Lateralized Fascia Dentata Lesion and Blockade of One Hippocampus: Effect on Spatial Memory in Rats

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ABSTRACT: Unilateral blockade of the dorsal hippocampus by tetrodotoxin makes it possible to form lateralized spatial memories, which rapidly transfer to the naive hippocampus when training continues with intact brain. Unilateral X-ray irradiation of newborn rats causes irreversible destruction of granule cells in the ipsilateral fascia dentata (FD). Possible compensation of poor learning in the lesioned hemisphere by commissural transfer of memories from the intact hippocampus was examined in seven rats with unilateral FD lesion, which were first trained in the Morris water maze to asymptotic performance (mean escape latency 6 ± 1 s). Subsequent testing during functional ablation either of the intact or of the lesioned hippocampus by tetrodotoxin revealed escape latencies 35 ± 8 s or 8 ± 1 s, respectively. Probe trial tests during inactivation of the intact and lesioned hippocampus showed target quadrant preference of $32 \pm 2\%$ or $54 \pm 3\%$, respectively. The results indicate: (a) that one intact hippocampus alone can support the water maze task, (b) that no, or only a very weak, memory trace is available in the lesioned hippocampus. It is concluded that the above results are due to the inability of the FD lesioned hippocampus to process the information received from the ipsilateral entorhinal cortex. *Hippocampus* 1998;8:647-650. © 1998 Wiley-Liss, Inc.

KEY WORDS: neonatal X-irradiation; tetrodotoxin; water maze; inter-hemispheric transfer

INTRODUCTION

Interhemispheric transfer (IHT) of lateralized engrams demonstrates that what is learned with one side of the brain can be not only retrieved and used by the other side, but even copied into the naive side. Unilateral functional blockade by tetrodotoxin (TTX) or lidocaine injection into

fimbria-fornix and dorsal hippocampus (Fenton and Bures, 1993, 1994) makes it possible to form lateralized spatial memories which can be rapidly transferred from the trained to the naive hippocampus after the latter recovers (Fenton et al., 1995). It was suggested that the transfer is mediated by interhippocampal commissural pathways activated when the task is performed with intact brain.

Focal X-ray irradiation of neonatal rats makes it possible to induce selective and massive reduction in the number of granule cells of the fascia dentata (Bayer and Altman, 1975). A single high dose of bilateral X-ray irradiation applied on the first postnatal day elicits a large loss of granule cells manifested by severe spatial navigation deficit in adult rats tested in the Morris water maze (Czurko et al., 1997).

Lateralized irradiation destroying the fascia dentata (FD) only in one hemisphere makes it possible to compare place navigation during unilateral functional ablation of the irradiated or of the intact hippocampus.

We asked whether the absence of the spatial learning deficit in the unilaterally irradiated rats can be due not only to the normal function of the intact hippocampus but also to spatial learning supported in the irradiated hippocampus by the commissurally mediated input from the intact hemisphere.

METHODS AND PROCEDURE

For detailed methodological description of the irradiation procedure, testing apparatus, behavioral tests, histology, and data analysis see Czurko et al. (1997). The TTX functional lesioning technique was described by Fenton and Bures (1993). Briefly, seven Long-Evans, newborn

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FIGURE 1. Camera lucida drawing of a representative coronal section showing the effect of unilateral irradiation and the injection sites. In the irradiated hemisphere, the dorsal blade of the dentate gyrus is greatly reduced, whereas the ventral blade is almost completely missing. The idealized drawing of the injection needle and the damage along the injection track indicates that the injections were in the dorsal hippocampus.

male pups received a single high dose of 6 Gy X-ray irradiation, while a lead-shield protected the whole body, except a small part of the cranium above the dorsal hippocampus on one side. The place navigation experiments started when the rats were four months old. The water maze was 200 cm in diameter and the swim paths were recorded with a computerized tracking system. Unilateral hippocampal blockade was achieved by injecting 10 ng of TTX diluted in 1 μ l of saline into the dorsal hippocampus [AP 3, L 2.5, V 3.5 according to Fikova and Marsala (1967)] through chronically implanted guiding tubes. At the end of the behavioral experiments the animals were given an overdose of pentobarbital and prepared for histology. Figure 1 shows a typical section demonstrating unilateral FD reduction and cortical lesions corresponding to the localization of the implanted guiding tubes above the injection sites. The cannulae were all above the hippocampus and there was usually evidence of some damage along the injection track. The effective spread of the drug was estimated to be about 1.4 mm in diameter (Zhuravin and Bures 1991) and the blockade lasted for several hours (Cahill et al. 1987, Zhuravin and Bures 1991).

