cats with TOCP neuropathy, and in 12 controls matched for age, initial weight, and conditions of handling. Each cat received an intraperitoneal injection of tritiated L-leucine (1.4 to 2.6 mc per kilogram of body weight, New England Nuclear). At intervals from 4 hours to 2 weeks later, they were anesthetized with pentobarbital, and killed by perfusion through the left ventricle first with saline and then with buffered 2 percent glutaraldehyde. Sections (5 μm) of a lumbosacral cord segment in continuity with its roots and ganglion were cut in a cryostat, mounted on glass slides, dipped in Kodak NTB-3 nuclear track emulsion, exposed for 2 or 4 weeks at 5°C, developed, and stained. The geometric centers of the anterior horn and of the ganglion were used as reference points from which respective distances along ventral and dorsal roots were measured. Fields containing no visible perineurium were selected, and silver grains were counted in 0.01-mm^2 areas at intervals of 0.25 mm along the roots on each of at least two slides prepared from each cat. To facilitate comparison within and between groups of cats, the counts were normalized by expressing them as a percentage of mean activity along each root. Curves expressing mean specific activity of 1-mm segments of nerve root ($\pm$ 1 S.E.) as a function of distance from nerve cell bodies are illustrated in Figs. 1 and 2.

One day after injection of tritiated leucine, the radioactivity in the ventral root of cats in all three groups was maximum adjacent to the spinal cord, whereas in the dorsal root maximum activity bordered the ganglion. In control cats, the peak radioactivity was displaced away from the cord in the ventral root at the rate of 1.6 $\pm$ 0.5 mm per day and away from the ganglion along the dorsal root at the rate of 1.3 $\pm$ 0.2 mm/day. Similar results were obtained with cats treated with TOCP; motor flow was 1.5 $\pm$ 0.4 mm/day and sensory flow was 1.5 $\pm$ 0.3 mm/day (with the exception that, in one cat killed 2 days after it received the isotope, maximum radioactivity remained adjacent to cord and ganglion). In contrast, in all seven cats treated with acrylamide and killed 2 or more days after receiving the isotope, maximum radioactivity remained at the border of the dorsal root ganglion in the dorsal root and, in five of the seven, at the edge of the spinal cord in the ventral root. In the other two acrylamide-treated cats, killed 3 and 7 days after receiving tritiated leucine, a distribution of radioactivity along the ventral roots similar to that found in control animals was obtained (Fig. 1).

We could not detect a flow rate of less than 0.1 mm/day or more than 100 mm/day. Systemic administration of the tritiated amino acid increased the background and decreased the sensitivity of the method, because Schwann cells, fibroblasts, and, to some extent, axons themselves, can incorporate leucine into protein (*3*). In 5-μm longitudinal sections of roots, radioactivity in Schwann cells and fibroblasts could not be distinguished from that within the axons. In normal and neuropathic cats killed 4 hours after receiving the isotope, uptake of radioactivity was uniform along the entire length of ventral and dorsal roots. Thus, no gradient in uptake of leucine by cells along the roots seemed to occur. Differences in rate of absorption from the peritoneal cavity probably did broaden the peaks observed and contributed to a variation in the absolute size of the peaks.

Although glutaraldehyde fixation binds free amino acids to macromolecules (*4*), this should not have introduced a significant error in cats killed a day or more after they had received radioactive leucine; by 24 hours essentially all leucine would have been metabolized (*5*). To verify this point, we perfused two controls with formaldehyde, which does not bind amino acids to tissue elements (*4*). No significant differences in flow rate or in ratio of peak height to average root activity were noted.

We have confirmed the existence of a protein fraction moving along axons from motor and sensory neurons at about 1½ mm/day. Evidence of such transport was absent in most of the cats made neuropathic by acrylamide, but was present in cats given TOCP. The axonal degeneration seen in acrylamide-treated cats could result from this alteration in axoplasmic flow, but another mechanism would have to be postulated for the similar lesions induced by TOCP.

