Orth *et al.* (*8*) briefly reported findings on a blind subject who appeared to have a "free-running" component of cortisol secretion despite a normal sleep-wake cycle. In several other studies, psychologically normal subjects not isolated from time cues, but with varying degrees of blindness, did not show evidence of "free-running" circadian rhythms (*9*). Nevertheless, our subsequent survey of 50 subjects with varying degrees of blindness revealed that 38 complained of a significant sleep-wake disorder. Of these, 20 reported that their symptoms were cyclic or episodic, 14 had no light perception, 18 were blind from birth, and 13 had retrolental fibroplasia (as did J.X.).

The syndrome suffered by J.X. is not necessarily restricted to the blind. When symptoms are less severe, the syndrome would rarely be suspected, and without sophisticated long-term monitoring or autorhythmometry (*10*) the diagnosis will be difficult to confirm. Nevertheless, the disorder might not be uncommon, and the social and economic impact of even minor symptoms might be substantial.

In view of the many publications which have demonstrated that animal (including human) biological systems are influenced by lunar rhythms (*11*), it is notable that J.X. maintains a circadian rhythm that cannot be significantly distinguished from the period of the lunar day (24.84 hours). Furthermore, throughout the ad-lib sleep study, there was a remarkable coincidence between his sleep onset and a local low tide.

Full details of this unusual case are in preparation (*12*).

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Memory Formation: Evidence for a Specific Neurochemical System in the Amygdala

Abstract. *β-Adrenergic antagonists injected into the amygdala complex of rats trained in a passive avoidance task produced time-dependent and dose-dependent decreases in retention of the task. In addition, the effects observed with β-adrenergic antagonists were both stereospecific and reversed by norepinephrine. The results support a role for an amygdala β-adrenergic system in memory processes.*

Clinical observations of retrograde amnesia following brain trauma (*1*), and permanent loss of long-term memory formation capabilities with temporal lobe resection in humans (*2*), have guided investigations with laboratory animals in which attempts have been made to elucidate the neuroanatomical and neurochemical substrates underlying memory formation. Although a wide variety of disruptive agents such as electroconvulsive shock, anesthetics, and protein synthesis inhibitors have been used in these investigations, the results generally agree that the sooner the agent is applied after an experience, the greater is the amnesia for the experience (*3*). This time-dependent retrograde amnesia gradient confirms and extends the earlier clinical findings of retrograde amnesia following human brain trauma. However, owing to the widespread and nonspecific effects of many of these amnesic agents (*4*), our knowledge of the specific neuroanatomical and neurochemical substrates underlying the memory process has not been greatly enhanced by their use.

Researchers have also investigated systematically the neuroanatomical and neurochemical systems involved in long-term memory formation in animals (*5*, *6*). Here we report that microinjections of β-adrenergic blocking agents into the amygdala of rats that have received a single training experience produce retrograde amnesia which is both time-dependent and dose-dependent. Furthermore, the amnesia produced by these agents is demonstrated to be stereospecific and re-

Table 1. Latencies on day 2. The control groups were as follows: 1, no surgery; 2, surgery only; 3, vehicle only.

Group	Dose (nmole)	*N*	Median (seconds)	Interquartile range (seconds)
Control groups				
1		17	268	139 to 576
2		9	279	80 to 411
3		8	216	145 to 315
Propranolol				
4	8.5	9	166	34 to 576
5	17.0	7	113	35 to 291
6*	34.0	9	30	11 to 180
10†	34.0 (6 hours delay)	9	135	87 to 397
12†	34.0 (dextro isomer)	8	235	126 to 322
Alprenolol				
7	8.5	6	155	45 to 297
8*	17.0	8	69	11 to 187
9*	34.0	9	44	8 to 74
11†	34.0 (6 hours delay)	8	246	166 to 414
13†	34.0 (dextro isomer)	6	182	134 to 302

*Mann-Whitney U tests (two-tailed) performed between these groups and the control groups (pooled data from groups 1 to 3) revealed significant differences ($P < .01$). †While each of these groups did not differ significantly from the pooled control data (groups 1 to 3), groups 10 and 12 each differed significantly from group 6. Likewise, groups 11 and 13 differed significantly from group 9.

versed by microinjections of *l*-norepinephrine.

Untrained male Sprague-Dawley rats ($N = 160$) were housed singly and assigned to one of 13 groups (*7*). Group 1 received no surgery. Groups 2 to 12 were surgically prepared with bilateral cannulas positioned at the dorsal surface of the amygdala complex. At the completion of the experiment cannula placement was verified histologically according to previously established criteria (*8*).

