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Retention Deficit Correlated with a Deficit in the Corticoid Response to Stress

Abstract. Previous research has indicated that intracerebral injection of potassium chloride, and the resultant electrical silence of the brain, lead to the attenuation of a previously conditioned emotional response; this response may reflect conditioned fear. The data reported here indicate that the normal mobilization of corticosteroids, perhaps a second index of emotionality, is also attenuated by such injections.

Avis and Carlton and later Hughes (1) demonstrated that temporary depression of the hippocampal electrical activity can produce a deficit in the retention of a previously conditioned emotional response (CER). The CER refers to the fact that an animal will suppress its ongoing behavior when a conditioned stimulus, previously paired with shock, is presented. Animals that have received intrahippocampal injections of KCl, which produce electrical silence of brain activity at the site of injection, do not show such suppression.

One question that these data pose is whether the deficit is a reflection of a general deficit in the ability to inhibit behavior or whether this behavioral deficit reflects a deficit in fear. If the latter were true, one would expect animals that had been given KCl to show a deficit in some independent measure of fear. Such a deficit would at least suggest that the relative absence of conditioned suppression was due to an attenuation of fear rather than to a general inability to inhibit ongoing behavior.

In an initial attempt to resolve this question, Avis (2) measured the cardiac deceleration, perhaps a measure of conditioned fear, that accompanies presentation of the conditioning stim-

ulus. He found that animals that have undergone electrical depression do not display such cardiac deceleration.

A second measure that may reflect emotionality is examined in the experiment reported here. The results point in the same direction: Intrahippocampal injections of KCl, and consequent electrical silence, attenuate the usual elevation in corticosteroids that accompanies presentation of a conditioned fear stimulus (3).

Nineteen male albino rats (Sprague-Dawley strain) were the subjects (4). Briefly, all animals were trained to lick from a water-filled tube and, several days later, were given four shocks, each preceded by a tone presentation. After intervening hippocampal injections (see below), the subjects were

tested for suppression of drinking in the presentation of the tone. The degree of drinking suppression was taken as a measure of conditioned fear. Immediately after this test session, the subjects were decapitated and blood was collected. The adrenals of each rat were also removed, weighed, and homogenized. The serum and adrenal samples were analyzed for corticosterone.

The details of the procedure are as follows. On day 1, animals deprived of water for 24 hours were placed in a response chamber with a water-filled drinking tube in one corner. All animals were allowed to make 110 licks. Three days later each animal was returned to the chamber, the drinking tube having been removed, and was given four tone-shock pairings, each separated by 1 minute. Each 15-second tone presentation was terminated by a 1-second, inescapable, 2-ma shock delivered to the animal's feet through the grid floor of the chamber. Twenty-four hours after the tone-shock pairings, all animals were anesthetized with pentobarbital (40 mg per kilogram of body weight) and chloral hydrate (165 mg/kg). Either KCl or saline was then bilaterally injected into the hippocampus. Two stainless steel, 23-gauge hypodermic tubes, insulated to within 1 mm of the tip and mounted on a Plexiglas holder, were used for electrical recording and injecting. With the aid of a stereotaxic instrument, these tubes were lowered into place. The placements were as follows: 5 mm posterior to bregma, 5 mm lateral to the midline suture, and 5 mm below the dorsal surface of the cortex (5). Monopolar recordings were made with the skin retractors serving as the indifferent electrode. Electroencephalographic (EEG) recordings were made with a Beckman type RB dynograph. For further procedural details see Avis and Carlton as well as Hughes (1).

The EEG recording began as soon as the electrodes were lowered into place. Once the EEG activity stabilized (5 to 10 minutes), a 30-gauge tube connected to a Gilmont microinjector was inserted through the electrodes. A 25 percent solution of KCl was injected into each side of the hippocampus. At least 11 minutes of electrical silence (little or no wave amplitude at an amplifier gain of 200 μ V) was induced in all cases. From 3 to 7.5 μ l of KCl were needed to produce this degree of electrical depression; comparable volumes of physio-

Table 1. Mean values for animals given KCl and saline.

Suppression	Plasma (μ g/100 ml)	Adrenal (μ g/100 g)
<i>Potassium chloride*</i>		
25.90 (10.68)†	18.37 (4.21)	3.09 (0.89)
<i>Saline‡</i>		
259.90 (20.81)	40.04 (7.19)	6.96 (0.96)

* Nine animals. † Standard error of the mean.

‡ Ten animals.

logical saline were given to the control animals.

