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EFFECTS OF HIPPOCAMPAL GRANULE-CELL AGENESIS ON ACQUISITION OF ESCAPE FROM FEAR AND ONE-WAY ACTIVE-AVOIDANCE RESPONSES[1]

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The head region of the hippocampus was irradiated with low-level X ray in infant rats. This reduced the total number of hippocampal granule-cells by an average of 84%. Four experiments extended the behavioral similarities previously noted between such hippocampal granule-cell agenesis and conventional hippocampal lesions. Irradiated and control rats were alike in the acquisition of a one-way avoidance response, although there was a trend of greater resistance to extinction in the irradiated group. The irradiated group displayed facilitated acquisition of an escape-from-fear response. When one-way avoidance was preceded by inescapable shock, the irradiated group was superior, suggesting that granule-cell loss, like hippocampal ablation, disrupts a tendency to remain immobile in the presence of stimuli related to inescapable punishment.

The multiplying precursors of the postnatally forming neurons of the cerebellar cortex are killed by exposure to low-level X ray without directly damaging its prenatally formed Purkinje cells (Altman & Anderson, 1971, 1972; Altman, Anderson, & Wright, 1968). Since the bulk of granule cells of the dentate gyrus of the rat hippocampus are formed postnatally (Altman & Das, 1965, 1966), exposure of the head region containing the hippocampus in infant rats should prevent the acquisition of these cells. It was established recently (Bayer, Brunner, Hine, & Altman, 1973) that with repeated doses of low-level X ray the population of hippocampal granule cells is reduced by an average of 85% without visible damage done to prenatally formed pyramidal cells of Ammon's horn. A thorough morphological analysis of the effects of hippocampal irradiation has yet to be accomplished. However, there are reasons to believe that because the pyramidal cells are unharmed, there is no modification in hippocampal efferents, although the mode of termination of afferents to the hippocampus may be altered due to the scarcity of granule cells in the dentate gyrus. Likewise, the overlying cortex and underlying diencephalic and telencephalic structures appear unaffected at the light-microscopic level, presumably because the neurons of these structures are all essentially formed prenatally. The possibility of a reduction of glia cells in these structures awaits examination. There are rostral (nucleus accumbens and olfactory bulb) and caudal (cerebellum) structures which are as radiosensitive as the hippocampus, but special care was taken to shield these regions from irradiation.

With these considerations in mind, the effects of irradiation should not be viewed as "destroying" the hippocampus, in the sense that an electrolytic or aspiration lesion does, but rather as producing a defect in its normal development, i.e., agenesis, by preventing the acquisition of its full complement of granule cells, which by way of their axons—the mossy fibers—play an important role in the intrinsic circuitry of the hippocampus.

In general, the present experiments continued to examine the behavioral similarities, and possible differences, between hippocampal destruction in adults and inter-

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ference with dentate gyrus development. A rat's performance on a passive-avoidance task is impaired by hippocampal damage produced by the conventional aspiration or electrolytic lesions (e.g., Blanchard & Fial, 1968; Isaacson & Wickelgren, 1962), and also by X-irradiation of the hippocampus during infancy (Bayer et al., 1973). Hippocampal damage is known to facilitate acquisition of a 2-way active-avoidance response (e.g., Isaacson, Douglas, & Moore, 1961), and this effect, too, is reproduced by hippocampal X-irradiation (Bayer et al., 1973). Dentate granule-cell reduction was also found to produce a deficit in spontaneous alternation to increase open field activity (Bayer et al., 1973), as does hippocampal damage (e.g., Means, Leander, & Isaacson, 1971).

The present experiments were concerned with the effects of granule-cell agenesis on the acquisition of fear-motivated responses in adult rats.

General Method

Subjects

The subjects were Purdue-Wistar male rats, cross-fostered at birth and raised 6–8 per litter. The subjects in each experiment were housed 2 to a cage and allowed continual access to food and water.

Radiation Procedure

Bayer et al. (1973) determined the location of the hippocampus in rat pups aged 2–18 days and described the procedure used in the present experiments to expose the hippocampus to X rays. Pups were immobilized in plastic tubes (see Altman, Anderson, & Strop, 1971) and were placed in a lead-shielded Lucite block holder. A slit in the holder, with a 1-mm. margin for error, allowed only that portion of the head containing the hippocampus to be directly exposed to X rays. X rays were delivered from a Maxitron 300-kv. unit at the rate of 50 r. per minute. Irradiation treatment began when the pups were 2 days old; 200 r. was delivered on Days 2 and 3, followed by 150 r. on Days 5, 7, 9, 11, 13, and 15. The control subjects, except where noted, were immobilized in the same manner as the irradiated subjects but were not exposed to X rays.

