Neonatal γ-Ray Irradiation Impairs Learning and Memory of an Olfactory Associative Task in Adult Rats

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Abstract

Adult neonatally γ-irradiated rats were compared with control animals in a non-spatial olfactory associative task using two different procedures. Irradiation induced a clear reduction in the total mean area of the olfactory bulbs and hippocampus but not of the orbital prefrontal cortex, diagonal band and cell layers of the entorhinal and piriform cortex. The γ-irradiation affected the granule cells of the olfactory bulbs and differentially altered the cell layers of the subfields of the ammonic fields and the dorsal and ventral blades of the dentate gyrus. In the CA1 ammonic field, dorsal and ventral blades of the dentate gyrus, the cellular loss was significant in comparison with control adult rats. The behavioural data indicated that irradiated rats were deeply disturbed in learning the odour-reward association, and substantially impaired in a reversal experiment, but not in the discrimination of the odours per se. The cellular loss in the olfactory bulbs, in the CA1 and in the ventral blade of the gyrus dentatus was positively correlated with the deficit in behavioural performance. The data support the findings that the hippocampal system participates in the odour-reward associations and facilitates the long-term storage of associations after learning is achieved in this olfactory associative task.

Introduction

Experiments with rats suggest that olfactory stimuli provide better access to higher cognitive processes than stimuli in other sensory modalities, even though controversies still exist (Rapp and Amaral, 1992; Eichenbaum and Otto, 1993; Lynch and Staubli, 1993; Reid and Morris, 1993; Slotnick, 1993). Indeed, odours provide important information about the environment, and many aspects of intraspecific social behaviour in rodents are governed by olfactory signals (Cheal and Spratt, 1971; Eisenberg and Kleiman, 1972). Rats trained with olfactory stimuli can rapidly acquire learning-set performance comparable to that achieved by primates in response to visual stimuli (Slotnick and Katz, 1974).

In a recent study, it was assumed that the link between the limbic system and the olfactory cortex was essential during the learning and storage of odour-reward associations (Roman et al., 1993b). Lesions of the horizontal diagonal band of Broca interrupting the efferent connections between the hippocampus and the piriform cortex (primary olfactory cortex) resulted in a severe deficit of the associations between olfactory and cue rewards. It is hypothesized here that disruption of the intrahippocampal circuitry will impair performance in a similar manner, demonstrating the need for the entire limbic system circuitry for the learning and memory of odour-reward associations. Until now, the role of the hippocampus in memory processing has been assessed using different behavioural tasks with conventional lesion techniques (aspiration, electrolytic, thermocoagulation) and lesions generated by multiple injections of ibotenic acid. However, some of these lesions damage not only the hippocampus but also extrahippocampal structures and their projections (Erschius and Wree, 1991; Jarrard, 1993; Bloc et al., 1994), and their physiological relevance is still being debated since results show both impaired and unimpaired performance (for review see Eichenbaum et al., 1992; Jarrard, 1993). In an attempt to clarify this situation, we studied a rat model in which the hippocampal circuitry was disrupted at birth by γ-irradiation, and addressed the question of whether an odour-reward association was impaired. Indeed the radiosensitivity of neurons at birth depends on their mitotic stage (Altman and Das, 1965). In the rat hippocampus, the majority of the granule cells, as well as their mossy fibres, develop postnatally, whereas other cell groups (e.g. pyramidal cells and interneurons) and afferent inputs are generated before birth (Hicks and d'Amato, 1966; Bayer and Altman, 1975; Represa et al., 1991). Thus, neonatal irradiation allows one to obtain adult rats whose hippocampal circuitry is irreversibly disrupted. However, as reported by Halasz (1986), postnatal irradiation of rats affects also the granular cell layer of the olfactory bulb. Taking into account a possible deficit created by a dysfunctioning of the olfactory system, two behavioural experiments were conducted. The first experiment was designed to determine potential impairments in learning and memory created by neonatal γ-irradiation. In the second experiment, similar irradiated rats underwent the same olfactory...
training, but with a procedure in which the hippocampal system does not seem to be involved. This last experiment was aimed at finding out whether the irradiated rats were able to discriminate between the odours like the control rats. The present paper addresses the issue of whether the entire hippocampal circuitry is critical for the learning and memory of odour-reward associations.

