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. Intergroup 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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7. A two-tailed Mann-Whitney U test was employed for all statistical analyses.
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 μg/100 ml for serum and 2.81 μg/100 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. *Injections 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

Table 1. Suppression of drinking (expressed in seconds) to tone during retention test. Numbers in parentheses indicate number of animals.

| Injection-test interval | Groups | Interval between training and injection | | | |
|---|---|---|---|---|---|
| | | 10 seconds | | 24 hours | |
| | | Median | Interquartile range | Median | Interquartile range |
| 4 day | Hippocampal NaCl | 367 (6) | 142–516 | 289 (4) | 130–439 |
| | Hippocampal KCl | 4 (9) | 2–35 | 5 (9) | 4–41 |
| | Cortical KCl | 600 (5) | 83–600 | 298 (5) | 161–401 |
| 21 day | Hippocampal NaCl | 358 (10) | 86–485 | 277 (5) | 148–304 |
| | Hippocampal KCl | 31 (9) | 2–71 | 259 (9) | 11–287 |

the cannulae were 27-gauge plugs which were removed at the time of injection and were replaced with injection tubes. A reference electrode of 21-gauge tubing was placed on the frontal bone of the skull and, along with the cannulae, was held in place by dental acrylic anchored to screws fixed to the skull.

Injections were made with two 27-gauge tubes, each packed with approximately 14 $\mu$g of KCl or NaCl crystals and cut to fit flush with the embedded end of the implanted 21-gauge cannulae. The injection tubes were inserted simultaneously into the hippocampi, were removed 3 minutes later, and were replaced with recording electrodes from which hippocampal electrical activity was bilaterally monitored. If the first injection did not produce disruption of ongoing activity for at least 30 minutes, additional injections were given until this criterion was met (*7*). Neural activity was monitored for 10 minutes before and up to 1 hour after the first injection.

On day 1 the animals, deprived of water for 24 hours, were placed in the training chamber and were allowed to emit 120 licks at a drinking tube. Four days later the animals, not deprived of water, were returned to the training chamber after the drinking tube had been removed and, after 60 seconds, were given four tone-shock pairings with an intertrial interval of 60 seconds; the tone (70 db, 1000 hz) was 10 seconds in duration and its termination coincided with the onset of footshock (2 ma for 1 second). After the training, animals were divided into two groups and were injected with KCl crystals into the hippocampus approximately 10 seconds or 24 hours later. Each group was further divided and was tested either 4 or 21 days after injection. On the test day the animals, deprived of water for 24 hours, were placed in the training chamber and were allowed to emit 100 licks. The tone was then presented and the time it took the animals to emit 10 additional licks during the tone was recorded. If the animals did not complete the 10 licks within 600 seconds, the test was terminated. After the testing, electrical activity of the hippocampus was monitored for approximately 10 minutes. Control animals received the same training, injection, and testing procedures; they were injected with NaCl crystals matched in weight with KCl crystals.

After the retention tests all animals were perfused with formalin and their brains were removed. To verify cannulae placement, frozen sections (40 $\mu$m) of the brains of representative samples from each group were stained with cresyl echt violet. Microscopic analysis confirmed that all cannulae were located in the ventral posterior hippocampus. The KCl and NaCl groups were indistinguishable on the basis of the size and place of the cannulae-induced lesion.

A sample electrohippocampogram is presented in Fig. 1. It can be seen that KCl produced both periods of electrical silence and periods of seizure activity. In nearly all cases the record began to return to normal within 1 hour after the first injection. In all cases recordings taken after the retention test showed complete recovery and did not differ from recordings taken before injections. The NaCl crystals, on the other hand, had virtually no effect on neural activity (*8*).

Table 1 presents the retention data; a Mann-Whitney U test (two-tailed) was used for statistical evaluation. The NaCl-control data indicate that the training procedure was effective in producing substantial suppression to the tone. Accordingly, in the groups injected with KCl a significant reduction in suppression times relative to the NaCl controls is taken to reflect impaired retention. In animals given KCl injections into the hippocampus 10 seconds after training, those ($P = .02$) tested 4 days as well as those ($P < .02$) tested 21 days after injection showed impaired retention. Similarly, animals given KCl injections into the hippocampus 24 hours after training showed impaired retention ($P = .05$) 4 days after injection. On the other hand, in contrast to the prolonged impairment shown by animals injected 10 seconds after training, animals injected 24 hours after training showed retention ($P > .05$) 21 days after injection. To assure that the impaired animals' ability to perform and to retain the conditioned response was not permanently disrupted by the