General Histological Results

All rats used in these experiments were sacrificed and perfused with buffered Formalin when 90 or 120 days old, and the brains were postfixed in Bouin's solution. Brains of 5 control and 6 irradiated rats (90 days old), randomly selected, were embedded in Paraplast and 6-μm. sections were cut in the coronal plane and stained with hematoxylin and eosin. The number of granule and pyramidal cells in matched sections (counted in a manner previously described by Bayer et al., 1973) for irradiated and control rats are presented below:

Control (Litter and rat numbers)	Dentate gyrus	Ammon's horn
C3-5	1,387	556
C6-1	1,531	619
C17-4	1,487	656
C15-2	1,702	599
C4-6	1,319	589
	$\bar{X}$ = 1,485	$\bar{X}$ = 604
Irradiated (Litter and rat numbers)		
WHB1-5	222	558
WHB2-1	234	657
WHB2-2	211	528
WHB2-3	244	651
WHB2-4	239	512
WHB3-1	246	560
	$\bar{X}$ = 233	$\bar{X}$ = 578

Compared with the controls, irradiation produced a mean reduction of 84% in the granule cells (Mann-Whitney $U = 0$, $p < .001$) and 5% reduction ($U = 11$, *ns*) in pyramidal cells. Photomicrographs of the normal and irradiated hippocampi are shown in Figure 1. The remaining brains were cut in the sagittal plane, stained, and examined microscopically in order to verify granule-cell loss in the dentate gyrus.

Experiment 1

The effects of surgical lesions of the hippocampal complex on one-way active-avoidance learning are at present ambiguous. Conflicting reports and vast differences in