Materials and methods

Subjects

Eighteen newborn male Wistar rats (mixed from different litters) from the breeding stock of the Laboratoire de Neurobiologie et Physiopathologie du Développement, University of Provence, were irradiated at Gy (600 rad) with a cobalt bomb (Centre d’Etudes Nucléaires, Fontenay-aux-Roses, France). Sixteen non-irradiated Wistar rats born on the same day (littermates) were used as controls. They were housed in an environmentally controlled vivarium on a 12 h light–12 h darkness cycle with lights on at 06.30 h; food and water were provided ad libitum. When, a few weeks later, they reached a weight ≥300 g, 3 days before the first training session, the animals were handled and weighed daily. All subjects were deprived of water 48 h before the first training session. On the following day, the rats were given water ad libitum for 30 min per day at 18.30 h.

Apparatus

The olfactory training apparatus was a rectangular box made of wire mesh (30 × 30 × 50 cm). A conical odour port (1.5 cm in diameter, 0.5 cm above the floor) was drilled horizontally through a triangular wedge of plexiglass, which was mounted in one corner of the cage. A circular (1 cm diameter) water port in the shape of a well was placed directly above the odour port; water port responses were monitored by a photoelectric circuit. Two flashlight bulbs, which could be turned on and off as conditions required, were placed outside the cage, one on each side of the odour and water ports, 10 cm above the floor.

Individual odours were delivered by forcing clean air (0.7 bars) through one of two 1000 ml Erlenmeyer flasks that contained 500 ml of water mixed (2 parts per thousand) with one of the chemicals or natural odorants (Sanofi Bio-Industries, Grasse, France). A ‘positive’ odour (S+) was paired with a ‘negative’ odour (S-) as follows: jasmine–strawberry, lily of the valley–vanilla, violet–pawpaw, rose–lemon, coconut–peach, and apricot–hazelnut, in that order. Non-odorized air was delivered by passing air through a flask that contained only water. Odorized and clean air streams were passed individually through tubes, which were put through the back of the sound-attenuating chamber and attached to the odour port. Water was delivered using a gravity feed system and was passed through a valve which, when opened, allowed 0.1 ml to be released into the water port. All experiments were conducted with an irradiated animal in one cage and a control animal in the other to allow training under the same conditions at the same time. All procedural and behavioural events were controlled and recorded by microcomputers (Commodore).

Experiment 1

Behavioural procedures

Phase 1: basic odour–reward associations. Animals were trained to make two odour–reward associations. Each of the two odours had to be associated with a specific reward; one odour was arbitrarily designated as positive and the other as negative, using a successive ‘go–no go’ paradigm.

Rats had to approach the odour and water ports to interrupt the light beam in front of them only when the positive odour was ejected for 10 s. Response to the odour designated as positive was rewarded with 0.1 ml of water. Response to the odour designated as negative resulted in a 10 s presentation of a non-aversive light. The water was only distributed with the response to the positive odour.

Individual trials were presented in a quasi-random fashion and one of the odours was presented for at most 10 s. A new trial was started only when the subject left the corner; if not the trial was delayed for 15 s after the termination of either water or light delivery or no response. A daily session was made up of 60 trials with an intertrial interval of 15 s. Animals were tested every day between 08.00 and 14.00 h.

Correct responses were ‘Go’ for the positive odour and ‘No Go’ for the negative odour. Incorrect responses were Go for the negative odour and No Go for the positive odour. The number of correct responses for both positive and negative odours was expressed as a percentage of the total number of odour presentations, thereby providing a global estimate of performance for the two groups. Training continued until a criterion of 80% correct responses for two consecutive sessions was reached by the control group. Latencies for positive (S+) and negative (S-) odours were recorded, representing the time elapsed between the beginning of a trial and its end when the rat responded to the odour; if a rat did not respond, then a latency of 10 s was scored. In addition, as the subject could delay the trials by responding during the intertrial interval, the cumulative time was considered. The cumulative time was the number of seconds added to the fixed 15 s intertrial interval divided by the number of intertrial intervals in the experiment (59). The latencies and the number of errors were analysed across sessions by blocks of ten trials.

