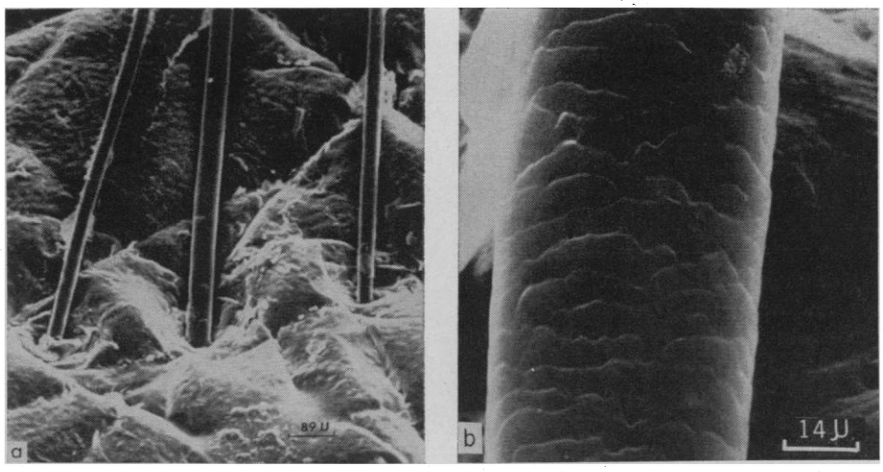

Fig. 3. (a) Polyethylene replica of the surface of human forearm skin (× 225), showing three hairs emerging from the skin. (b) The middle hair of (a) shown at higher magnification (× 1400).

rectangular brass frame (17 by 38 mm). A layer of polyethylene pellets (Union Carbide DYLT) was placed on the surface of the negative Silastic mold which was then put in a vacuum oven in which the temperature was slowly raised to 180°C. The polyethylene melted and flowed evenly over the surface of the Silastic and at the same time the oven was evacuated to about 10 torr until the air bubbles on the surface of the polyethylene had collapsed (about 5 minutes). The vacuum was then opened to air. Intermittent evacuation was repeated until essentially all entrapped air was removed from the polyethylene-Silastic interface.

The entire mold was removed from the oven and cooled to room temperature. The polyethylene was separated from the Silastic, and this first positive cast containing nonreplicated hair and loose debris removed from the surface of the Silastic was discarded. A second cast for viewing in the SEM was made in an identical manner.

In preparing the cast for SEM micrography, 9-mm punches were taken from the center and were mounted on specimen blocks with silver-containing cement (electronic grade). These punches were shadowed at 2 to 3 × 10^{-5} torr with 7 to 8 cm of 8-mil Au-Pd wire wrapped around a tungsten filament at a distance of 12.5 cm from the specimens. (Average thickness of Au-Pd on the surface of the replicas was approximately 150 Å.) The block was inclined at an angle of 45° toward the electron beam of a Jeolco JSM-2 scanning electron microscope which was usually operated with a beam accelerating voltage of 25 kv.

The faithfulness of replications made by this method is illustrated by a comparison of a replica of guinea pig skin with a glutaraldehyde-fixed and frozen-dried specimen taken from adjacent sites of the body (Fig. 1). The shape and appearance of the epidermal cells in the replicated and fixed specimens are similar. Minute detail and dimensions are also preserved in the replica of human forearm skin, as illustrated by the loosened polygonal cells (Fig. 2b) which average about 30 to 40 µ in width, a measurement in agreement with values reported for fixed specimens (*4*). At this higher magnification, both the underlying, still firmly attached, epidermal cells (appearing as protuberances) and the loosened, overlying squamous cells are visible. Because of the greater depth of field, the squamous cells, which appear flat when viewed through a light microscope, are seen in their true three-dimensional form.

In other replicas of human forearm skin containing hair fibers, not only are the same epidermal patterns faithfully reproduced, but so are the details of the hair shafts (Fig. 3, a and b). The size and spacing of the cuticle scales on these hairs are the same as those observed on actual hairs by other microscopic techniques.

This new replication technique, used in combination with metal shadowing and scanning microscopy, provides a very useful addition to existing methods for the study of the skin surface.