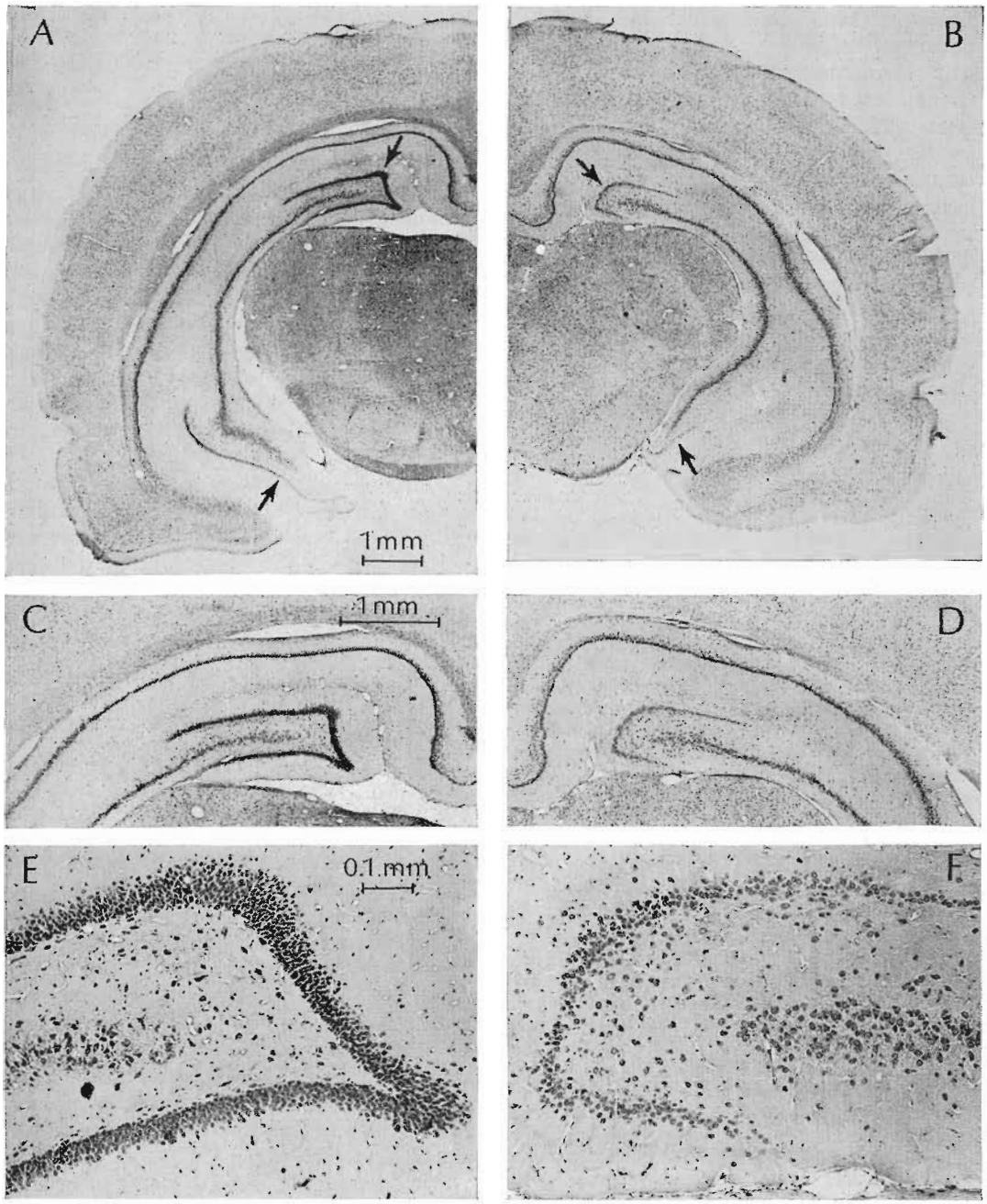

FIGURE 1. Photomicrographs of frontal sections from control (left) and representative X-irradiated brains (right) at 3 levels of magnification. (Arrows in A and B point to the granule cells of the dentate gyrus.)

experimental design and procedure, as well as size and type of lesions, impose considerable difficulty of interpretation and provide at best only tenuous support for the view that hippocampal ablation impairs one-way avoidance acquisition.

Coscina and Lash (1969, 1970), McNew and Thompson (1966), and Olton and

Isaacson (1968) have concluded that hippocampal-lesioned rats show impaired one-way avoidance acquisition relative to normal rats. However, only Coscina and Lash (1969) obtained a significant difference between rats with large hippocampal lesions and rats with neocortical lesions. Coscina and Lash (1970) subsequently reported no one-way avoidance deficit relative to neocortical controls for rats with large aspirated lesions.

The present experiment investigated the effect of dentate granule-cell loss on acquisition and extinction of a one-way avoidance response.

Method

Subjects

Two groups of subjects were formed by randomly selecting 12 rats which had been irradiated during infancy and 12 nonwrapped control rats.

Apparatus

The apparatus was a shuttle box (Lafayette, No. 85102) with electrical circuitry which automated presentation of the conditioned and unconditioned stimuli (CS and US), opening and closing of the guillotine door, and all time intervals. The shock compartment was lined with white construction paper and had a 6-w. light mounted in the lid behind white translucent Plexiglas. The safe compartment was lined with black construction paper. The US was a constant-current .25-ma. scrambled shock.

Procedure

On Day 1 the subjects were handled in groups of 2 for 5 min. each. On Day 2 each subject was allowed to explore the apparatus for 10 min. with the guillotine door open. On Day 3 a trial was initiated by placing a rat in the shock compartment. The CS came on and the guillotine door opened automatically 2 sec. after the subject was placed on the shock grid. The US came on 6 sec. after CS onset and remained on until the subject made an escape response. Both avoidance and escape responses terminated the CS. Following a response, the rat was confined to the safe compartment for 10 sec. and then placed in its home cage to await the next trial. The rats were run in squads which varied in size from 3–7 subjects, depending upon how many subjects had reached criterion either in acquisition or extinction. Hence, the intertrial interval varied from about 2–5 min. Each subject received 10 trials per day to a criterion of 9 avoidance responses (not necessarily successive) in 10 trials. Each subject was given 10 extinction trials per day beginning on the day after it attained the acquisition criterion. Extinction continued to a criterion of 5 successive failures to leave the shock side within 60 sec.

Results

Two control rats and one irradiated rat were dropped from the experiment after failing to attain the acquisition criterion following 90 acquisition trials. The control rats required an average of 14.2 trials and the irradiated rats 13 trials before starting a chain of 9 out of 10 avoidance responses ($U = 51.5$, *ns*). An inspection of response latencies following CS onset during acquisition also failed to reveal a difference between the irradiated and control groups (data not shown).

During the extinction phase, the irradiated group required 46 trials to reach a criterion of 3 successive failures to respond within 60 sec. The control group required 23.9 trials to reach the same criterion. This difference approaches significance ($U = 29$, $.05 < p < .10$). The irradiated rats required 49.5 trials to reach a criterion of 5 extinction responses while the control group required 32.7 trials ($U = 32.5$, *ns*).