The disappearance of a migrating peak along ventral and dorsal roots in acrylamide neuropathy may be explained in either of two ways. (i) Formation of the proteins destined for transport may be blocked. Administration of puromycin, an inhibitor of protein synthesis, before administration of the radioactive amino acid causes a diminution in the amount of labeled protein appearing in nerve roots (*5*). (ii) Alternatively, owing to an abnormality in the transport mechanism itself, labeled proteins that are formed may not be transported normally within axons. The accumulation of radioactivity in segments of roots close to the cell bodies of origin in cats treated with acrylamide suggests that protein synthesis has occurred and that the defect is in the transport process itself.

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Electroconvulsive Shock Effects on a Reactivated Memory Trace: Further Examination

Abstract. *Rats showed amnesia for conditioned fear training if given an electroconvulsive shock immediately after training. Retention was unimpaired, however, when the electroconvulsive shock treatment was given 1 day after training immediately after the presentation of the stimulus used in the fear conditioning training. These results support the view that electroconvulsive shock disrupts memory trace consolidation but does not disrupt a recently reactivated memory trace.*

Electroconvulsive shock (ECS) produces retrograde amnesia (*1*). The evidence from numerous ECS studies indicates that retrograde amnesia is obtained only if the treatment is administered shortly (that is, within a few hours) after training.

Misanin *et al.* (*2*) reported results suggesting that memory is disrupted by ECS given 24 hours after training, pro-

vided that the memory trace is "reactivated" immediately prior to the ECS. These findings suggest that ECS may affect memory not only by disrupting initial consolidation, but also by affecting traces which are in transit from long-term store into active memory. This conclusion, if valid, would alter current concepts of memory storage.

We attempted to replicate the experiment of Misanin *et al.* (*2*). We included several additional control groups (Table 1). Although most of the results of this study confirmed those reported by Misanin *et al.*, the critical effect was not replicated. Electroconvulsive shock given 24 hours after training—but immediately after "trace reactivation"—did not produce amnesia.

The subjects were 100 male Sprague-Dawley rats (220 to 300 g) from commercial sources (*3*). They were housed individually and fed a restricted diet (five pellets of Purina laboratory chow) each day, 1 hour after training.

Fear conditioning and drinking training were given in a light gray rectangular wooden box (30 by 17 by 18 cm) with a grid floor and illuminated by a 15-watt light. A drinkometer circuit automatically recorded licks whenever the subject licked a drinking tube located at one end of the box. The box was placed in a white, sound-attenuated compartment. A 20-cm speaker was suspended approximately 20 cm above the box. The speaker delivered an 80-decibel white-noise conditioned stimulus (CS) (as measured from inside the drinking chamber). During trace reactivation, or control operations for this procedure, the light was turned off, and a black plywood box which covered the walls and grid floor was placed inside the drinking chamber.

Ten days before the beginning of the experiment, wound clips were attached to the rear of the rats' pinnas. The rats were first given 5 days of adaptation in which they were placed, individually, in the black box, and alligator-clip electrodes (through which ECS was subsequently delivered) were attached to the wound clips. After 1 minute, the rat was returned to its home cage. After the last adaptation trial, the animals were deprived of water, and 24 hours later (day 1) each rat was placed in the drinking chamber and allowed to make 110 licks. If the rat did not lick within 5 minutes it was directed to the drinking tube. One hour later, water was available in the home cages for 10 minutes. The procedure on the next day (day 2) was identical, except that there was no prompting. Latency to the first lick and latency to 110 licks were recorded. Water was present in the home cage for the next 24 hours. No gloves were used in handling the rats on days 1 and 2.

The rats were randomly assigned to one of nine groups with 10 to 13 rats in each. However, no more than one of the several subjects with highly deviant lick latencies was placed in any single group (*5*). The following day (day 3) all subjects received their first treatment (Table 1). Groups 1 through 5 were treated in the same manner as groups 1 through 5 in the study by Misanin *et al.* Groups 2 through 6 received the some treatment. Each rat was placed (with the experimenter wearing gloves) in the drinking chamber, and alligator clips were then attached to the wound clips. A metal plate covered the hole over the drinking spout. After 47 seconds, the CS was presented for 10 seconds, and immediately upon its offset a 1.3-ma scrambled shock was delivered through the grids for 3 seconds. Group 1 received the same treatment but was given an ECS (0.5 second, 50 ma) immediately after the foot shock. Group 7 received the CS (but no foot shock) followed by an ECS 3 seconds later, group 8 received only the CS, and group 9 received only the foot shock 57 seconds after the animals had been placed in the apparatus. All rats were then immediately returned to their home cages. No water was given on day 3.

