mized females during treatment with estradiol showed that amounts of volatile components (peaks 1 through 6) were at least eight times greater (Fig. 1B). To determine whether the increased production of these constituents in animals treated with estrogen was responsible for the change in the secretions' behavioral properties, we trapped peaks 1 through 5 (80° to 130°C) into ice-cold ether; the material for the trapping procedure was obtained from a pool of 48 vaginal washings collected from three donor females. The trapped fraction was then applied daily to the sexual skin of four recipient females, each paired with a different male; an immediate, marked stimulation of the sexual activity of the males resulted in each case (Fig. 2). The four males made a total of ten mounting attempts during 22 tests before treatment compared with 213 mounting attempts during 26 tests with treatment (t-test, $P < .001$). There were no ejaculations during tests before treatment, whereas 14 occurred during treatment (χ^2 test, $P < .001$). In three pairs, stopping treatment resulted in an immediate return to baseline, but this did not occur in the remaining pair until the fifth test after treatment ended; similar carry-over effects have been reported previously (*6*). It should be emphasized that the recipient females were unreceptive and frequently refused the mounting attempts of males for which intromission was sometimes very difficult because of the atrophic state of the females' genital tracts. In one case (Fig. 2), where the female's persistent refusals generally prevented intromission and ejaculation, the male masturbated to ejaculation during several tests with treatment; here, the stimulation and expression of male sexual excitement in the presence of a totally unreceptive female was noteworthy.

These results establish that highly active copulins which sexually stimulate these male primates are trapped with peaks 1 through 5. Using gas chromatography and coinjections with authentic substances, together with mass spectrometry, we have made preliminary identifications of peaks 1 through 6 as follows: 1, acetic; 2, propionic; 3, isobutyric; 4, butyric; 5, isovaleric; and 6, isocaproic acids. The amounts of these acids in vaginal samples can therefore be calculated from the chromatograms (*10*). A synthetic mixture of authentic acids was then prepared to correspond in concentration and composition to the acids present in estrogen-stimulated vaginal secretions. When tested, this mixture possessed behavioral activity that was identical to that of the material trapped with peaks 1 to 5, a finding which excludes the possibility that a trace amount of an unknown substance was responsible for the activity of the fractions (*11*). The identification of these sexual pheromones, the first in any primate species, as simple aliphatic acids provides both a useful tool in behavioral research and also a specific olfactory input for neurophysiological studies on rhinencephalic-hypothalamic integration. The need for comparative studies in related primate species including the human is now obvious.

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References and Notes

1. C. H. Conaway and M. W. Sorenson, *Symp. Zool. Soc. London* **15**, 471 (1966); G. A. Doyle, A. Pelletier, T. Bekker, *Folia Primatol.* **7**, 169 (1967); B. Harrison, *Malayan Natur. J.* **17**, 218 (1963); A. Jolly, *Lemur Behavior* (Univ. of Chicago Press, Chicago, 1966); C. S. Evans and R. W. Goy, *J. Zool. Proc. Zool. Soc. London* **156**, 181 (1968).
2. D. R. Ilse, *Brit. J. Anim. Behav.* **3**, 118 (1955); A. Jolly, in *Social Communication among Primates*, S. A. Altmann, Ed. (Univ. of Chicago Press, Chicago, 1967), p. 2; J. J. Petter, in *Primate Behavior*, I. DeVore, Ed. (Holt, Rinehart & Winston, New York, 1965), p. 292; *Linnean Soc. Biol. J.* **1**, 77 (1969).
3. C. R. Carpenter, *J. Comp. Psychol.* **33**, 113 (1942); K. R. L. Hall, R. C. Boelkins, M. J. Goswell, *Folia Primatol.* **3**, 22 (1965); P. Jay, in *Behavior of Nonhuman Primates*, A. M. Schrier, H. F. Harlow, F. Stollnitz, Eds. (Academic Press, New York, 1965), p. 525; J. van Lawick-Goodall, *Anim. Behav. Monogr.* **1**, 161 (1968); H. Rahaman and M. D. Parthasarathy, *Primates* **10**, 149 (1969).
4. R. P. Michael and G. S. Saayman, *J. Endocrinol.* **41**, 231 (1968).
5. R. P. Michael and E. B. Keverne, *Nature* **218**, 746 (1968); R. P. Michael, in *Recent Advances in Primatology*, C. R. Carpenter, Ed. (Karger, Basel, 1969), vol. 1, p. 101.
6. R. P. Michael and E. B. Keverne, *Nature* **223**, 84 (1970).
7. R. P. Michael, J. Herbert, J. Welegalla, *J. Endocrinol.* **39**, 81 (1967); R. P. Michael and J. Welegalla, *ibid.* **41**, 407 (1968).
8. Either 2 ml of ether or 2 ml of ether containing 500 μg of cholesterol was used in applications to the sexual skin for control purposes during the period before treatment.
9. E. B. Keverne and R. P. Michael, *J. Endocrinol.*, in press.
10. *n*-Pentanol (3.8 mg/liter) was added to the extracting ether. The size of the P peaks depends upon the concentration of extracts occurring during preparatory procedures; this enables all peak areas to be related to amounts present in original extracts.
11. R. F. Curtis, J. Ballantine, E. B. Keverne, R. W. Bonsall, R. P. Michael, *Nature*, in press.
12. We thank Drs. R. F. Curtis and J. Ballantine for preliminary identifications by mass spectrometry. This work was assisted by grants from the Foundations' Fund for Research in Psychiatry and the Population Council.