Discussion

The acquisition results do not support the view that interference with hippocampal functioning impairs one-way active-avoidance acquisition. As noted above, the discrepant findings of lesion studies may be due to subtle procedural differences, size, completeness or type of lesion, or most notably, damage to other structures, e.g., the neocortex. The results of the present experiment (in which presumably only the hippocampus was affected by the treatment) may provide a more reliable indication of the minimal role played by the hippocampus in one-way active-avoidance acquisition.

The ambiguity that exists concerning performance of rats with hippocampal lesions in one-way avoidance acquisition also holds for extinction of avoidance responding. In 2-way avoidance, Isaacson et al. (1961) found that rats with hippocampal lesions were more resistant to extinction during successive acquisition and extinction sessions. Lovely, Grossen, Moot, Bauer, and

Peterson (1971) did not run animals to a criterion but proposed that there were no differences in extinction of a shuttle response if baseline performance level is taken into account. Ackil, Mellgren, Halgren, and Frommer (1969) also concluded that rats with hippocampal lesions were no more resistant in 2-way extinction, although they used a much less stringent criterion than the present experiment. While the apparent increase in resistance to extinction found in the present experiment did not reach significance, the trend was consistent with a reported effect of hippocampal lesions on extinction in appetitive tasks (Jarrard, Isaacson, & Wickelgren, 1964).

Experiment 2

Various hypotheses have been offered to explain the inferiority of hippocampal-lesioned animals on passive-avoidance tasks where the subject must return to an area on which it was just shocked. One suggestion has been that hippocampal damage elevates grid-shock threshold (Blanchard & Fial, 1968); another, that it interferes with response inhibition or the tendency to freeze when the animal is required to return to a place where it was previously punished (Douglas, 1967, 1972). Still another assumption is that hippocampal damage reduces emotional reactivity to shock (Blanchard & Fial, 1968) or that it interferes with the rat's ability to form associations between noxious events and cues reminiscent of noxious events (Blanchard & Fial, 1968; Olton & Isaacson, 1968). The latter hypotheses imply that the lesioned rats are less fearful than normals.

Miller (1948) and McAllister and McAllister (1962a) have shown that normal rats will learn to perform an instrumental reaction to escape a stimulus previously paired with shock. This escape is assumed to be motivated by a classically conditioned fear reaction and reinforced by reduction of fear as a result of response-contingent CS termination. Experiment 2 tested the view that loss of dentate granule cells disrupts the ability to form associations involving stimuli which predict shock. The escape-from-fear task was used because unlike the assessment of fear in conditioned emotional response and passive-avoidance situations, the measure of fear does not depend upon the subject's ability to withhold a response. If granule-cell agenesis reduces the rats' emotional reaction to shock or its ability to form associations involving stimuli which predict shock, hippocampal-irradiated rats should show an impaired escape from fear.

Method

Subjects

Two major groups were formed by randomly selecting 12 animals irradiated during infancy and 12 controls.

Apparatus

The apparatus was similar to that described by McAllister and McAllister (1962a). It consisted of a rectangular wooden box with 2 compartments separated by a 3.5-cm.-high hurdle and manually operated 5 × 7 cm. guillotine door. Both compartments were 23.5 × 10.5 × 9.5 cm. The white shock compartment had a grid floor of 3-mm.-diam. brass rods spaced 11 mm. apart. The safe compartment had a plywood floor and was painted black.

A 75-w. light was mounted inside the lid of the shock compartment behind white translucent Plexiglas. A speaker, wired to a tone generator, was located approximately 10 cm. behind the shock compartment. The CS, either the light or a 64-db., 1.5-kHz. tone, could be activated by raising the guillotine door, or the CS and US could be activated by a series of interval timers with the door closed. Opening the guillotine door also activated a Hunter Klockounter, which stopped when the floor of the safe compartment was depressed.

Procedure

Pretraining and fear conditioning. This phase of the experiment began when the rats were approximately 70–90 days old. On Day 1 the subjects were handled in groups of 2 for 5 min. On Day 2 each subject was allowed to explore the apparatus for 10 min. with the guillotine door raised. Fear conditioning began on Day 3 and consisted of 35 CS–US pairings (forward conditioning) administered in the white shock compartment with the guillotine door closed. The CS was either a light or a tone, and the US was a .5-ma. scrambled shock of .5-sec. duration. A delay conditioning procedure was used such that the CS was presented for 6 sec. and terminated with US onset. A constant 30-sec. intertrial interval was maintained throughout fear conditioning. The 4 groups were defined by a 2 × 2 factorial combination of X ray vs. control rats and light vs. tone CS.