On the next day (day 4), all rats were placed in the black box (the ex-

Table 1. Latency (seconds) and lick rate (licks per minute) before and during CS on test day (day 5). All groups received their first treatment on day 3. Group 1 received CS, foot shock, and ECS. Groups 2 through 6 received CS and foot shock. Group 7 received CS and ECS, group 8 received only CS, and group 9 received only foot shock. On day 4, all groups were placed in the black box; groups 2 and 6 received both CS and ECS, group 3 received only CS, group 4 received only ECS, and groups 1, 5, 7, 8, and 9 received neither. Groups 2 and 6 were "reactivation" groups tested on days 5 and 6 respectively.

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Time taken by each subject for the first 100 licks (second)								
67	128	279	227	221	330	18	25	126
77	194	292	287	241	413	50	30	247
121	198	294	382	247	464	80	40	276
151	347	317	482	520	499	80	49	435
194	480	319	501	573	543	85	53	440
202	567	409	512	581	767	97	54	675
257	699	487	684	872	780	112	75	728
266	740	587	1263	960	1260	130	146	857
286	1012	592	3417	1174	3600*	249	151	926
347	1107	840	3600*	1604		378	153	1089
431		1020						
		2274						
		3600*						
Median latency								
202	524	487	506	577	543	91	54	558
Median lick rate								
0.49	0.19	0.21	0.20	0.17	0.18	1.10	1.85	0.18
Median terminal lick rate								
4.76	3.64	2.56	4.54	4.0	5.88	2.60	2.63	5.88
Time taken by each group for the next ten licks (second)								
21	462	275	384	327	600*	348	3	4
62	37	215	45	575	600	2	3	276
220	398	600*	6	310	312	2	21	195
65	240	180	323	435	600*	24	15	3
4	427	25	128	250	382	125	2	19
8	600*	302	600*	235	480	156	10	13
4	326	27	600*	600*	600*	23	374	357
176	600*	595	190	127	600*	80	24	94
6	600*	145	600*	600*	600*	212	2	24
2	600*	9		600*		13	59	70
309		600*						
		600*						
Median latency								
21	444	245	323	381	600	52	12	47
Median lick rate								
0.48	0.02	0.04	0.03	0.03	0.0	0.19	.77	0.21

* Indicates the cutoff time. † Indicates additional control groups. These groups were not included in the study by Misanin *et al.* (*2*).

perimenter did not wear gloves), alligator clips were attached, and the rats were retained in the box for 30 seconds. The CS was then presented for 2 seconds to some groups in order to reactivate the fear associated with it on the previous day. Groups 2 and 6 received CS followed immediately by ECS, group 3 received only the CS, and group 4 received only the ECS. The remaining groups, 1, 5, 7, 8, and 9, received neither the CS nor the ECS. The rats were returned to their home cages, and 1 hour later water was available for 10 minutes. Precautions (*2*) were taken to ensure that some aspects of the procedures on day 4 were distinct from those on day 3 (that is, handling without gloves rather than with gloves, water absent rather than present during the previous 24 hours, and distinctive differences in the compartments).

On day 5, eight of the groups were returned to the drinking chamber and allowed to make 100 licks. Group 6, whose previous treatment was identical to that of group 2, was tested on day 6 (48 hours after reactivation) so that its subsequent comparison with group 1 (given ECS immediately after training) would not be confounded by differing intervals of time between ECS and retesting (*2*). After the 100th lick, the CS came on and remained on for 10 minutes, or until the rats made an additional ten licks. Latencies to the first lick, the first 100 licks, and the subsequent ten licks were automatically recorded as on day 2. The latency to 100 licks was used as an index of fear elicited by the chamber, and the latency to ten licks during the CS was used as an index of fear elicited by the CS (Table 1). During the retest, the rats typically did not lick for a period of time and then made 100 licks at a fairly constant rate. For groups 1 through 7 there was no correlation between terminal lick rate (that is, the rate following the initial lick) and the rate during the CS. Thus, in these groups, rates during the CS were not affected by rate before CS onset. However, in groups 8 and 9 there were high correlations ($r = +.82$ and $+.66$, respectively) between terminal rates in the situational phase and rates during the CS.

