lins or their analogs have been shown to exert profound effects upon behavioral systems. Nevertheless, there has been relatively little direct evidence concerning the role of endogenous opiate systems in normal behavioral regulation. If endogenous opiate systems are normally active in reducing pain, then blockade of opiate receptors should enhance pain sensitivity. To test this possibility, we examined the effects of naloxone, a specific blocker of opiate receptors, on sensitivity to pain as measured by the tail-flick test. Twelve male albino (Holtzman) rats were given a single tail-flick test according to the general procedures outlined above. After baseline testing, six animals were injected with naloxone (2 mg/kg, subcutaneously) and the other six received subcutaneous injections of the saline vehicle alone. After drug treatments, tail-flick latencies were redetermined (3-minute intertrial interval) over the course of the subsequent 20 minutes. We found that while baseline latencies did not differ between the two groups and while saline produced no significant change in latencies, naloxone induced a significant decline in the latency to tail-withdrawal relative both to baseline values before injection (mean baseline latency, 4.42 seconds; latency after injection, 3.39 seconds; t, 5.81; 5 d.f.; $P < .01$) and saline control latencies (mean latencies after saline injections, 4.50 seconds; after naloxone, 3.39 seconds; t, 3.37, 10 d.f., $P < .01$). Thus, blockade of opiate receptors in otherwise untreated animals increased their sensitivity to the thermal stimuli used in the present test, indicating that opiate systems may act normally to suppress sensitivity to certain classes of stimuli. This suggestion is supported by a report in which a different measure of thermal sensitivity (the hot plate test) was used (*16*), although other workers have challenged this view because of their failure to obtain enhanced pain reactions to electric shock after naloxone administration (*17*). In view of our findings it seems highly possible that endogenous opiate systems may exert differential tonic influences on different sensory modalities.

The demonstration that the naturally occurring opiate-like peptide methionine-enkephalin or its potent analog [D-Ala^2]-methionine-enkephalin can produce analgesia, whereas the opiate receptor blocker, naloxone, induces hypalgesia, supports the suggestion that endogenous opiate systems may function to regulate tonic sensitivity of central pain systems. The previous demonstration that physical stress can induce a release of endogenous enkephalin and a corresponding marked analgesia in the rat further indicates that the tonic activity of opiate systems can be modulated by environmental influences.

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Angiotensin Injected into the Neostriatum After Learning Disrupts Retention Performance

Abstract. *Angiotensin II, injected into the dorsal neostriatum of rats 5 minutes after they had learned a passive avoidance task, disrupted the retention of the task 24 hours later. Identical neostriatal injections given 22 hours after learning (2 hours before retention) were without effect on retention performance. Ventral neostriatum or posterior thalamus were ineffective sites for injection of angiotensin. Injection of thyrotropin releasing hormone or lysine-8-vasopressin into the dorsal neostriatum was ineffective. These findings indicate a possible role for endogenous angiotensin in the neostriatum on retention performance and suggest potential involvement in mnemonic processes.*

The nigro-neostriatal system has recently been implicated in learning and memory processes. Lesions and electrical stimulation of the neostriatum or the substantia nigra (*1*) produce deficits in the acquisition or retention of active and passive avoidance tasks or both. Manipulation of the synaptic transmitters within this system also affects learning and memory (*2*). Thus the nigro-neostriatal system, in addition to its well-known role in motor functions, may also be involved in memory mechanisms.

Recent evidence indicating that peptides play an important role in learning and memory (*3*) prompted us to investigate the effects of angiotensin II, a peptide that is endogenous to the nigro-neostriatal system. In particular, this octapeptide is present in the neostriatum along with its precursors and metabolic enzymes (*4*); angiotensin-converting enzyme is present, indeed, in highest concentrations in the neostriatum (*5*). This enzymatic system is isolated from circulating angiotensin II by the blood-brain barrier (*4*), suggesting a separate functional role for this peptide in the brain. Although other peptides affect avoidance learning when injected intracerebrally

(*6*), there have been no reports on the intracerebral effects of angiotensin II on the retention of a simple avoidance response.