Phase 2: new–previous olfactory training and reversal. Eight days after phase 1, the animals were given new–previous olfactory training. Each day, animals were trained on a new odour pair with only ten trials per session. Then they were tested for retention 24 h later in ten trials. After the first session of this paradigm, in which the rats learned the first new odour pair, they were tested on each subsequent day on the previous trained odour pair before being trained on a new odour pair. During this training, five odour pairs were used. After the retention of the fifth odour pair, the training was pursued until the 60 trials had elapsed. Twenty-four hours later animals were submitted to a reversal session. This reversal session consisted of reversing the valence of the previously learned odours. In this second phase, only the latencies were recorded.

Experiment 2

Behavioural procedure

The procedure used in this experiment was exactly the same as that used in experiment 1, except that the intertrial interval was decreased from 15 to 5 s.

Histological procedures

After training, all of the rats’ brains were dissected and frozen in isopentane (−60°C). Sections (20 μm) were cut in the frontal plane from the olfactory bulbs to the ventral part of the hippocampus with a cryostat (−17°C). The brains of the ten irradiated and three non-irradiated rats in experiment 1 and those of eight irradiated and three non-irradiated rats in experiment 2 were analysed. For the irradiated
rats, only unilateral sections were analysed, half from one side and half from the other side from different animals and that for both irradiated groups. For both groups of control animals, bilateral sections were used to make the comparison with the irradiated brains. The measure of the areas (in mm²), observed with a light microscope, was quantified with a computer-assisted image analyser (Biolab).

**Phase 1: Olfactory bulbs, orbital prefrontal cortex, diagonal band, piriform cortex, entorhinal cortex, and hippocampus**

Olfactory bulbs and orbital prefrontal cortex. The unilateral olfactory bulbs and orbital prefrontal cortex of the irradiated rats were compared with the bilateral olfactory bulbs of non-irradiated rats. The rostro-caudal coordinate with respect to bregma was +5.2 mm (Paxinos and Watson, 1986).

Diagonal band. The areas of the diagonal band were measured at the bregma 0.0 mm level.

Entorhinal cortex. Like the piriform cortex, the entire entorhinal cortex was difficult to delineate, so only the area of the cell layers was considered in the histological analysis. The −5.2 mm rostro-caudal level was used to make the comparison between the irradiated and non-irradiated brains.

Piriform cortex. To compare irradiated and non-irradiated brains, the surfaces of the cell layers in the piriform cortex were analysed at six rostro-caudal levels (−1.8, −2.0, −2.3, −3.0, −3.6, −4.0 mm).

Hippocampus. The hippocampi including the dentate gyrus were analysed at seven different rostro-caudal levels (−1.8, −2.0, −2.3, −3.0, −3.6, −4.0, −5.0 mm).

**Phase 2: Olfactory bulb granular ammonic and dentate gyrus cell layers**

Olfactory bulb granular cell layer. This cell layer was quantified at the rostro-caudal coordinate +5.2 mm. The limits of the different hippocampus cell layers, as suggested by Schwerdtfeger (1984), are shown on the left side of Figure 6E.

Ammonic cell layers. The cell layers of the hippocampus were examined independently. The CA1 cell layer was clearly distinguishable only from the rostro-caudal coordinate −2.3 mm. Thus, only five levels were considered (−2.3, −3.0, −3.6, −4.0, −5.0 mm). For a similar reason, the same levels were kept for layers CA3a and c. Cell layer CA3b allowed a more rostral analysis, and seven levels were measured (−1.8, −2.0, −2.3, −3.0, −3.6, −4.0, −5.0 mm).