Inspection of latency scores for the 100 licks before CS onset reveals a time-dependent effect of ECS on the fear conditioning. Rats given ECS immediately after fear conditioning (group 1) drank significantly faster than those given ECS after 24 hours (group 4, $P < .01$) or those given no ECS (group 5, $P < .001$). However, the amnesic effect of immediate ECS appears to be incomplete: Rats in group 1 licked more slowly than rats in groups 7 and 8, which received the CS but did not receive the foot shock (group 1 versus groups 7 and 8 combined, $P < .001$). Moreover, the treatment on day 4 did not affect the subsequent drinking of rats given the foot shock on day 3 since there were no differences between groups 2 through 6 and 9. And all these groups exhibited more fear than group 1 (1 versus 2, $P < .05$; 1 versus 3, $P < .001$; 1 versus 4, $P < .001$; 1 versus 5, $P < .01$; 1 versus 6, $P < .001$; and 1 versus 9, $P < .01$).

Fear elicited by the apparatus appears to be primarily dependent upon the foot shock, since there were no differences in fear exhibited by groups given CS-foot shock pairing on day 3 (2 through 6) and the group given foot shock alone (*9*).

Analyses of the latency scores after CS onset revealed that immediate ECS significantly decreased the fear associated with the CS. Group 1, which received immediate ECS, drank significantly faster than groups 2 through 6, which were exposed to the same conditioning stimuli on day 3 (1 versus 2, $P < .001$; 1 versus 3, $P < .05$; 1 versus 4, $P < .05$; 1 versus 5, $P < .001$; and 1 versus 6, $P < .001$). The ECS effect appears to be complete since the performance of group 1 was indistinguishable from that of groups which received no fear conditioning at all (groups 7 and 8). This result is not surprising since the onset of the CS was much closer in time to the ECS than the presentation of the apparatus cues on day 3. This finding thus provides further evidence of the temporal effectiveness of ECS; that is, the closer the ECS follows the experience, the greater the disruptive effect (*1*).

It appears that the depressing effect of the CS on licking on the test day is due to its pairing on day 3 with foot shock. Inspection of the data for ten licks shows that in group 5 (CS + foot shock) drinking was significantly slower than in group 8 (CS alone) ($P < .001$). In group 9, which was given foot shock alone on day 3, drinking was significantly faster during the CS than in groups to which CS was given prior to foot shock (2 through 6, $P < .01$). Moreover, the drinking rate of group 9 was comparable to those of groups 7 and 8, which did not receive a foot shock. Thus, the depressed drinking rates following CS onset in groups 2 through 6 are due to the prior association of the CS and foot shock on day 3. The high correlations in groups 8 and 9 between terminal lick rate and lick rate during the CS suggest that licking is controlled by the same factors in the two cases; that is, CS onset has little effect on lick rate in these groups. Group 7, however, does not fit this pattern, and the reason for this is not clear. The high lick rates of groups 7 and 8 indicate that the alligator-clip application, the handling, and the ECS treatments were not punishing.

The most significant finding of this study is that the administration of an ECS immediately after the reactivation procedure on day 4 was totally ineffective in reducing subsequent fear of the CS. In fact, the reactivated groups (2 and 6) tend to exhibit more fear to the CS. Thus, we did not replicate the critical findings of Misanin *et al.* (*2*).

Our findings indicate that the CS elicited fear after it was paired with a foot shock. However, ECS was not effective in disrupting such reactivated fear, even though ECS did produce amnesia when administered immediately after the original training. Our findings provide no support for the hypothesis that ECS affects recently reactivated memory traces. We have been unable to discover any procedural details that could explain our failure to replicate the findings of Misanin *et al.* (*2*). A more detailed analysis of their data (that is, terminal lick rates) may provide some clarification.

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4. Misanin *et al.* (*2*) did not specify the light intensity. Their fear-conditioning chamber was a Plexiglas box lit by a 10-watt bulb.
5. Unequal numbers in the groups resulted from several factors. A larger number of rats was placed in ECS groups in anticipation of losses; two rats were discarded because they did not display full tonic seizures; five rats died after the seizure.
6. Statistical analyses followed the Mann-Whitney U test (all probabilities are two-tailed). Correlations were determined by the Pearson correlational analysis.
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