Escape-from-fear conditioning. Twenty-four hours after fear conditioning each subject received 20 escape-from-fear trials. No shocks were delivered during this phase of training. Each trial consisted of raising the guillotine door, which produced CS onset, and allowing the rat to escape from the fear-eliciting stimuli to the safe compartment. If the subject did not cross to the safe side within 60 sec., it was gently guided through the door by hand. The rat's weight on the floor of the safe compartment terminated the CS, and the subject was then confined to the safe compartment for 10 sec. The subjects were run individually with a 30-sec. intertrial interval.

Experimental training was conducted in 2 replications with half of the subjects in each group assigned to each replication. The second replication began 2 days after the first one had finished.

Results

The data from the 2 replications were combined, and a 2 × 2 × 20 between–within analysis of variance, including CS (light vs. tone) and X ray (irradiated vs. control) as factors, was performed on reciprocals (1/sec) of the hurdle-jumping latencies over the 20 escape-from-fear acquisition trials. The analysis failed to reveal any difference between the 2 CS conditions ($F < 1$).

Figure 2 shows the acquisition performance of the X-irradiated and control subjects. Since the CS effect was not significant, the data were collapsed across this condition. It can be seen that the control subjects initially escaped faster than the irradiated subjects but that the latter subjects responded numerically faster by Trial Block 2. Moreover, the control subjects reached a performance asymptote very early in training, whereas the irradiated subjects escaped increasingly faster over successive trial blocks. These observations were supported by a significant X ray Treatment × Trial Blocks interaction ($F = 3.16$, $df = 4/88$, $p < .01$), which when partitioned into simple effects of groups at each trial block, showed that irradiated and control subjects did not differ on Trial Blocks 1, 2, or 3 (all Fs < 1), while the irradiated subjects were faster than the controls on Trial Block 5 ($F = 5.93$, $df = 1/59$, $p < .05$). The simple effect of groups at Trial Block 4 fell slightly short of significance ($F = 3.09$, $.05 < p < .1$). Simple effects of trial blocks were found to be a significant source of variance for irradiated subjects ($F = 7.99$, $df = 4/88$, $p < .001$), while the escape speed of controls did not change over trial blocks ($F < 1$).

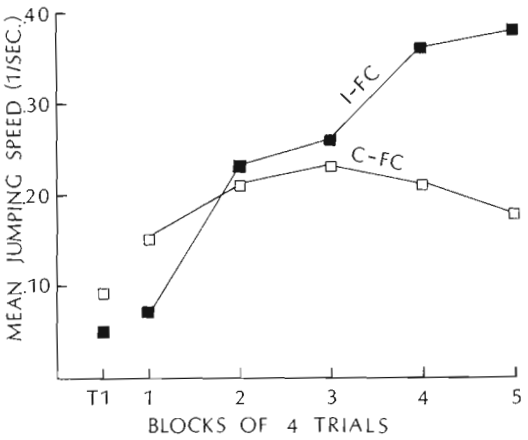

FIGURE 2. Mean reciprocal jumping speed in escape-from-fear instrumental conditioning trials. (Abbreviations: I-FC = irradiated-forward conditioning; C-FC = control-forward conditioning.)

Discussion

Contrary to the hypothesis that animals with hippocampal deficiency have difficulties in associating a noxious event with antecedent stimuli, this result suggests that the subjects with granule-cell agenesis were more responsive than controls in an escape-from-fear task. This does not necessarily imply that this group was *more* fearful, since normal animals may have the tendency to freeze rather than to run in this situation as in many other aversive situations. But this result, together with the result of Experiment 1 showing no deficit in one-way avoidance, suggests that hippocampal deficiency does not lead to reduced emotional reactivity.

EXPERIMENT 3

This experiment was undertaken (*a*) to determine whether the enhanced performance level of the irradiated subjects in the escape-from-fear situation reflects true conditioning of fear to the CS and (*b*) to examine the effect of altered shock threshold in the irradiated subjects. Thus, with some modifications, Experiment 2 was repeated

with the inclusion of hippocampally irradiated subjects given backward conditioning during the fear-conditioning phase. The purpose of a backward-conditioning group was to assess the relative contribution of sensitization and conditioning to general apparatus cues to the performance level of X-irradiated rats. The strength of fear conditioning was examined as a function of .25-ma. shocks delivered to half of the subjects and .5-ma. shocks to the other half.

