Hippocampal injections of KCl and shock-induced bradycardia in the rat*

HARRY H. AVIS

Rutgers—The State University, New Brunswick, N.J. 08854

Rats given hippocampal injections of 25% solution of KCl 24 h after tone-shock pairings do not show bradycardia when presented subsequently with the tone alone. Animals given the same training but with saline injections do show cardiac deceleration. KCl injections themselves have no effect on heart-rate response to a tone. The results are discussed in terms of previous research indicating that KCl injections also produce a retention deficit in a CER paradigm, the suggestion being that the deficit in the CER paradigm is mediated by a failure to retain fear conditioning.

Various investigators (Avis & Carlton, 1968; Hughes, 1969; Kapp & Schneider, 1971; Auerbach & Carlton, 1971) have shown that injections of KCl into the hippocampus of the rat 24 h after training produces a retention deficit in a CER paradigm. In this paradigm, animals are given four tone-shock pairings in a chamber in which the animals had previously learned to approach a water-filled tube. On subsequent testing, the presentation of the tone suppresses the licking response of the rat. Hippocampal injections of KCl cause a deficit in this suppression. The deficit does not appear to be a proactive performance deficit, since Avis & Carlton (1968) and Hughes (1969) present evidence suggesting that animals injected with KCl and then given tone-shock pairings do not show a CER deficit. Thus, the data suggest that deficit may be mediated by a deficit in the retention of the fear response.

An autonomic measure of reactivity to the tone might provide further evidence for the fear hypothesis. If the Ss given KCl injections do not show the same autonomic reactivity to the tone as do animals that have received saline injections, but instead resemble animals that had not been shocked, it might be reasonable to conclude that the tone had no more consequence to the KCl-injected animal than to the unshocked animal.

It has been shown that presentation of a tone previously paired with shock produces bradycardia (DeVietti & Porter, 1970; Fitzgerald & Teyler, 1970). Thus, it appears that heart-rate deceleration might be a useful measure of reactivity to the tone.

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SUBJECTS

Twenty-four male albino rats of the Sprague-Dawley strain, 200-250 g at the start of the experiment, were used as Ss. The animals were housed individually with food and water available throughout the experiment.

APPARATUS

A Sanborn dc recorder (Model 320) was used to record the electrocardiogram. Dime electrodes were used, and Microdot cable was soldered to the dimes and connected to the Sanborn recorder. During the recording, the Ss were placed in a restraining cage (8½ x 4 x 4 in.), which was placed inside the Gerbrands student box (9½ x 8½ x 7½ in.), which in turn was housed in a sound-attenuating chamber (22 x 16½ x 13½ in.).

PROCEDURE

The Ss were assigned randomly to one of four groups: no-shock saline (NS-Sal), no-shock KCl (NS-KCl), shock saline (Sh-Sal), and shock KCl (Sh-KCl). On Day 1, all Ss were placed in the restraining cage and initial baseline ECG recordings were taken. Recording was accomplished by attaching the dime electrodes to ¼-in.-wide electrical tape and wrapping the tape around the shaved chest area of the rat so that the electrodes were against the ventral surface of the body. Both the electrodes and the chest area of the rat were coated with electrode paste.

After 3 min of recording, the Ss were removed from the restraining cages. Animals in Groups NS-Sal and NS-KCl were returned to their home cages. Animals in Groups Sh-Sal and Sh-KCl were placed without electrodes in the demonstration box and given four tone-shock pairings. The tone was of 15-sec duration, and its termination was coincident with the delivery of a 1-sec 2-mA shock delivered to the animal's feet through the grid floor of the chamber.

On Day 2, all animals were anesthetized with pentobarbital (40 mg/kg) and chloral hydrate (165 mg/kg). Either KCl or saline was then injected bilaterally into the hippocampus. Two stainless steel 23-ga hypodermic tubes, insulated to within 1 mm of the tip and mounted on a Plexiglas holder, were used for recording and injection. 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. Monopolar recordings were made with a Type RB dynograph.

The EEG recording began as soon as the electrodes were lowered into place. Once EEG activity had stabilized, a 30-ga tube connected to a Gilmont microinjector was inserted through the electrodes. Groups NS-KCl and Sh-KCl were injected with a 25% solution of KCl into each side of the hippocampus. A sufficient volume (3.0-6.5 microliters) of solution were given to induce at least 11 min of electrical silence, since this appears to be the minimum amount of silence necessary to produce a retention deficit in the CER paradigm (Avis & Carlton, 1968). Saline control groups were given equivalent volumes of physiological saline.

On Day 6, the Ss were returned to the restraining cage and placed in the sound-attenuating chamber. After 2 min of recording, the tone was presented for 1 min. After termination of the tone, ECG recording continued for 1 min more. Heart rate was monitored at six intervals: (1) the first and last 15 sec of the familiarization period, (2) 15 sec after the start of the test session, (3) 15 sec prior to tone onset, (4) the first 15 sec of tone, (5) 15 sec prior to tone offset, and (6) the 15 sec following tone offset.