EMIL O. BERNSTEIN
Gillette Research Institute, Rockville, Maryland 20850
CHASE B. JONES
Gillette Toiletries Company, Boston, Massachusetts 02106

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Paradoxical Fear-Increasing Effects of Tranquilizers: Evidence of Repression of Memory in the Rat

Abstract. *Conditioned suppression of feeding, an index of fear, was increased rather than decreased by the administration of benzodiazepine tranquilizers or amobarbital. The drug-induced increase in conditioned fear varied directly with the intensity of the shock used in fear conditioning. The drugs had no fear-increasing effect in unshocked controls or in rats made amnesic by electroconvulsive shock given immediately after fear conditioning. These observations in animals are reminiscent of clinical reports that intraveneous amobarbital facilitates the recall of repressed traumatic experiences. The retrieval of painful memories may be inhibited or repressed in animals as well as in humans. In both cases, tranquilizers may counteract repression by disinhibition of the act of retrieval.*

Minor tranquilizers are used in clinical practice to alleviate symptoms of anxiety and tension (*1*). Laboratory studies in animals generally parallel these indications of anxiety-reducing activity in man (*2*, *3*). In the experiments reported here, however, tranquilizers increased rather than decreased conditioned fear in the rat. These paradoxical fear-increasing effects sug-

gest that, under certain conditions, the fear-reducing action of tranquilizers may be counteracted by another action. On the basis of our data, we propose that tranquilizers can increase fear by facilitating the retrieval of painful memories.

Fear was measured by the conditioned suppression of feeding behavior (*4*). There were three stages in the experimental procedure. In preliminary training, the rats learned to drink a milk solution (1 part of Borden's Eagle Brand sweetened condensed milk to 2 parts of tap water) from a glass drinking tube mounted on a wall of a clear Plexiglas cubicle, 37 cm on each side. Licks on the tube were recorded with a touch-sensitive relay. A clock started when the door of the chamber was closed and stopped after the rat had made 100 licks. Daily training sessions were given until 90 to 95 percent of the rats had made 100 licks within 50 seconds (typically, 7 days). Rats that did not meet the latency criterion were eliminated from the study. In the second stage (fear conditioning), the animals were placed in the chamber as before, but the drinking tube was removed. After 15 seconds, a 1-ma shock was delivered for 1 second through the grid floor. The animals were immediately returned to their home cages. Unshocked control groups were given a 15-second placement in the chamber instead of a fear-conditioning trial. Seven days after fear conditioning, the rats were tested for conditioned suppression of the drinking response (retention test), by determining, exactly as in preliminary training, the time to make 100 licks. Animals that failed to complete 100 licks in the 900-second retention test were assigned a score of 900 seconds.

Drugs (or saline) were injected intraperitoneally 30 minutes before the retention test. The drugs and doses (mg/kg) were: sodium amobarbital, 30; Wy 4036, a new benzodiazepine tranquilizer, 0.5 and 2 (*5*); chlordiazepoxide hydrochloride, 15 and 25; diazepam, 4; oxazepam, 20; chlorpromazine hydrochloride, 3; and scopolamine hydrochloride, 1. The doses of the tranquilizers caused marked passive avoidance deficits in the test described by Geller and Seifter (*6*).

Drinking latencies in the test chamber usually decreased from about 400 seconds on the first training trial to about 40 seconds on the seventh. After a single shock, the mean drinking latency of undrugged rats in the retention test increased about 200 seconds (Table 1). The increase was not due to forgetting, extinction, or some kind of warm-up effect. Unshocked animals, which also were susceptible to these factors, drank as rapidly on the test day as they had on the last day of training, despite the 7-day interruption of daily drinking trials during the retention interval. Since the two groups differ only with respect to exposure to shock, we conclude that the increase in drinking latency in the shocked group is due to conditioned suppression or fear.

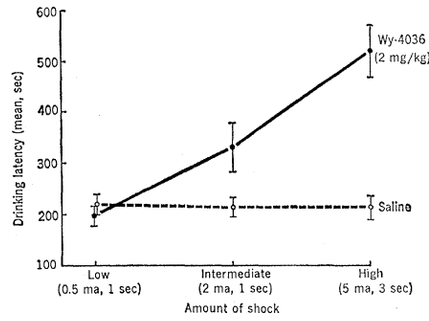

Fig. 1. Differential action of Wy 4036 on the drinking latencies of rats conditioned with shocks of different intensities and tested 1 week after conditioning. In the high-shock group, the difference between mean drinking latencies of rats given drug or saline is significant at $P < .05$. There were 10 rats in each of the low-shock subgroups and 12 in each of the intermediate and high-shock subgroups. The bars indicate standard errors.