Method

Subjects and Apparatus

Twenty-four rats (controls not-wrapped) selected as in the previous experiments were assigned to 3 groups of 8 rats each. Two additional groups each containing 6 subjects were added at a later date. The apparatus was the same as in Experiment 2.

Procedure

Pretraining and fear conditioning. Treatment on Days 1 and 2 was the same as in Experiment 2. On Day 3, 35 CS–US pairings were delivered as in Experiment 2 except that only a light CS was used and the US was either a .25-ma. or .5-ma. scrambled shock of .5-sec. duration. Shock offset was simultaneous with CS onset for the backward-conditioning subjects.

The 3 main groups in this experiment consisted of irradiated and control subjects given forward fear conditioning CS–US pairings (Groups I-FC and C-FC) and an irradiated group given backward-conditioning pairings (Group I-BC). Within each group, half of the subjects received low shock and half received high shock. No backward-conditioning control subjects were run because the main purpose in running the backward-conditioning subjects was to determine whether the facilitated performance of irradiated subjects was due to conditioning of fear to the CS or a heightened reactivity to shock. Two additional groups were run to assess the possibility that irradiated subjects are aided in this task by an above-normal activity level or baseline response rate. These subjects were tested in a manner identical to the I-FC and C-FC subjects except that the shock source was turned off. Since these subjects did not receive shock-conditioning trials, they may be identified as Groups I-NC and C-NC.

Escape-from-fear conditioning. This phase was conducted as in the previous experiment except that acquisition consisted of 10 trials per day for 3 days, and the subjects were run in squads of 4 subjects each, resulting in a 3–4 min. intertrial interval.

The entire experiment was conducted in 3 segments with only 2 of the subgroups (high or low shock) being represented in each segment. In the order in which they were run, the 3 segments contained (*a*) the subjects in Groups I-FC and C-FC given .5-ma. shock, (*b*) the I-FC subjects and C-FC subjects given .25-ma. shock, and (*c*) the I-BC subjects given .25- and .5-ma. shock. After these subjects were finished the I-NC and C-NC subjects were run.

Results

Two analyses were performed to determine if there was any effect of US intensity on escape-from-fear acquisition. The first analysis involved Groups I-FC and C-FC; a $2 \times 2 \times 3 \times 10$ between–within analysis of variance, including X-ray and US intensity as between factors and days and trials as within factors, was performed on the reciprocal jumping latencies. This analysis revealed no effect of US intensity ($F < 1$) and no interactions with US intensity (largest $F = 1.14$, $df = 18/216$ for Trials $\times$ Days $\times$ US Intensity interaction). The second analysis was a Groups $\times$ Trials $\times$ Days analysis of variance performed on jumping speeds comparing the I-BC subjects given .25- vs. .5-ma. shock. There was no significant difference between these subjects ($F < 1$) and no differences in the rate of acquisition within days ($F < 1$) or trials within days ($F < 1$). Thus, the data are presented in Figure 3 for the 3 main groups collapsed across US intensity, and all subsequent analyses were performed without US intensity as a factor. Figure 3 also shows the performance of the 2 nonconditioning groups.

It can be seen that the performance of Groups I-FC and I-BC began to diverge after the second trial block. Further, Group I-BC initially performed better than the control subjects but then showed a decline in escape speed to the level of the controls. The 2 nonconditioning groups do not appear to differ from each other and appear to be intermediate to the C-FC and I-BC groups. A Groups $\times$ Trials $\times$ Days analysis of variance yielded significant effects of groups ($F = 4.26$, $df = 4/31$, $p < .01$) and Groups $\times$ Days ($F = 3.55$, $df = 8/62$, $p < .005$). Subsequent individual comparisons between groups corroborated what can be seen in Figure 3. Group I-FC was significantly faster than all other groups except