Subjects were adult male albino Holtzman rats (200 to 250 g), implanted with a single 24-gauge guide cannula aimed at the head of the neostriatum. On the 4th day after surgery the rats were weighed and then placed on the platform of the passive avoidance test chamber described previously (*7*). Briefly, the chamber was 27.5 by 27.5 by 31.5 cm and the platform, 8 cm wide and 9 cm high, was against one wall of the chamber. The rats received 0.5 ma of foot shock through the grid floor whenever they stepped off the platform. All rats initially stepped down, with a mean latency of 1.8 seconds. The acquisition session of the passive avoidance task lasted until the rats remained on the platform for 2 minutes without stepping down.

The rats were then removed from the chamber and placed in their home cage for 5 minutes. Following this delay each animal received a single intracranial injection of 1.0 μl of a test solution (*8*) delivered in a saline vehicle at the rate of 1 μl per 4 minutes. The injection was given according to previous recommendations and procedures (*9*). The animals were then returned to their home cages and their behavior was observed. Twenty-four hours after injection the rats were again placed on the platform in the test chamber for retention testing. All conditions were the same as those on the acquisition day except that the grid bars did not deliver foot shock when the rat stepped off the platform. If the animal remained on the platform for 3 minutes he was placed in his home cage and tested the next day. Testing continued until the rat stepped off the platform on two consecutive days during the 3-minute retention trial. The length of time each rat remained on the platform was recorded. These data, then, furnished two performance measures shown in Table 1: (i) percentage of retention disruption—the proportion of rats in each group that stepped down to the grid floor in the 3-minute test session on the first retention day—and (ii) mean latency to first descent—the average time the rats in each group remained on the platform during retention testing (*10*). These two measures indicate the willingness of the rats to stand on the grid floor that they had previously learned to avoid. Thus the shorter the time spent on the platform, the greater the indication of retention disruption. Animals were killed and cannula placement was determined by histological examination.

Angiotensin II injected directly into the dorsal neostriatum caused disruption of retention performance. The percentage of retention disruption in the groups that received 1 μg and 100 ng of angiotensin II was significantly greater ($P < .05$) than in the saline- and operated-control groups (see Table 1). In addition, the mean latency to descent was significantly shorter ($P < .01$) for the groups receiving 1 μg (211 seconds) and 100 ng (215 seconds) of angiotensin II than it was for the saline-control (509 seconds) and operated-control (643 seconds) groups.

The anatomical location of lesions or injections within the neostriatum (dorsal versus ventral) is an important variable related to the subsequent behavior of the rat (*11*). In order to determine whether a comparable effect would hold for intracranial injections of angiotensin II, another group of rats was implanted with cannulae in the neostriatum 0.5 mm lateral and 1.0 mm ventral to the dorsal neostriatal groups and injected with 100 ng of angiotensin II. There was no significant difference between this group (mean latency, 666 seconds) and a group with similarly placed cannulae in the ventral lateral neostriatum and injected with saline (mean latency, 667 seconds) or the control groups previously mentioned that were injected in the dorsal neostriatum (Table 1). The mean latency of the group that received 100 ng of angiotensin II in the dorsal neostriatum was significantly shorter than the mean latency of this group (ventral neostriatum) ($P < .01$).

Table 1. Percentage retention disruption and mean latency (in seconds ± standard error of the mean) for each treatment and control group. *N*, number of subjects; A-II, angiotensin II; TRH, thyrotropin-releasing hormone; LVP, lysine-8-vasopressin.

Group	*N*	Percentage of retention disruption	Mean latency (seconds)
A-II 1 μg	9	78	211 ± 88
A-II 100 ng	15	67	215 ± 73
A-II 10 ng	9	33	581 ± 173*
A-II 1 ng	9	22	558 ± 159*
Saline control	15	20*	509 ± 100*
Operated control	11	18*	643 ± 159*
A-II Posterior thalamus	11	18*	471 ± 87*
A-II 2 hours before retention	12	17*	480 ± 123*
A-II Ventral lateral neostriatum	9	33	666 ± 189*
TRH (100 ng)	15	27*	410 ± 102*
LVP (100 ng)	15	27*	552 ± 113*

*Significantly different from groups receiving 1 μg or 100 ng of angiotensin II ($P < .05$).

Since the posterior thalamus has been implicated as a site of action of peptides in influencing avoidance behavior (*5*), we injected a group of rats in this region with 100 ng of angiotensin II to test the effect of the octapeptide on this peptide-sensitive brain area. Neither performance measure, however, indicated a significant difference between the group injected in the posterior thalamus (mean latency, 471 seconds) and a group injected with saline in the posterior thalamus (mean latency, 590 seconds) or the control groups injected in the dorsal neostriatum (Table 1). Both performance measures indicated a significant difference between the group that received 100 ng of angiotensin in the posterior thalamus and the group that received the same dose of angiotensin in the dorsal neostriatum ($P < .05$). Thus, with the brain injection sites used in this study, only the dorsal neostriatum was found to be an active site for angiotensin II disruption of retention.