If tranquilizers reduce fear, then administration of the drugs shortly before the retention test should decrease the drinking latencies of shocked animals. Surprisingly, however, drinking latencies were increased rather than decreased by Wy 4036 and amobarbital (Table 1). Drug specificity is suggested because neither chlordiazepoxide nor chlorpromazine increased the latency of drinking. The increase cannot easily be attributed to direct suppression of drinking as a result of sedation or motor incapacitation, because neither Wy 4036 nor amobarbital significantly increased the drinking latencies of unshocked animals. Furthermore, even a sedative dose (25 mg/kg) of chlordiazepoxide did not significantly augment the drinking latencies of shocked animals. Finally, we found that Wy 4036 and a closely related tranquilizer, oxazepam, increased drinking latencies after repeated-dose as well as after single-dose administration.

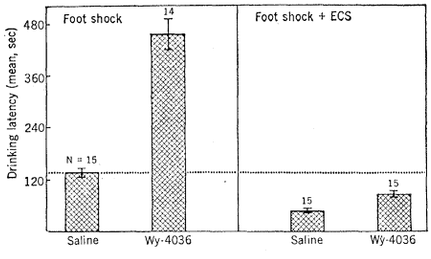

Fig. 2. Administration of electroconvulsive shock (ECS) immediately after foot shock on the day of fear conditioning abolishes the increase in conditioned suppression induced by Wy 4036. The bars indicate standard errors.

In the repeated-dose experiments, either a tranquilizer or scopolamine was injected every day to different groups of rats 30 minutes before each training and test session (including the day of fear conditioning and on each day of the retention interval). Studies in both rat and man show that the depressant effects of benzodiazepine tranquilizers are rapidly tolerated, although their anxiety-reducing effects persist and even increase (*7*). Similarly, in our tests, the first few doses of all drugs interfered with drinking (Table 2). However, these suppressant effects disappeared rapidly as dosing continued, and, in the last two trials of training, the drugs even facilitated drinking. Nevertheless, after fear conditioning, the tranquilizers increased the latency of drinking. Drug specificity again is suggested because scopolamine had no such effect and even tended to reduce conditioned suppression in the retention test.

Taken together, the results of the single-dose and repeated-dose experiments make it unlikely that tranquilizers increase the drinking latencies of fear-conditioned rats by some depressant side effect. The results suggest, rather, that tranquilizers somehow intensify fear in these tests. Direct observation of the animals supports this idea. The drugged animals assumed an exaggerated crouching and freezing posture, defecated, and urinated.

These paradoxical fear-increasing effects of tranquilizers may be related to their effects on memory retrieval in humans. In the studies of Grinker and Spiegel (*8*), amnesic patients suffering from war neuroses could recall, after intraveneous injection of amobarbital, the traumatic events they had experienced during combat. Grinker and Spiegel assumed that the amnesia was caused by an active inhibitory process (repression) and that amobarbital permitted recall of traumatic memories by disinhibition. Although this interpretation is controversial (*9*), tranquilizers, by

analogy, may increase conditioned suppression in our rats by facilitating the retrieval of the shock memory. According to this idea, the retrieval of painful memories may be inhibited or repressed in animals as well as in man (*10*). Hence our undrugged rats may have failed to exhibit maximum suppression because the memory of the painful shock was partially repressed. Under the disinhibitory influence of tranquilizers (*3*), recall of the shock experience may be enhanced, and conditioned suppression may thereby be increased. These experiments thus appear to provide a model of repression of memory.

As one test of these ideas, we compared the effects of tranquilizers on groups of rats conditioned with shocks of different intensities. Because it is assumed that the strength of repression in man depends on the intensity of the original traumatic experience, we assumed that the amount of repression in our rats would depend on the intensity of the conditioning shock. Thus, repression should be strong in rats conditioned with a high intensity of shock and weak in rats conditioned with a low intensity. These differences in repression should tend to counteract the differences in conditioned fear associated with the different intensities of shock. Hence, we predicted that the function between conditioned suppression and shock intensity would have a relatively flat slope in rats treated with saline. By counteracting repression, tranquilizers might reveal the differences in conditioned fear associated with the different levels of shock. Therefore, we predicted that the intensity function for drugged animals would have a relatively steep slope.

Figure 1 shows the differential effects of Wy 4036 on the conditioned suppression of groups of rats that received either low, intermediate, or high intensities of shock on the day of fear conditioning. As predicted, the tranquilizer increased the mean drinking latency of the strongly shocked group more than it increased those of groups that had received shock of intermediate or weak intensities. In fact, animals conditioned with weak shock tended to drink more rapidly under Wy 4036 (*11*).