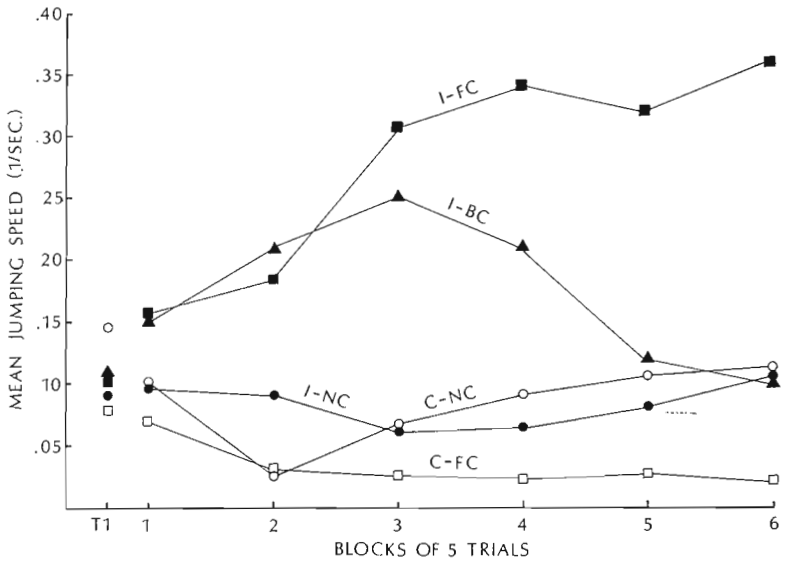

FIGURE 3. Mean reciprocal crossing latencies in escape-from-fear instrumental conditioning trials. (Abbreviations: I-FC = irradiated-forward conditioning; I-BC = irradiated-backward conditioning; I-NC = irradiated-nonshock; C-FC = control-forward conditioning; C-NC = control-nonshock.)

Group I-BC (smallest $F = 8.15$, $df = 1/31$, $p < .01$ for I-FC vs. I-NC; $F = 2.68$, $df = 1/31$ for I-FC vs. I-BC). However, additional comparisons showed that Group I-FC was faster than I-BC on Trial Block 4 ($F = 4.35$, $df = 1/31$, $p < .05$) and Trial Block 6 ($F = 4.97$, $df = 1/31$, $p < .05$), while this difference approached significance on Trial Block 5 ($F = 3.56$, $df = 1/31$, $.05 < p < .10$). In addition, the subjects in Group I-BC escaped significantly faster than those in Group C-FC ($F = 5.20$, $df = 1/31$, $p < .05$). No other comparisons approached a conventional level of significance. Thus, as in Experiment 2, hippocampal granule-cell agenesis facilitated escape-from-fear performance.

Discussion

The apparent failure of the normal rats to acquire the escape-from-fear reaction in Experiment 3 and the depressed performance of these subjects in Experiment 2 was consistent with the range of response level reported by McAllister and McAllister (1962b) for the relatively low US intensities employed in these experiments (.25- or .5-ma.). Moreover, with the shock intensities employed here, strength of fear conditioning did not vary with US intensity. This was true even of the irradiated rats, which were very sensitive to the escape-from-fear procedure. However, the facilitated performance of the irradiated subjects does not appear to be due to a higher baseline activity level or to heightened reactivity to shock in the absence of true conditioning. Moreover, these data clearly suggest that irradiated rats are not deficient in the acquisition of fear responses or the ability to form associations between shock and cues that predict shock. Indeed, they acquired an instrumental reaction reinforced by the reduction of fear.

The performance of the backward-conditioning subjects in Experiment 3 was not unlike results which had been reported for normal rats given backward conditioning and probably reflects the conditioning of fear to less temporally discrete cues, e.g., place and color (McAllister & McAllister, 1962b). The performance of these subjects further attests to the sensitivity of the irradiated rats to fear conditioning under the present procedures.

EXPERIMENT 4

Blanchard and Fial (1968) reported that inescapable shock presentations led to the development of immobility responses which

interfered with the subsequent acquisition of an escape response. While no measures of freezing responses were recorded in the present experiments, hippocampal damage has been shown to disrupt the acquisition of freezing responses (Blanchard, Blanchard, & Fial, 1970). A similar disruption of freezing in hippocampally irradiated rats could account for the facilitated escape-from-fear performance of these subjects. That is, the failure of control rats to acquire the escape-from-fear response may have been due to the acquisition of immobility responses during the fear-conditioning phase, in which the subjects received inescapable shocks.

If the above view is correct, control rats given inescapable shock paired with a CS prior to the introduction of an avoidance contingency would be expected to show impaired acquisition of a one-way avoidance response relative to hippocampally irradiated rats. The present experiment tested this hypothesis.

Method

Subjects and Apparatus

Fifteen irradiated and 16 nonwrapped control subjects were selected as in the previous experiments. The apparatus, CS, and US were the same as in Experiment 1.