We have shown that angiotensin II affects passive avoidance retention when injected 5 minutes after training. Since we observed retention disruption 24 hours after the injection, the disruption could be linked to events occurring just after learning, at the time of angiotensin injection, or 24 hours later, during the retention test. Therefore, a group of rats was injected with 100 ng of angiotensin II in the dorsal neostriatum 22 hours after the completion of acquisition of the passive avoidance task (2 hours before retention testing). Neither performance measure indicated a significant difference between this group (mean latency, 480 seconds) and groups receiving saline injected 22 hours or 5 minutes after learning. The group that received 100 ng of angiotensin II in the dorsal neostriatum showed a significant retention impairment relative to this 22-hour group ($P < .05$) on both performance measures. Thus, angiotensin II is unlikely to produce the disruption of retention by interfering with retrieval processes, since injections closer in time to the retention test have less effect than when the octapeptide is injected 5 minutes after learning.

Angiotensin II is a powerful dipsogenic agent (*9*, *12*). While testing rats in-

jected with 100 ng of angiotensin II into the dorsal neostriatum, three rats, which were deleted from the study, were found to drink persistently beginning about 8 minutes and ending about 20 minutes after injection. These rats demonstrated no retention disruptive effect, stepping down on the 6th, 11th, and 12th days, respectively. We concluded, therefore, that the disruption of retention reported here was not a function of the dipsogenic effect of angiotensin II.

In order to determine whether other peptides would have an effect similar to angiotensin II we investigated the effects of dorsal neostriatal injections of thyrotropin-releasing hormone (TRH) and lysine-8-vasopressin (LVP) on this task. Peripherally administered TRH has antidepressant effects on mice (*13*) and on normal (*14*) and disturbed (*15*) humans. Although this hormone, peripherally injected, does not change brain levels of dopamine, brain L-dopa is increased by 50 percent and a proposed site of its action is the neostriatum (*13*), although it is not endogenous to the neostriatum (*16*). Subcutaneous and intracranial injections of LVP increase the resistance to extinction on active or passive avoidance tasks, or both (*3, 17*).

In our study, then, a group of 30 rats were trained, and 5 minutes later they were injected with either TRH or LVP (*7*) in the dorsal neostriatum. Neither performance measure indicated a significant difference between these groups and the control group injected in the dorsal neostriatum (Table 1). The group that received 100 ng of angiotensin II in the dorsal neostriatum showed a significant retention impairment relative to these groups ($P < .05$) on both performance measures. Thus, retention disruption produced by angiotensin II is not a nonspecific effect related to the injection of any peptide, since TRH or LVP at the same dosage were ineffective.

The observed passive avoidance retention deficit caused by angiotensin II may be interpreted from several viewpoints. One view is that processes involved in learning, attention, and registration were disrupted by intracranial injection of angiotensin II. Because of the 5-minute interval between learning and the onset of injection, however, it is unlikely that angiotensin II interfered with such processes. A second view is that angiotensin II enhanced locomoter activity; hence the animal would more likely step down on the retention test. But if angiotensin II injected 5 minutes after learning augmented the tendency to step down 24 hours later, then the same or an enhanced effect should also have been seen in the group injected with angiotensin 2 hours before retention testing. Since it was not, it is unlikely that increased activity produced the passive avoidance retention deficit. Furthermore, TRH increases activity in the open field (*15*) but did not produce a retention deficit in this study. A third view is that angiotensin produced the retention deficit as a consequence of vasoconstriction of neostriatal blood vessels. If this were the case, the group injected with angiotensin II 2 hours before retention testing might have been expected to demonstrate a retention deficit as well. In addition, LVP, a vasoconstrictive agent, did not produce disruption, suggesting that blood pressure alterations may not be important to the retention disruption. A fourth view, and one which we provisionally advocate, is that angiotensin may be acting as a neuromodulator (*18*), altering the neuronal activity occurring after learning that is necessary for adequate retention performance. Although the present data do not provide evidence as to the mechanism of this alteration, it may be worthwhile to explore the influence of angiotensin on neostriatal synaptic transmission.

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