Mean drinking latencies of the subgroups tested with saline did not increase with increases in the intensity of the conditioning shock (although unconditioned reactions to the different shocks were obviously different). This observation of a flat intensity function is unusual but compatible with the idea that the unpleasantness of an experience determines the extent to which the memory will later be repressed.

Table 1. Effects of tranquilizers on the drinking latencies of shocked and unshocked rats in a retention test 1 week after fear conditioning. Drugs or saline were injected intraperitoneally 30 minutes before the test. The mean drinking latency of all rats on the last training trial prior to fear conditioning was 42.6 ± 1.9 seconds. Number of rats is shown in parentheses.

Drug	Dose (mg/kg)	Drinking latency (mean in seconds) ± S.E.	
		Shocked	Unshocked
Saline		233.3 ± 15.1 (87)	45.6 ± 6.5 (38)
Amobarbital	30	522.3 ± 97.6 (9)*	38.1 ± 5.2 (8)
Wy 4036	0.5	409.5 ± 64.2 (14)*	35.2 ± 4.8 (14)
	2	425.2 ± 40.5 (48)*	64.8 ± 11.1 (17)
Chlordiazepoxide	15	216.1 ± 46.7 (9)	40.5 ± 3.7 (9)
	25	311.2 ± 95.1 (9)	78.8 ± 27.7 (5)
Chlorpromazine	3	266.9 ± 53.5 (12)	35.6 ± 7.4 (10)

* Significantly different from saline at $P < .05$.

Table 2. Effects of chronic administration of tranquilizers or scopolamine on the conditioned suppression of drinking. Different groups of rats were injected each day with a drug or saline. After 7 days of milk-drinking training, the rats were shocked on day 8 and then tested for conditioned suppression on day 11.

Drugs	Dose (mg/kg)	No. of rats	Drinking latency (mean in seconds) ± S.E.							
			Training trial							Test
			1	2	3	4	5	6	7	
Saline		23	332.8	131.7	124.7	89.4	73.7	50.0	43.9	249.8 ± 27.6
Wy 4036	2	25	495.0	209.5	116.2	63.9	32.8	30.1	28.7	541.3 ± 53.1*
Oxazepam	20	11	497.6	289.6	224.8	163.3	96.9	37.2	27.0	514.1 ± 112.6*
Diazepam	4	9	432.0	290.6	90.1	36.3	30.9	27.8	24.6	345.6 ± 87.3
Scopolamine	1	9	535.3	315.7	175.7	72.8	57.4	37.3	29.3	172.5 ± 29.9

* Differs from saline at $P < .05$.

To test in another way the idea that tranquilizers facilitate the retrieval of painful memories, we determined the effectiveness of the drugs in rats made amnesic by electroconvulsive shock (ECS). There is evidence that storage of the painful shock experience in permanent memory could be retarded or prevented if ECS were given immediately after the fear-conditioning trial (*12*). Abolition of the painful memory should have rendered the tranquilizers ineffective in our test because we assumed that the drugs acted by releasing an intact memory from repression.

Electroconvulsive shock (40 ma for 0.6 seconds, via ear clips) was administered in the test chamber to 30 rats within 1 second after the delivery of the foot shock. Another 29 rats were treated similarly, but no current was passed through their ear clips. One week later, half of each group was tested for conditioned suppression after being given injections of either Wy 4036 or saline. The saline–ECS group drank significantly faster than the saline–no ECS group ($t = 3.48$, d.f. $= 28$, $P < .001$) (Fig. 2). This result is evidence that ECS produced an amnesic effect in our test. As we predicted, Wy 4036 was largely ineffective in animals made amnesic by ECS; the mean drinking latency of the Wy 4036–ECS group was 86.9 seconds versus 455.3 seconds for the Wy 4036–no ECS group ($t = 5.05$, d.f. $= 27$, $P < .001$). Indeed, the Wy 4036–ECS animals exhibited less conditioned suppression than the saline–no ECS controls, although the difference just failed to achieve statistical significance ($t = 1.86$, d.f. $= 28$, $P < .1$).

