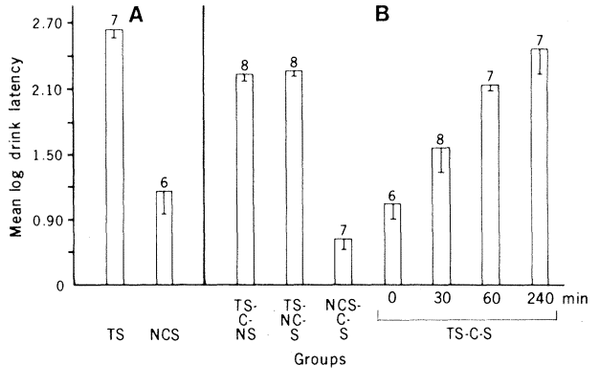

Fig. 1. Mean (−1 standard error) drink latencies in the presence of the tone. (A) Retention performance 4 days after tone-shock (TS) or noncontingent shock (NCS). (B) Retention performance on day 5 in groups given AC stimulation (S) or no stimulation (NS) at different intervals after memory reactivation (as a result of presentation of training cues, C) or no memory reactivation (NC) on day 4. The number of animals in each group is shown above each bar.

We have interpreted the effects of stimulation of the AC after memory reactivation in terms of a modification of a memory retrieval process because (i) animals given noncontingent shock were insensitive to the effects of the stimulation; (ii) previous research in our laboratory failed to demonstrate any motivational or reinforcement effects of AC stimulation with the present parameters, as rats failed to modulate the stimulation in a tilt-box task, failed to press a bar for the stimulation, and failed to acquire a position habit either to escape or to receive the stimulation (*10*); and (iii) retrograde amnesia was produced by stimulating the AC and was a time-dependent disruption of an already consolidated memory trace.

We have also found that stimulation of the AC at the same intervals used in the present experiment, but beginning immediately after conditioning, produced the same pattern of results; that is, no effect of AC stimulation was reflected in the 50-second response times, but an amnesia gradient as indicated in the drink latencies was obtained in the presence of the tone. However, the age of the memory is largely irrelevant to the demonstration of an amnesia gradient. This conclusion is consistent with the hypothesis that the critical variable in modifying memory is the degree to which the memory is active at the time of treatment (*11*). We conclude that stimulation of the AC alters memory retrieval rather than memory consolidation.

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3. Electrodes were made of 0.25-mm stainless steel wire (Plastic Products Co., Roanoke, Va.). Stereotaxic coordinates were 1.00 mm posterior to bregma, 4.00 mm lateral, and 9.25 mm vertical [L. J. Pellegrino and A. J. Cushman, *A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967)]. The electrodes were implanted while the rats were anesthetized with sodium pentobarbital. The electrode assembly was fixed to the skull with dental screws and acrylic cement.
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6. Stimulation was provided by a pulse generator stimulator (BERL) equipped with a micro-current amplifier adjusted to deliver 5 μa, 60 hertz, of continuous biphasic stimulation with each pulse (interpulse interval, 1 msec) for 60 seconds. Stimulation did not result in seizure activity in neurons as indicated by electroencephalo-

graphic recordings obtained in subsequent experiments.
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8. Within each group, the majority of the AC placements were located with about equal frequency in the vicinity of the medial or cortical nucleus, although some were located in or near the basal medial and basal lateral nuclei of this structure. There did not seem to be any relation between placement within the AC and test performance.
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Enkephalin-Induced Depression of Single Neurons in Brain Areas with Opiate Receptors—Antagonism by Naloxone

Abstract. *Enkephalin, applied microiontophoretically, depressed spontaneous and glutamate-induced firing of single neurons in frontal cortex, caudate nucleus, and periaqueductal gray matter, where enkephalin and high concentrations of opiate receptors are found. Many of the depressions were blocked by the specific narcotic antagonist naloxone. The data are compatible with a neurotransmitter or neuromodulator role for this new brain pentapeptide.*

Stereospecific opiate binding sites have been demonstrated in mammalian brain (*1*). An endogenous morphine-like substance can be extracted from mammalian brain or cerebrospinal fluid and has a distribution similar to that of the receptors (*2*). Hughes *et al.* (*3*) identified their substance as a mixture of two pentapeptides, H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH, which they named methionine-enkephalin and leucine-enkephalin, respectively (*4*). These substances have been synthesized (*3*) and, like the endogenously extracted morphine-like material, have a high affinity for the opiate receptor. Their pharmacology is similar to that of the opiates on in vitro opiate receptor model systems such as the mouse vas deferens and guinea pig ileum (*1–3*). Methionine-enkephalin is more potent than leucine-enkephalin in these systems. In this report the term enkephalin refers to the methionine terminal material.