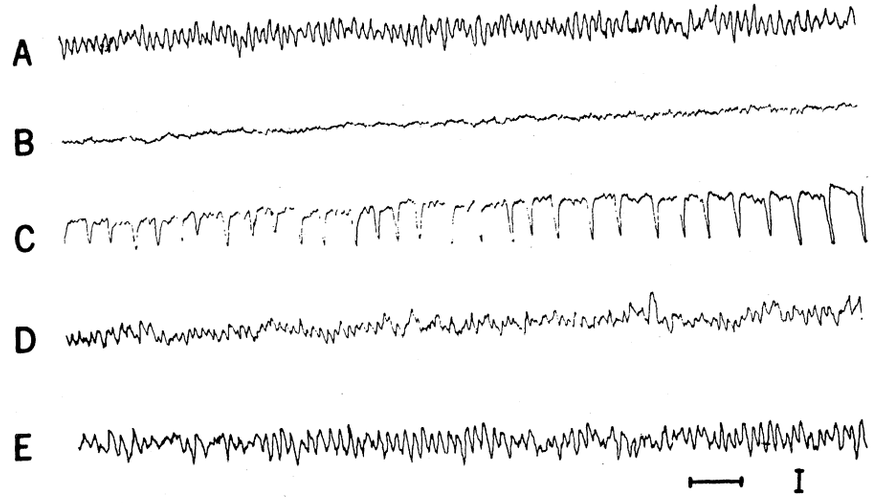


Fig. 1. Unilateral electrohippocampogram of a freely moving animal taken before, during, and after injections of KCl into the hippocampus. (A) Hippocampal theta rhythm recorded 10 minutes before injection; (B) neural depression observed during the injection period; (C) seizure activity observed during the injection period; (D) partial recovery of neural activity recorded 60 minutes after the first injection; and (E) full recovery of neural activity recorded 10 minutes after the retention test. Calibrations, 1 second and 500 $\mu$v.

injection, they were retrained and 4 days later were tested; without exception they showed retention.

It is possible that the difference in lick rate during the tone between animals injected with NaCl and those injected with KCl reflects differences in thirst or motor ability rather than amnesia. Supplementary measures rule out both possibilities: animals injected with NaCl and those injected with KCl did not differ on daily water intake monitored in the home cage (*9*) nor on ability to lick, as measured in terms of time to complete ten licks before the tone ($P > .05$ in both cases). These data, taken together with the fact that the animals injected with NaCl and those injected with KCl were subject to the same lesion produced by the cannulae and the same mechanical stimulation produced by the crystal, provide cogent evidence that KCl impairs retention.

The possibility remains that impaired retention could have been produced by KCl-induced disruption spreading to sites outside the hippocampus (*10, 11*). That this tends not to be the case, at least regarding spread to the cortex, is indicated by data from two additional groups that received KCl injections in the cortex after training. The surgery for these groups was designed to equate them with the hippocampal groups on the size and place of the hippocampal lesion yet allow for injection to the cortex. To accomplish this, the cannulae were lowered into the hippocampus, then were redrawn and fixed at the surface of the cortex. These groups received the same training, injection, and testing procedure as the hippocampal groups—one group received KCl crystals in the cortex 10 seconds after training, the other group received KCl crystals in the cortex 24 hours after training, and both groups were tested 4 days after injection (*12*). The retention data, presented in Table 1, indicated that the two groups did not differ ($P > .05$) and that each group showed significantly greater retention than its corresponding hippocampal group ($P < .05$ in both cases).

The results indicate that recovery from the amnesic effects of KCl is selective—animals 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. To interpret these results it is reasonable to assume that hippocampal injections of KCl have two disruptive effects on memory: a storage effect that is specific to short-term memory and is permanent; a retrieval effect that is specific to long-term memory and is temporary.

The retrieval effect presupposes that KCl produces neural abnormalities that prevail for at least 4 days after injection, thereby interfering with performance during the retention test, but that subside within 21 days after injection, thereby permitting performance during the test. Although the behavioral data bear this out, the neural data do not: the electrohippocampograms show no signs of disruption during the impaired retention, which suggests that the retrieval effect may be due to subtle neurochemical alterations that are not manifested in the electrophysiology. A similar neural-behavioral dissociation, but with cortical KCl, has been reported for an unconditioned response, hypesthesia (*13*). The storage effect, on the other hand, is consistent with the notion of memory consolidation and indicates that memory does change as a function of time after training.

Neither the permanent nor the temporary amnesic effects are unique to this study (*14*), but what is unusual is that both effects were obtained with a time-dependent parameter (that is, training-injection interval) closely related to selective recovery from amnesia in humans. Our data not only parallel those from humans but suggest that the source of the interference is related to both storage and retrieval effects produced, at least in part, by disruption of neural activity in the hippocampus.

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6. Hippocampal coordinates were 5 mm posterior to bregma, 5 mm lateral to the midline suture, and 5 mm below the dorsal surface of the skull. Placements were based on D. Albe-Fessard, F. Stutinsky, S. Libouban [*Atlas Stereotaxique du Diencephale du Rat Blanc* (Editions du Centre National de la Recherche Scientifique, Paris, 1966)].
7. From two to five injections were needed to meet criterion. Comparison among animals that received different numbers of injections revealed no differences in retention scores. Although some animals showed slight clonus during the later stages of depression, their retention scores did not differ from those of animals treated with KCl, which showed no overt seizures.
8. Several animals injected with NaCl showed a slight reduction in neural activity but no seizure activity. Retention in these animals did not differ from that in the other NaCl controls.
9. Water intake was monitored with a milliliter-calibrated drinking tube 1 day before injection and 2 days before the retention test.
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11. Recordings from the overlying cortex of additional animals during injections of KCl into the hippocampus showed sporadic seizure bursts. Similar spread of seizures produced by electrical stimulation has been noted by others [Green (*10*)].
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