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One-Trial Learning and Biphasic Time Course of Performance in the Goldfish

Abstract. *Goldfish* (N = *408) spontaneously swam against flowing water into a calm-water well. After a single trial punished by brief electric shock, the fish avoided the well, as indexed by increased latencies of reentry. Avoidance declined during the first minute after shock, then rose to a peak 1 hour later. The biphasic time course is compatible with the two-store theory of memory formation.*

Many phenomena of learning and retention have been demonstrated in fish (*1*). Goldfish have been useful in memory research that correlates deficits in the retention of active shuttlebox avoidance with inhibition of brain protein synthesis (*2*). The many trials required for such learning, however, mask the onset of consolidation of a memory trace and hamper precise determination of the consolidation gradient.

A useful one-trial learning method for fish is not generally available (*3*). We describe here a one-trial technique that uses electric shock to suppress a strong spontaneous behavior of the fish. Goldfish swim against a flow of water into a region of calm water. A single brief shock causes them to retreat into the water flow and to delay reentry into the calm-water well.

We tested 526 goldfish (Lambourne Industries Fish Hatchery, San Fernando, Calif.), measuring 3 to 6 cm from snout to caudal peduncle, during October to December 1970. Groups of 48 fish were kept for 2-weeks' acclimation in 40-liter tanks filled with aged tap water at 24° ± 1°C. Training and testing were done in a plastic foam trough, 38 cm long and 7.5 cm wide. Its floor was molded in a spinal curvature that ended in an overflow; the opposite end was formed into a calm-water well, 8 cm long and 3 cm below the adjacent curved floor. The outlet tube of a water-recirculating system

Table 1. Aftershock avoidance of upstream swimming of fish into calm-water well.

Interval between shock and retention test (min)	Control groups			Noncontingent shock groups			Experimental groups		
	(*N*)	Median latency (sec)	Median duration (sec)	(*N*)	Median latency (sec)	Median duration (sec)	(*N*)	Median latency (sec)	Median duration (sec)
Preshock	66	3.0	22.0	54	2.0	22.0	360	3.0	22.0
0.33	20	4.0	20.5	11	3.0	18.0	52	16.5*	4.0
1	10	2.0	19.0	11	2.0	26.0	68	6.5	11.0
2	12	3.0	18.5	11	2.0	24.0	30	15.0*	2.0
4	12	3.0	18.0	11	2.0	21.5	80	20.0*	4.0
10	12	4.0	20.0	10	2.0	16.0	54	19.0*	4.0
30							24	21.5*	3.0
60							52	20.0*	3.5
1-hour retest	14	3.5	20.5	10	1.0	23.0			
4-hour retest	20	4.0	23.0	22	7.0	14.0	24	15.5*	5.0
1-day retest	20	2.0	20.0	22	2.0	15.0	48	9.0	3.0
3-day retest	12	4.0	18.0				45	9.0	2.5

* Values significantly different from preshock and control latencies (P, .006, Mann-Whitney U test).

was fastened at the well edge above the water surface, so that water flowed down the curved floor at 5 liter/min to the overflow. During flow, the water depth was 4 cm in the flow chamber, 2.5 cm at the well edge, and 5.5 cm in the well. Two wire mesh electrodes from a Grass S4 stimulator at the front and end walls of the well could deliver electric shock in monophasic pulses of 9 to 12 volts, at an in-water gradient of 1.0 to 1.2 volt/cm.