The suggestion has been made that the opiate receptors in brain do not exist by chance but are there to receive an endogenous ligand, presumably enkephalin, which is released by a specific neural system (*2*, *5–8*). We have examined the effects of microiontophoretically applied enkephalin and morphine on single neurons in various brain areas reported to be rich both in enkephalin and in opiate receptors and have obtained support for this suggestion. Enkephalin depressed the firing rate of many neurons in these brain areas by a mechanism that could be blocked by the narcotic antagonist naloxone, but did not depress neurons in an area comparatively devoid of opiate receptors.

Male Sprague-Dawley rats (230 to 260 g) were used in these experiments. The rats were anesthetized with urethane (1.2 g/kg, intraperitoneally) and rectal temperature was monitored with a thermistor probe and maintained at 36.5° ± 0.5°C by means of a heating pad under the abdomen. Six-barrel glass microelectrodes were placed into the caudate nucleus or periaqueductal gray (PAG) region (Fig. 1) according to the atlas of König and Klippel (*9*). Neurons were studied in frontal and posterior cerebral cortex also (see Fig. 1). Immediately before use, the electrode barrels were filled (*10*) with the following solutions: NaCl (5*M*, one barrel for recording and one barrel for current balancing), monosodium-L-glutamate (0.5*M*, *p*H 9), enkephalin hydrochloride (*11*) (3.75 m*M* in 0.03*M* NaCl, *p*H 4), morphine sulfate (0.05*M* in 0.03*M* NaCl, *p*H 4), and naloxone hydrochloride (0.2*M*, *p*H 4). Glutamate was passed as an anion, while enkephalin, morphine, and naloxone were passed as cations. The unit recording and microiontophoretic techniques have been described previously (*12*). The technique of automatic balancing of current at the tip of the microelectrodes was used to prevent or minimize current effects (*13*).

The results reported here for enkephalin were obtained from 63 neurons (spontaneously firing, glutamate-accelerated, or glutamate-activated) in the caudate nucleus, the PAG, or the cerebral

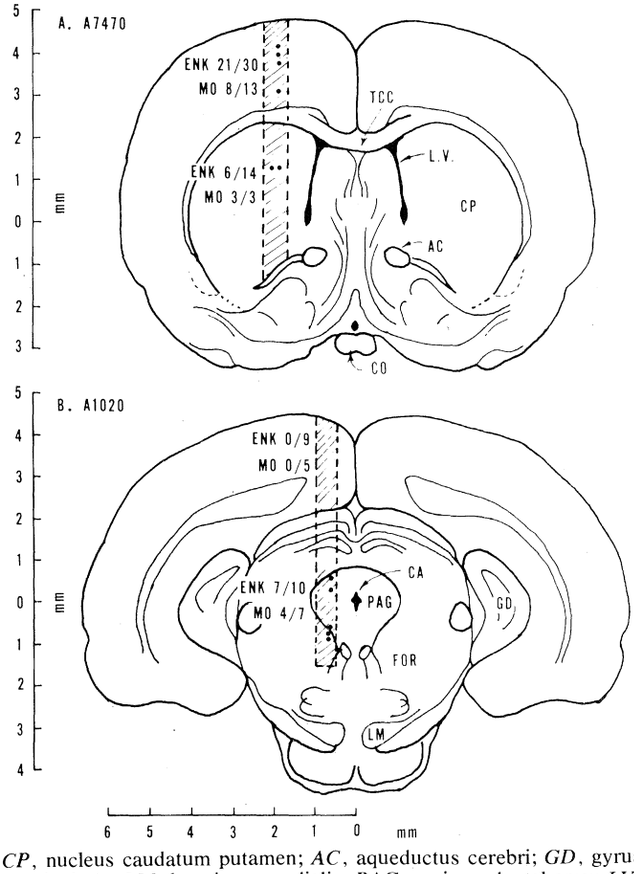

Fig. 1. Frontal sections of rat brain showing location of studies with multibarrel microelectrodes. Hatched areas indicate regions where cells were recorded and tested with microiontophoretic enkephalin. These sections were drawn from the atlas of König and Klippel (*9*). (A) Section at anterior 7470 μm including the caudate nucleus; (B) section at anterior 1020 μm including the PAG matter. The number of cells depressed by morphine and enkephalin over the total number of cells tested in each case is indicated just outside each hatched area. Filled circles in hatched areas show approximate locations of cells on which depression of activity by enkephalin was antagonized by naloxone. Abbreviations: *TCC*, truncus corporis callosi; *CA*, commissura anterior; *CO*, chiasma opticum; *CP*, nucleus caudatum putamen; *AC*, aqueductus cerebri; *GD*, gyrus dentatus; *FOR*, formatio reticularis; *LM*, lemniscus medialis; *PAG*, periaqueductal gray; *LV*, lateral ventricle; *ENK*, enkephalin; and *MO*, morphine.