The animals were all tested for retention of the CER 4 days after the operation. The subjects deprived of water for 24 hours were placed in the chamber and allowed to make 100 licks. The tone was then presented and a record was made of the time it took the animals to make ten additional licks. The tone presentation and the session ended after the completion of the ten licks or, if the animals did not emit ten licks within 300 seconds, the test was arbitrarily terminated. No shocks were delivered during the test session.

After two experimental and four control animals had been tested, the test procedure was slightly modified. All animals were placed in the chamber for 15 minutes. Once the tone was activated after the animal's 100th lick, it was not terminated until the end of the 15 minutes; the time required to emit the first ten licks during the tone was, however, recorded. This modification was introduced to make the length of the tone presentation comparable for the experimentals and controls.

Immediately after the test session, each animal was decapitated. Trunk blood was collected and centrifuged to obtain plasma. The adrenals were removed and weighed after decapitation. The fluorescence method of Hilf *et al.* (6) was employed to determine corticosterone levels in the plasma and homogenized adrenals of each animal.

There was no overlap between the experimental and control groups in terms of conditioned suppression. All animals injected with KCl took less time (mean, 25.90 seconds) than animals injected with saline did (mean, 259.90 seconds) to make ten licks in the presence of the tone ($P < .002$) (7). These data are presented in Table 1.

Because there were no differences in plasma and adrenal corticosterone levels of animals injected with KCl between those exposed to the original procedure and those exposed to the modified procedure, all animals injected with KCl were considered together in the statistical analysis. Data for animals injected with saline were combined for the same reason. Corticosterone levels for experimentals and controls are given in Table 1. Inter-group differences for both plasma and adrenal corticosterone are significant: $P < .02$ and $P = .02$. The relevant basal levels are given below (8).

These data, in combination with those on cardiac deceleration reported by Avis, suggest (i) that electrical silence induced between conditioning and testing attenuates the normal physiological response to a fear-provoking stimulus and (ii) that this attenuation of fear may be the basis of the lack of behavioral suppression originally reported by Avis and Carlton.

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8. Basal values of serum and adrenal corticosterone from a different set of KCl and saline animals were determined 24 hours after CER testing. Animals were decapitated within 15 seconds of being picked up. Because their basal values did not differ, the data from the KCl and saline animals were pooled. It was found that these mean basal values ($12.90 \mu\text{g}/100 \text{ ml}$ for serum and $2.81 \mu\text{g}/100 \text{ ml}$ for adrenals) were not statistically different from the values for the KCl animals in the present study. These basal values were, however, significantly lower than the values for the saline animals that are given in Table 1. It should also be noted that these basal values are similar to those reported in the literature [V. Critchlow, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963); S. Levine and F. R. Brush, *Physiol. Behav.* **2**, 385 (1967)].
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Selective Recovery from Retrograde Amnesia Produced by Hippocampal Spreading Depression

Abstract. *Injectons of potassium chloride into the hippocampus after learning produce temporary disruption of neural activity and retrograde amnesia. Recovery from the amnesia is selective—rats recover from amnesia of events that occurred 24 hours before injection but do not recover from amnesia of events that occurred 10 seconds before injection.*

Potassium chloride (KCl) injected into the hippocampus produces temporary disruption of hippocampal neural-electrical activity (1). If such injections are given to rats from 1 to 21 days after conditioning, the animals show amnesia from which they eventually recover (2). Consistent with the animal data, clinical reports on the amnesic effects of head trauma in humans have also noted recovery, but in the human case recovery is selective: memory of events that occurred minutes before the trauma often does not recover (3). The present study, in which hippocampal injections of KCl were used, established an experimental prototype of the clinical data by first replicating the finding (2) that amnesia of long-term memory is temporary and then extending the analysis to show that amnesia of short-term memory is relatively permanent. To accomplish this we injected KCl into the hippocampus of rats 10 seconds or 24 hours after conditioning and assessed recovery from amnesia by testing

the animals either 4 or 21 days after injection.

The subjects were 71 male albino rats of the Sprague-Dawley strain weighing 275 to 325 g. Training consisted of a conditioned emotional response procedure (4) in which a tone was paired with footshock; retention was assessed by the degree to which the tone, in the absence of footshock, later suppressed (measured in seconds) drinking behavior. Injections of KCl crystals into the hippocampus of free-moving rats were used to disrupt neural activity (5). The neural disruption in this case is temporary and is characterized by a reduction in the amplitude of electrical activity interspersed with high-amplitude seizure discharges.

Three to five days before the start of the experiment the animals were anesthetized and stainless steel 21-gauge cannulae, insulated except for the cross section of the tip, were implanted bilaterally into the ventral posterior area of the hippocampus (6). Inserted into