We assumed that the spatial learning deficit caused by bilateral FD destruction will be absent during examination of the unilaterally irradiated rats because one intact hemisphere can support place learning. In addition, after excessive pretraining, the memory traces implementing place navigation in the Morris water maze, which are presumably formed in the intact hippocampus, can perhaps be transferred into the irradiated hippocampus by transcommissural information flow. If this happens, the presence of both memory traces will be manifested by equal retrieval of the overlearned task, tested during functional ablation of either hippocampus.

The aims of this experiment were: (a) to examine the spatial learning ability of the unilaterally irradiated rats; (b) to test the retrieval of an overlearned place navigation task during functional ablation of the irradiated or of the nonirradiated hippocampus,

and (c) to verify the possibility that the missing FD input of the irradiated hippocampus can be replaced by commissural input from the intact hippocampus.

The unilaterally irradiated rats were first pretrained to navigate to the hidden escape platform. Retrieval of this task was then tested during unilateral functional inactivation either of the control or of the irradiated hippocampus.

During the six-day long pretraining phase all rats were trained in 12 daily trials to escape from four starting points to an underwater platform hidden in the southeast quadrant of the pool. The purpose of this phase was to improve the animals' performance to the truly asymptotic level of goal-directed navigation. Retrieval was tested during the four subsequent days: On days 7 and 9, unilateral TTX injections were made into the intact and irradiated hippocampus, respectively. No TTX was applied before retrieval testing on days 8 and 10 in order to demonstrate the reversibility of the preceding TTX treatment. Learning capability of the irradiated and intact hippocampus was examined by repeating the same sequence of tests on days 11 to 14. In addition, immediately following the last trial on days 6, 7, and 9, all animals were started from the north and given a 60-s probe trial with the escape platform removed.

Pretraining Phase (Days 1–6)

All rats improved gradually in their ability to find the hidden platform and after four days of training, reached the asymptotic level of performance (Fig. 2). The average escape latency was 6.5 s on the last day of pretraining. One-way ANOVA of the average latencies of daily blocks revealed a highly significant effect of days [$F(5,30) = 85.55$, $P < 0.0001$] indicating fast learning. Tukey's post hoc comparisons showed no significant differences ($P > 0.05$) among the final three days of pretraining. These data demonstrate that unilaterally irradiated rats can master the task as well as control animals, if they are allowed to use both hemispheres.

First Retrieval Tests (Day 7–10)

The first injection of TTX into the nonirradiated hippocampus (1st TTX-CO) severely impaired the rats' performance (Figure 2). Most rats just swam along the wall and only a few managed to swim into the pool and find the platform by chance. The average escape latency on this day (day 7) was 45 s. The first injection of TTX into the irradiated hippocampus (1st TTX-IR) caused a less severe navigation impairment. All rats searched in the target quadrant and eventually found the platform after an average 21-s escape latency (day 9). One-way ANOVA with repeated measures comparing the average latencies of the last pretraining day (day 6) with the first TTX-CO day (day 7) and TTX-IR day (day 9), as well with the second TTX-CO day (day 11) and TTX-IR day (day 13) revealed a significant effect of the experimental conditions [$F(4,24) = 14.41$, $P < 0.0001$]. Newman-Keuls multiple comparisons showed significant differences between days 6 and 7 ($P < 0.01$), 6 and 9 ($P < 0.05$), as well as 7 and 9 ($P < 0.01$).