Procedure

The pretraining and fear-conditioning procedures were the same as in Experiment 3. The avoidance contingency was introduced on Day 4. All subjects were run in the same manner as in Experiment 1 except that no extinction trials were given.

This experiment was conducted in 3 segments. In the first segment, 4 control and 4 irradiated rats were run; in the second, 8 control rats were run; and in the third, 8 irradiated rats were run. One control subject was dropped from the experiment for failure to learn after 90 acquisition trials.

Results

The mean number of trials to the acquisition criterion of 9 avoidance responses in a block of 10 trials was 25.8 for the control group and 11.5 for the irradiated group. A 2-tailed Mann-Whitney comparison showed that the control subjects required more trials than the irradiated rats to acquire the avoidance response ($U = 44$, $p < .01$).

Discussion

Although no measures of freezing responses were recorded, these results conform to the expectation that the control rats would acquire some response during the fear-conditioning phase which would interfere with the subsequent acquisition of an avoidance response. Indeed, the control rats in this experiment required more trials to reach the same acquisition criterion as their counterparts in Experiment 1, while the irradiated rats required fewer trials than their counterparts in Experiment 1.

In agreement with Blanchard and Blanchard (1969b), then, it appears that inescapable shock leads to the development of responses which are incompatible with running to cues which predict shock. Further, loss of dentate granule cells, like conventional hippocampal damage, disrupts the acquisition of such responses and may even heighten reactivity to cues associated with noxious events as suggested by Lovely et al. (1971).

The finding that CS–US pairings prior to avoidance training retarded acquisition in control rats is not in agreement with some recent experiments showing that this procedure facilitated acquisition of a one-way avoidance response (Anisman & Waller, 1971, 1972). However, Anisman and Waller employed only 10 CS–US pairings compared to 35 in the present experiment. More important, however, is the fact that the facilitating effects of preacquisition CS–US pairings were assessed by contrasting this treatment with a latent inhibition group, i.e., subjects given 10 CS-alone presentations, a treatment which should retard acquisition. Nakamura and Anderson (1968) found that conditioned emotional response pretraining did markedly reduce responding in an active-avoidance situation. Moreover, Blanchard and Blanchard (1969a) observed freezing responses following a single inescapable shock. This pattern of results suggests that CS–US pairings prior to avoidance training should disrupt acquisition.

General Discussion

Exposure of a brain region to 1–2 doses of low-level X ray results in selective elimina-

tion of its proliferative cells. If regeneration of the proliferative cells is interfered with by supplementary doses of X ray (Altman, Anderson, & Wright, 1969), the acquisition of the neurons to which these cells give rise may be altogether prevented, resulting in a selective agenesis of these cell constituents. In the cerebellar cortex such a treatment does not have any pathological effects, as determined by electron microscopy (Altman & Anderson, 1972), but the termination of afferents and their synaptic relationships are drastically modified. The consequences of granule-cell agenesis on the organization of the circuitry of the developing hippocampus have yet to be analyzed morphologically and physiologically. However, to the extent that the effects produced in the hippocampus are comparable to the agenesis of interneurons in the cerebellar cortex with a similar schedule of X-irradiation, no pathological changes or direct damage to the pyramidal cells of Ammon's horn are expected.

The behavioral results of the present study, like an earlier one (Bayer et al., 1973), in general support the idea that the behavioral effects of granule-cell agenesis parallel those reported for hippocampal ablation. First, in agreement with several well controlled studies of hippocampal ablation and one-way active-avoidance acquisition, granule-cell loss had no detrimental effect. Second, hippocampal removal or irradiation apparently disrupts immobility responses normally acquired during inescapable shock presentations. One must be careful in drawing conclusions about fearfulness or about learning ability since it may only be the probability of emission of mobility and immobility responses in some shock situations which is affected by hippocampal functioning. It may be that normal rats appear to be better learners or more fearful in situations where a tendency to be immobile is the performance measure, e.g. passive avoidance and conditioned emotional response tasks, and only seem less able to learn and less fearful when an immobility tendency interferes with the performance measure, e.g. 2-way active avoidance, escape-from-fear, and avoidance following prior inescapable shock.

In other words, the above tasks reveal performance differences which may not necessarily reflect fear or learning. There does not seem to be any precedent for assuming that either freezing or running is the dominant response in the presence of fear-eliciting stimuli or that one is indicative of a greater fear than the other. Until the relationship between a well defined concept of fear in rats and the tendency to run or remain immobile has been clarified, it would seem premature to speculate about the effects of hippocampal damage on fear.

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