One week after surgery all groups were trained in a passive avoidance task (*9*). On day 1, each animal was placed into the lit compartment of a two-compartment apparatus. As it stepped into a dark compartment through an open door which then closed, the animal received a footshock (1 ma, 2 seconds). Retention of passive avoidance was measured 24 hours later (day 2). An increase in latency (time between start of trial and entry into dark compartment) indicated retention of the training experience (*10*). Group 1 (no surgery) and group 2 (surgery only) served as control groups. Group 3 (vehicle control group) received a 0.5-μl injection of a Krebs-Ringer phosphate solution (*11*) bilaterally in the amygdala complex immediately after training. Groups 4 to 6 received bilateral injections of the β-adrenergic blocking agent *dl*-propranolol (8.5, 17, or 34 nmole, respectively) immediately after training, and groups 7 to 9 received identical bilateral injections of a second β-adrenergic blocking agent, *dl*-alprenolol (8.5, 17, or 34 nmole). Groups 10 and 11 received 34-nmole injections of either *dl*-propranolol or *dl*-alprenolol 6 hours after training, and groups 12 and 13 received 34-nmole injections of the dextro isomer of either propranolol or alprenolol immediately after training (*12*). The volume and rate of infusion for all injections was 0.5 μl per minute.

Retention test data are shown in Table 1. A Kruskal-Wallis one-way analysis of variance (*13*) performed on the latency data obtained on day 2 for all groups revealed a significant difference among groups (*14*). Since an additional Kruskal-Wallis one-way analysis of variance performed on the latency data obtained on day 2 for the control groups (groups 1, 2, and 3) revealed no significant difference among these groups, the data from these groups were pooled for further statistical analyses. Subsequent two-tailed Mann-Whitney U tests (*13*) indicated that, in comparison with the control groups, injections of 34 nmole of *dl*-propranolol or of either 17 or 34 nmole of *dl*-alprenolol immediately after training produced a significant retention deficit ($P < .01$), while lower doses of these two blocking agents did not significantly affect retention. The similar magnitude of effect on retention for equivalent doses of *dl*-propranolol and *dl*-alprenolol parallels results obtained with these two agents in other adrenergic systems, indicating that they are approximately equipotent β-adrenergic blocking agents (*15*).

Table 2. Latencies on day 2. The groups were as follows: 1, no surgery; 2, vehicle only; 3, combined *l*-norepinephrine (25 nmole) and *dl*-propranolol (34 nmole); 4, *l*-norepinephrine (25 nmole).

Group	N	Median (seconds)	Interquartile range (seconds)
1	9	355	158 to 563
2	9	334	128 to 491
3*	10	175	81 to 496
4*	10	201	144 to 264

*Did not differ significantly from the pooled data from groups 1 and 2.

The dose-related decrease in retention obtained at the higher doses of *dl*-propranolol and *dl*-alprenolol appeared to be time-dependent because if the injection of the 34-nmole dose was delayed for 6 hours after training retention was not impaired relative to controls. In addition, the retention deficit obtained with *dl*-propranolol and *dl*-alprenolol administered immediately after training was stereospecific because 34 nmole of the dextro isomer of either propranolol or alprenolol had no significant effect on retention 24 hours later.

The possibility that nonspecific effects caused by local anesthetic properties of the drugs accounted for the results is unlikely, because the dextro isomer had no effect on retention (*16*). Furthermore, electroencephalogram recordings taken from the sites of injection after the administration of either *dl*-propranolol or *dl*-alprenolol (34 nmole) in additional groups of animals did not reveal any abnormal afterdischarge activity. This absence of abnormal afterdischarge activity excludes the possibility that these drugs produce retention deficits by inducing the spread of abnormal electrical activity from the site of injection to extra-amygdala areas.

Our next experiment provided a further test of the hypothesis that the effects obtained with *dl*-propranolol and *dl*-alprenolol in our first experiment were due to the specific β-adrenergic blocking activity of these two agents. Since the β-blocking activity of propranolol and alprenolol is competitive and reversible it would be predicted that increased adrenergic activity immediately after training might at least partially reverse the effects on retention produced by the administration of the β-adrenergic antagonists after training. We therefore assessed the effects on retention of injections of *dl*-propranolol in combination with *l*-norepinephrine.