Fish were tested individually in a trial before and a trial after the shock; the latencies of entry and the duration of stay in the calm-water well during flow were recorded to the nearest 0.5 second. In the 30-second preshock trial, each fish was required to meet the criterion of entering the well within the first 10 seconds and to remain there for a combined total of at least 18 seconds; 480 of all fish tested (91 percent) met this criterion. For the learning trial 30 seconds later, the water flow, the shock, and a light over the flow chamber were turned on simultaneously for a 5-second period. Each fish spent the interval after the shock in a beaker filled with aged tap water; fish of the 0.33-minute test group were held in a net in the beaker for 3 to 5 seconds during the period immediately after the shock. Fish were returned to the flow chamber at least 10 seconds before testing. A brief flow of water oriented the fish toward the well, before the postshock test reintroduced flow and light for 30 seconds. Groups of 10 to 15 fish, and replications, were run independently at each of the first-hour intervals after the shock (Table 1); some groups were retested 4 hours, 1 day, or 3 days later. All fish survived the 9- to 12-volt shock; 2 of 20 fish died 1 day after a noncontingent 90-volt shock.

Fish avoided the well when tested shortly after the one-trial shock experience. Characteristically, they either swam against the flow holding their position or repeatedly swam toward the well only to turn back abruptly over its edge. Of all fish tested 0.33 minute after shock, 44 percent made skirmishes in the well which lasted longer than 2 seconds, and only 31 percent stayed in the well longer than 10 seconds, although 2 minutes earlier, in tests before the shock, all fish had done so. Little increase in test latencies was observed in fish of control groups that were not shocked. Fish that were shocked noncontingently outside of the trough, in a dissimilar plastic container but under the same shock conditions as those in the well, did not avoid the well in tests at any of the intervals after the shock. The latency data of these control groups did not differ statistically from each other nor from the preshock data of shocked fish (Table 1).

After shock, median latencies of entry into the well were a nonmonotonic function of the interval after the shock. At 0.33 minute after shock or after 4 minutes or more, the fish avoided the well; when tested after a 1-minute interval, 72 percent of the fish swam into the well within 10 seconds, as if amnesic to the preceding shock encounter. The 1-minute-group latencies were only slightly longer than preshock latencies, but they differed significantly from latencies at 0.33 minute or at 2 to 60 minutes after shock (P, .006, Mann-Whitney U test). The percentage of fish with latencies of 8 seconds or more after the shock was significantly reduced in the 1-minute group, as compared with the 0.33-, 2-, 4-, 10-, and 60-minute groups ($P < .01$, chi-square test). In retests 1 or 3 days later, fish entered the well more rapidly but remained there for not more than 3 seconds (median).

A similar early time course has been observed in mice with one-trial avoidance tasks (*4*, *5*). Irwin (*4*) presented a biphasic time-latency curve for hurdle-crossing of mice which seems congruent with our latency data for goldfish. It was characterized by a high initial latency with rapid decay to a low at 5 minutes, followed by a slow rise in latencies to a peak at 90 minutes and subsequent decline to baseline at 24 hours. Cherkin (*6*) has reported a similar biphasic retention curve in the chick.

Divergent interpretations of this apparent retention deficit have been proposed. One has supported a retrieval-failure interpretation (*7*), another the incubation of conditioned fear, that is, the systematic increase in fear over time after shock (*8*). Since many motivational components feed into the expression of an avoidance response (*5*), it appears difficult to dissociate the cues inducing "fear" from those triggering retrieval of the learned behavior. We observed in our studies that goldfish swam into the shock region during the 10-second period before the test after shock but avoided the well shortly after the water flow was turned on for the test. This incidental observation makes it unlikely that "fear" accounts for the avoidance behavior at 0.33 or 4 minutes but not for the brief, temporary amnesia at 1 minute after shock. The observed fall of the latency curve could also be in-

dicative of the decay of an early memory phase and the subsequent consolidation of a memory trace (*6*). Conceiving the fish's latency as an index of memory retention, the one-trial method can be a concise technique to determine how memory itself varies over time.

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References and Notes

1. M. E. Bitterman, *Amer. Psychol.* **20**, 396 (1965).
2. B. W. Agranoff and P. D. Klinger, *Science* **145**, 952 (1964); J. J. Brink, R. E. Davis, B. W. Agranoff, *J. Neurochem.* **13**, 889 (1966); A. Potts and M. E. Bitterman, *Science* **158**, 1694 (1967).
3. A film demonstration by I. Beritashvili, Georgian Academy of Sciences, Tbilisi, U.S.S.R., shows one-trial learning in goldfish. The translation of the published report [I. Beritashvili, *Vertebrate Memory* (Plenum, New York, 1971)] is not yet available.
4. S. Irwin, A. Banuazizi, S. Kalsner, A. Curtis, *Psychopharmacologia* **12**, 286 (1968).
5. F. Robustelli, A. Geller, M. E. Jarvik, *Psychon. Sci.* **20**, 129 (1970).
6. A. Cherkin, *Commun. Behav. Biol.* **5**, 379 (1971).
7. B. S. Klein and N. E. Spear, *J. Comp. Physiol, Psychol.* **72**, 498 (1970).
8. J. P. J. Pinel and R. M. Cooper, *Psychon. Sci.* **6**, 125 (1966).
9. We thank A. Von Thun for suggesting the basic water-flow technique and R. O. Meinecke for exploratory experimentation.