After the completion of the experiment, the animals were perfused with saline and Formalin solution and the brains were removed. After a period of time, the brains were frozen and sectioned (50-micron sections) and stained to verify electrode placements. All electrodes were located in the ventral posterior hippocampus. The sections of brains of animals injected with KCl could not be distinguished from those injected with saline.

RESULTS

All statistical comparisons were made between Intervals 3 and 4, i.e., between the intervals 15 sec before and 15 sec after tone onset. There was a significant heart-rate decrease upon presentation of the tone in the Sh-Sal group ($p < .01$, Mann-Whitney U test). The Sh-KCl group, however, did not

Table 1
Heart Rate Per 15-Sec Epochs at Selected Times During Training and Testing*

Group	Pretest		Test				
	1a	1b	2	3	4	5	6
N-S Sal	115	118	130	128	127	129	131
N-S Sal	118	115	127	122	122	121	122
N-S Sal	116	115	123	123	121	121	119
N-S Sal	117	120	126	125	125	124	126
N-S Sal	119	114	122	123	123	120	120
N-S Sal	117	116	130	130	128	128	127
Sh-Sal	126	123	125	127	122	123	123
Sh-Sal	115	116	123	125	120	120	120
Sh-Sal	110	106	98	105	98	99	101
Sh-Sal	116	115	123	121	110	114	114
Sh-Sal	113	115	121	123	113	116	114
Sh-Sal	106	105	124	124	113	119	115
NS-KCl	120	122	133	130	129	128	125
NS-KCl	125	121	133	130	132	131	130
NS-KCl	118	117	129	122	123	124	123
NS-KCl	115	108	124	127	128	125	122
NS-KCl	120	117	127	126	125	126	123
NS-KCl	118	119	125	127	128	126	125
Sh-KCl	113	113	117	117	115	118	118
Sh-KCl	115	117	129	128	127	127	126
Sh-KCl	126	125	121	120	112	117	118
Sh-KCl	119	118	127	122	121	120	121
Sh-KCl	108	113	123	120	119	119	120
Sh-KCl	118	123	130	128	131	130	130

*The tone was presented during Epoch 4.

differ from the NS-KCl and NS-Sal. These results indicate that electrical silence induced by injections of KCl into the hippocampus eliminates the heart-rate change that occurs when a tone previously paired with shock is presented (Table 1).

DISCUSSION

It should not be assumed that the bradycardia seen in this study is, in fact, a classically conditioned heart-rate change, since several factors could be involved, including sensitization due to the previous presentation of shock. Nonetheless, it is significant to note that whatever the cause of the heart-rate change in the Sh-Sal group, no change is seen in the Sh-KCl groups. In fact, the Sh-KCl group is indistinguishable from the NS-Sal, indicating that, as measured by the heart-rate change, the tone no longer evokes the same autonomic response when hippocampal KCl injections are given.

Auerbach & Carlton (1971) have recently presented evidence supporting the hypothesis that the KCl-induced behavioral deficit may, in fact, reflect a failure to retain the fear response. Their procedure was essentially similar to that of Avis & Carlton (1968). Animals were trained to approach a water-filled tube. Subsequently, they were given four tone-shock pairings.

Twenty-four hours later, they were given hippocampal injections of either saline or KCl. Animals that had received KCl did not show the tone-induced suppression of licking. Auerbach & Carlton (1971) also measured corticosteroid levels of adrenal glands and blood plasma. They reported that animals given KCl injections showed significantly lower levels of plasma and adrenal corticosteroids than animals shocked and injected with saline. To the extent that corticosteroid changes and heart-rate decreases are measures of fear in the present situation, it appears that KCl injections disrupt the retention of the conditioned emotional response itself.

Although direct evidence is not available in this study, it should not be assumed that the deficit seen is produced by depression of electrical activity in the hippocampus alone. Avis (1969) has shown that a similar injection of KCl solution into the hippocampus produced a decrease in electrical activity in all areas of the brain that were monitored; therefore, no primacy of the hippocampus for the site of this effect need be assumed. On the other hand, this effect is not likely to be mediated KCl-induced lesions, since the physical spread of KCl is limited to the hippocampus

(Avis, 1969), and, in the present study, histology indicated that the lesions produced in the KCl group were indistinguishable from those of the saline group.

The present results appear to contradict those of Mendoza & Adams (1969) and Hine & Paolino (1970). Mendoza and Adams reported that ECS given shortly after a single buzzer-shock pairing did not modify the bradycardia seen on subsequent testing 24 h later. Similarly, Hine and Paolino reported that rats given ECS after a one-trial passive avoidance test did show heart-rate changes similar to those animals not given ECS, even though a clear behavioral deficit is seen. The reason for this discrepancy is unknown, but may lie in the relative ineffectiveness of ECS as compared to hippocampal injections of KCl; ECS is not effective unless given almost immediately after training, but KCl injections, on the other hand, produce a retention deficit when given as long as 24 days after training (Hughes, 1969).

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