Untrained Sprague-Dawley rats ($N = 50$) served as subjects. Surgical, behavioral, histological, and data analysis procedures were identical to those in the previous experiment. Prior to training, the animals were assigned to one of four groups: control groups 1 (no surgery) and 2 (vehicle only); group 3, which received bilateral amygdala injections of *l*-norepinephrine (25 nmole) combined with *dl*-propranolol (34 nmole); and group 4, which received bilateral amygdala injections of *l*-norepinephrine (25 nmole). All injections were given immediately after training. A Kruskal-Wallis analysis of variance performed on the data obtained on day 2 (Table 2) revealed no significant differences among groups (*14*). Comparison of group 3 (*l*-norepinephrine and *dl*-propranolol) with group 6 of the previous experiment (*dl*-propranolol alone, 34 nmole; Table 1) revealed a significant difference between the two treatments ($P < .05$). This reversal of the *dl*-propranolol retention deficit by injection of the adrenergic agonist *l*-norepinephrine in combination with *dl*-propranolol provides further support for the argument that the stereospecific amnesic effect observed in the first experiment was produced by the activity of these β-adrenergic blocking agents on an adrenergic-sensitive system in the amygdala (*17*).

Our results support the interpretation that β-adrenergic blockade in the amygdala of rats disrupts long-term memory formation in passive avoidance conditioning. While the possibility that drugs injected via the intracerebral cannula method produce their effects by spreading to extra-amygdala regions cannot be ruled out, autoradiographic studies (*18*) of intracerebral amygdala injections of [^{14}C]propranolol of twice the volume used in these experiments indicated that the drug was largely contained within the amygdala complex. Furthermore, others have reported that intraventricular injections of *dl*-propranolol at doses equal to and above those used in the present experiments have no effects on retention of passive avoidance conditioning (*19*),

suggesting that extra-amygdala spread into the ventricles is not responsible for the effects described herein.

Recent reports from other laboratories confirming the presence of norepinephrine in amygdala nuclei of rats (*20*) and studies on the localization and characterization of β-adrenergic receptors in the amygdala complex (*21*) strongly support the possibility that our results indeed reflect the effects of local manipulation of a specific β-adrenergic-sensitive neurochemical system within the amygdala of rats.

Previous observations that low-level electrical stimulation of the amygdala after training disrupts long-term memory formation have implicated this neuroanatomical region in the memory process (*5*).

Furthermore, pharmacological studies have indicated that the integrity of whole brain norepinephrine systems is necessary for long-term memory formation (*6*). Our results thus confirm and extend these previous findings by implicating an amygdala adrenergic system in long-term memory formation.

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Increasing Frequency of Thyroid Goiters in Coho Salmon (*Oncorhynchus kisutch*) in the Great Lakes

Abstract. *Coho salmon collected during the 1976 spawning runs from Lakes Michigan, Ontario, and Erie had overt goiter frequencies of 6.3, 47.6, and 79.5 percent, respectively. These represent significant increases over the frequencies observed in previous years. Epizootiological data suggest that environmental goitrogens (possibly pollutants) may be involved in the etiology of the thyroid disorder.*

Coho salmon (*Oncorhynchus kisutch*) is a highly prized sport and commercial marine fish. In the late 1960's the species was successfully introduced into the Great Lakes (*1*), and it has provided a spectacular multimillion-dollar sports fishery to North American anglers. Since it was introduced into the Great Lakes, several investigators have reported thyroid hyperplasia (goiters) in this species (*2–4*). Recently, Drongowski *et al.* (*3*) and Sonstegard and Leatherland (*4*) described severe hypothyroidism associated with thyroid hyperplasia in coho from Lakes Michigan and Ontario. While the hypothyroid condition (goiters) found in the coho salmon is undoubtedly due in part to the low availability of iodine in the Great Lakes Basin (*5, 6*), we report studies here which strongly suggest the involvement of environmental goitrogens (possibly pollutants) in the etiology of the thyroid disorder. In addition, our data (Table 1) suggest an increase in the frequency of occurrence of overt goiters over that in previous years.

Table 1. Goiter frequencies of Great Lakes coho salmon. Frequencies were determined from sexually mature fish examined during the fall spawning run. The number of fish examined is given in parentheses. Abbreviation: T.R., this report.

Lake	Year	Frequency (%)	Reference
Erie	1972	44 (117)	(*2*)
	1976	79.5 (117)	T.R.
Ontario	1975	24 (51)	(*4*)
	1976	47.6 (63)	T.R.
Michigan	1973	1 (100)	T.R.
	1976	6.3 (111)	T.R.

Sexually mature coho salmon were collected during the fall spawning runs from Lakes Michigan, Ontario, and Erie. The presence of goiters was determined by retracting the operculum of each fish and examining the base of the gill arches for evidence of swelling or nodules (see Fig. 1). Fish with one or more distinct nodules more than 1 cm in diameter were recorded as having overt goiters. The frequency of overt goiters ranged from 6.3 percent in Lake Michigan to 47.6 percent in Lake Ontario to a striking 79.5 percent in Lake Erie.

If low iodine availability were the sole factor contributing to goiter develop-