Figure 3 shows the results of the probe trials performed at the end of pretraining (day 6), on the first TTX-CO day (day 7), and

FIGURE 2. Left side: acquisition in unilaterally irradiated rats without functional ablation. Right side: retrieval of place navigation during functional inactivation either of the intact (TTX-CO) or of the irradiated (TTX-IR) hippocampus, separated by training days without injection (abscissa). Ordinate: mean escape latencies ($\pm$ SEM). Abscissa: days of training.

on the first TTX-IR day (day 9). The animals had a significant preference for the goal quadrant ($P < 0.01$) on the last day of pretraining and on the first TTX-IR day, but not on the first TTX-CO day ($P > 0.05$). Rats navigating with their irradiated hippocampus did not remember the target location and spent an equal amount of time in each quadrant.

FIGURE 3. Results of the probe trials at the end of the last day of pretraining (without TTX) and at the end of those days when TTX was injected for the first time either into the intact (TTX-CO) or into the irradiated (TTX-IR) hippocampus. Ordinate: Mean percentage of time ($\pm$ SEM) spent in the four quadrants.

Second Retrieval Tests (Days 11–14)

During the second functional blockade of the intact hippocampus by TTX (Fig. 2), average escape latency decreased by 10 s, but the Newman-Keuls test indicated that this difference is not significant. Though some rats improved to less than 20-s average escape latency on the second TTX-CO day (day 11), other rats were still circling along the wall of the tank. On the other hand, TTX injection into the irradiated hippocampus caused no impairment during the second retrieval test (second TTX-IR), and rats reached an average escape latency of 8.2 s on that day. The Newman-Keuls test revealed no significant difference between the second TTX-IR day and the last day of pretraining.

Histological examination showed that X-ray irradiation reduced the total volume of the granule cell layer to 40% of control value. In addition, granule cell density decreased by 45% in the dorsal and by 25 to 30% in the ventral hippocampus. There was also a substantial reduction in the number of hilar neurons in the irradiated hippocampus.

DISCUSSION

The behavioral data indicate that the unilaterally irradiated rats were able to acquire and retrieve place navigation in the Morris water maze with the intact brain as efficiently as nonirradiated rats with similarly implanted cannulae aimed at the hippocampus (Francis Turner et al. 1997). Retrieval tests performed during

functional ablation of the irradiated hippocampus increased escape latency from 5 s to 21 s during the first and to 8 s during the second TTX inactivation, i.e. to values closely analogous to those reported after unilateral TTX injection into the hippocampus of similarly pretrained nonirradiated rats (17 s and 8 s, Fenton and Bures, 1993) which displayed, however, similar impairment caused by blockade of either hippocampus. This is in sharp contrast to the strikingly asymmetric effect of the TTX injection in the irradiated rats. The escape latencies were twice longer during first TTX blockade of the nonirradiated than of the irradiated hippocampus, and this ratio increased to four times during the second TTX injection.

The initial weak impairment of place navigation elicited by the first inactivation of the irradiated hippocampus suggests that it may contribute, in spite of the damage, to the overall performance, e.g. by serving as an auxiliary output available through commissural pathways to the contralateral hippocampus with intact entorhinal input (Olton et al., 1982). Sudden elimination of this additional output probably participating in the procedural aspects of the task may account for the initial deterioration which is, however, rapidly compensated so that already the second blockade does not elicit a significant impairment.

The efficient compensation of the FD lesion is perhaps due to the fact that the lateralized irradiation was applied immediately after birth, and the developing brain had ample opportunity to optimize the available connections of the intact hippocampus with the contralateral hemisphere. The contribution of the neocortex ipsilateral to the lesioned hippocampus could be tested by comparing the place navigation impairment caused by functional blockade of similar cortical areas in the intact and lesioned hemisphere.

The failure of the place navigation engrams acquired by the intact hippocampus to spread into the lesioned hippocampus can be partly due to the limited capability of the latter to process ipsilateral entorhinal input. As shown by Fenton and Bures (1994) effective interhippocampal transfer does not occur spontaneously but only after several transfer trials have been performed with both hemispheres intact, i.e. it requires convergence of the relevant entorhinal and commissural inputs to the naive hippocampus. Hippocampus lacking the FD input cannot profit from the

commissural connections with the trained hippocampus performing the place navigation task, because the commissural signals are not supported by the corresponding entorhinal input. The present study does not answer the question, however, whether FD is necessary for commissural retrieval of information which has been received by the lesioned hippocampus before FD elimination.

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