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Electroencephalographic and Behavioral Alterations Produced by Δ^1-Tetrahydrocannabinol

Abstract. *The administration of small doses of Δ^1-tetrahydrocannabinol to cats with indwelling electrodes produced a disruption of both the electroencephalogram and behavior. Some of these alterations, including the appearance of a high-voltage slow wave electroencephalogram in the awake and moving animal, have been observed in cats that had been administered other drugs known to cause hallucinogenic states in man.*

Several recent reports (*1–4*) have dealt with the disruptive influences exerted by active constituents of *Cannabis* on various behaviors in different species. In one of these studies, Lipparini *et al.* (*4*) showed that the disrupted discriminative behavior observed in cats after the administration of Δ^1-tetrahydrocannabinol was accompanied by an increased electroencephalographic (EEG) synchronization.

Scheckel *et al.* (*3*) pointed out that many earlier investigations with *Cannabis* showed inconsistencies due primarily to problems encountered in determining the actual amount of active ingredients in the crude extracts. In recent years, however, advances in cannabinoid chemistry (*5*) have led to the isolation of the major active component of marijuana and to the elucidation of its structure. In the present experiments, this constituent, namely Δ^1-tetrahydrocannabinol (THC), was found to alter behavior and induce a concommitant EEG disruption in the freely moving cat.

Under halothane anesthesia, stainless steel monopolar electrodes were stereotaxically placed in regions of the ventromedial hypothalamus, midbrain reticular formation, basolateral amygdala, and ventral hippocampus. Ball electrodes were placed over the dura above the coronal, suprasylvian, and lateral gyri, which are usually considered to correspond to the frontal, association, and occipital areas of the cerebral cortex, respectively. A stainless steel screw over the frontal sinus served as a reference. All electrode placements conformed to the system of coordinates developed by Snider and Niemer (*6*).

At least 1 week was allowed for recovery from the surgical procedure, following which each animal was placed in a recording cabinet (Lehigh Valley Electronics) for a 1-hour period on each of six consecutive days. After habituation to the cabinet, each cat was subjected to three daily 60-minute control sessions during which electrical activity was recorded for 100-second intervals from all placements at 5, 10, 15, 30, 45, and 60 minutes, and displayed on a model VI Grass electroencephalograph.

Each experimental session consisted of a 90-minute period, the first 30 minutes serving as an additional control. During this predrug period, electrical activity was sampled at 10, 15, 20, and 30 minutes. Then THC dissolved in 3 ml of polyethyleneglycol was administered intraperitoneally, and electrical activity was sampled at 5, 10, 30, 45, and 60 minutes after drug administration. Each of six cats received three dosages of THC, namely, 0.5, 1.0, and 4.0 mg/kg. The order of drug administration was counterbalanced, and from 4 to 7 days elapsed between doses. In an additional experimental session, each animal received 3 ml of the diluent alone. During all sessions, protocols were kept of each animal's behavior.

After having been administered the drug, all animals showed a mydriasis which persisted for as long as 2 hours after an experimental session. While we are unable to state precisely the moment of onset of this response, it was present 5 minutes after THC (at all dose levels), and the pupils of all animals were fully dilated at 30 minutes. The corneal reflex was normal.

All cats displayed variable amounts of locomotor activity during control and predrug sessions, and all dosage levels of THC had a quietening effect on all animals with two exceptions: one cat spent the latter 30 minutes of a session after the 4-mg dose walking around the cabinet with occasional leaps in the air, and another animal spent most of the experimental session after the 1-mg dose walking around the enclosure. In all other sessions, the animals assumed either a standing, sitting, or prone position which they maintained throughout the experimental period. Although the tranquilizing effect of the drug made the study of motor deficits difficult, we did observe alterations in postural attitudes which included bobbing and weaving of the head, swaying from side to side, and an ataxic, broad-based gait. If an animal was placed in a standing position, it tended to retain a stance, and displayed extremely poor coordination in altering this attitude.

With all dosages of THC, the animals stared into space, and often appeared to be following stimuli with their eyes even though no moving stimulus was discernible by the experimenter. Scheckel *et al.* (*3*) assumed that similar behavior in the monkey was indicative of visual hallucinations; however, there is no evidence to support this contention.

All drug dosages produced an increase in synchrony from all electrode placements in the quiet but awake animal (only one animal slept during the latter part of an experimental session).