On the basis of the foregoing observations, we suggest that the act of memory retrieval has some of the properties of behavioral acts or operant responses (*13*). Specifically, we propose that the retrieval of memories may be facilitated by reward and inhibited by punishment, just as behavioral responses are (*14*). This idea suggests a simple mechanism for the repression of painful memories. As the memory of a painful experience is aroused, the incipient memory activates an inhibitory or punishment process which shuts off the act of retrieval. Tranquilizers interrupt this negative feedback process by blocking the punishment system (*3*) and thus release the painful memory from repression. Facilitated recall of positive

memories may be accomplished by a complementary process. In this case the incipient positive memory activates the reward mechanism, which facilitates the act of retrieval.

LARRY STEIN
BARRY D. BERGER
Wyeth Laboratories,
Philadelphia, Pennsylvania 19101

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15. We thank J. Monahan, D. Massaro, and D. Leath for technical assistance.

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Behavioral Regulation of Hypothalamic Temperature

Abstract. *Animals will work to produce changes in hypothalamic temperature. The two main inputs, skin and hypothalamic temperatures, combine to control this behavior. Specifically, the rate at which rats work for changes in hypothalamic temperature is proportional to the sum of the weighted displacements of skin and hypothalamic temperatures from their respective neutral values.*

Animals work for changes in skin temperature in response to displacements of either skin or hypothalamic temperature (*1*, *2*). For example, rats work to turn on a draft of cool air when either skin or hypothalamic temperature is increased.

I now report that animals will also work for changes in hypothalamic temperature. The rate at which rats press a lever to produce reductions in hypothalamic temperature increases when either skin temperature (T_s) or hypothalamic temperature (T_{hy}) is increased above neutrality. When both inputs are varied, T_s and T_{hy} are additive, and T_s is given more weight than T_{hy} in the control of this behavior. Application of an additive linear model to the behavioral data permits direct quantitative comparison of the control systems for physiological and behavioral thermoregulatory responses.

Investigation of the interaction of T_s and T_{hy} in the control of behavioral adjustments of T_{hy} was made possible by the development of a general-purpose system (*3*) for studying thermoregulatory behavior in small mammals. The system incorporates a continuous flow of air over the body surface to control T_s and a water-perfused thermode implanted in the hypothalamus to control T_{hy}. Heat exchangers in conjunction with constant-temperature circulators provided stable control of air and water temperatures.

The animal is given control over either T_s or T_{hy} by arranging for its responses to activate a solenoid-operated valve in either the air or the hydraulic portion of the system. By pressing a lever, the animal produces an abrupt change in the temperature of either the air flowing over its body surface or of the water perfusing its hypothalamic thermode, which causes an abrupt change in either T_s or T_{hy}. Thus the animal's choices between two values of T_s can be studied while T_{hy} is held constant, or its choices between two values of T_{hy} can be studied while T_s is held constant.

Thermodes (*1*, *3*) consisting of a concentric arrangement of 18- and 22-gauge thin-wall stainless steel tubes were implanted in two adult, male Sprague-Dawley rats (T7 and T9). The thermodes were placed in the anterior medial preoptic areas. The following stereotaxic coordinates with the skull level between bregma and lambda were used, namely, 1.0 mm anterior to bregma; 0.5 mm lateral to the center of the superior sagittal sinus; and 8.5 mm down from the surface of the dura. Stainless steel machine screws in the frontal and parietal bones and dental acrylic cement were used to anchor the thermodes in place. A small (0.3 mm) thermistor bead attached to the outer surface of the thermodes 1 mm from the tip was used to measure T_{hy}.

About 1 week after the animals underwent surgery, the location of the thermodes was evaluated functionally. The animals were mildly restrained in still air at 25°C, and changes in T_s and rectal temperature caused by thermal stimulation of the hypothalamus were recorded. Both animals showed normal increases in T_s and decreases in rectal temperature when the hypothalamus was warmed. This indicates that the thermodes were located in or near the temperature-sensitive area and that the temperature sensitivity of the area had not been impaired by the placement of the thermode. In one animal, rat T7 (Figs. 1 and 2), the location of the thermode has been verified anatomically (*4*). In this animal, damage to the preoptic area was unilateral and was restricted to the anterior portion of one medial preoptic nucleus. The thermode was in contact with both medial preoptic nuclei, and the thermistor measured preoptic temperature.

Next, the animals were trained to work for reductions in ambient temperature. The temperature of the air flowing through the test chamber was normally warm (39°C), and the animal could reduce it to a neutral value (29°C) for 15 seconds by pressing the lever. After the animals mastered this task, as indicated by their stable rate of responding for reductions in ambient temperature, they were transferred to working for reductions in T_{hy}.