Dentate gyrus cell layers. Two cell layers could be distinguished in the dentate gyrus—the dorsal cell layer or dorsal blade and the ventral cell layer or ventral blade. The dorsal blade was more posterior and was clearly distinguishable from the rostro-caudal coordinate (−2.3 mm). Five levels were analysed (−2.3, −3.0, −3.6, −4.0, −5.0 mm). The ventral blade was distinguishable from the start of the hippocampus, and the same levels as for the CA3b cell layer were analysed.

**Data analysis**

Statistical analysis was performed with the SPSS/PC+ Statistics 4.0 software (SPSS Inc, Chicago, IL). Data were analysed by repeated MANOVA measures for behavioural as well as histological data. Subsequent analyses of variance were computed for each session and each rostro-caudal coordinate.

**Results**

Two irradiated rats were irradiated mainly unilaterally in both experiments. Thus, all the histological and behavioural analyses excluded these two irradiated animals.

![Figure 1](https://via.placeholder.com/150)

**Behavioural results**

**Experiment 1**

Basic odour-reward associations. The percentage of correct responses by irradiated and non-irradiated animals showed that both groups improved their performance across the six sessions (Fig. 1A). However, MANOVAs indicated that there was a significant difference between the performance of the two groups (F1,16 = 23.47; P < 0.001) and even over sessions (group × session interaction, F5,80 = 5.73; P < 0.01). Subsequent ANOVAs revealed that this difference appeared starting from the third session (F1,16 = 7.28; P < 0.02 for session 3; F1,16 = 15.15; P < 0.02 for session 4; F1,16 = 32.1; P < 0.001 for session 5; F1,16 = 17.95, P = 0.001 for session 6). Thus, even if irradiated rats were able to learn the task, their level of
performance was always lower than that of the control group. Furthermore, the irradiated group did not reach the criterion of 80% correct responses in session 6.

Training performance analysed by S+ and S− latencies for the two groups is presented in Figure 1B. A MANOVA computed on these data yielded a significant group difference ($F_{1,18} = 4.28; P < 0.05$), while the improvement over sessions followed an identical learning process (MANOVA, group × session interaction, $F_{5,170} = 1.53$; not significant). Moreover, the differences in performance (S+ versus S− latency) were statistically significant for each group. Indeed, MANOVA revealed a reinforcement × session interaction for the control group ($F_{5,90} = 32.28; P < 0.01$) as well as for the irradiated group ($F_{5,70} = 15.35; P < 0.01$). However, repeated ANOVA measures indicated that a significant difference between S+ and S− values was reached starting from the third session for the control group ($F_{1,18} = 8.42; P = 0.01$), while this difference appeared only by the fourth session for the irradiated group ($F_{1,14} = 11.68; P = 0.01$). The performance differences between the two groups appeared when the S− values rather than the S+ values were considered. Indeed, a MANOVA computed on S+ values did not show a group × session interaction ($F_{5,80} = 0.31$; not significant) while the analysis on the S− indicated a statistical difference ($F_{5,80} = 3.8; P < 0.01$). Subsequent ANOVAs on each session revealed that this difference appeared starting from the third session ($F_{1,16} = 6.02; P < 0.05$).

These results, detected by examining the latency values, indicated that both the irradiated and the control rats responded more rapidly to the S+ stimulus. In fact, the deficit was due to the S− trials, although both groups were able to increase, over the sessions, the time necessary for a negative response to S− stimuli.

The analysis of the cumulative times decreased for both groups over the sessions (Fig. 1C) but they were lower for irradiated rats, without reaching a significant difference in comparison with control rats (MANOVA, group × session interaction, $F_{5,80} = 1.25$) except during the first session, when the irradiated rats were statistically significantly different from the control rats (ANOVA, $F_{1,16} = 5.8; P < 0.05$). This last observation indicates that the irradiated animals complied more accurately with the intertrial interval during which the animals had to avoid responding. The decrease in the cumulative times for both groups indicated that, over the sessions, all the animals learned to leave the corner where the stimuli and reward were distributed in order to respond only when an odour was ejected. This adaptive behaviour was slightly better for the irradiated rats.

A detailed analysis of changes in latencies over the sessions by blocks of ten trials (Fig. 2A, B) indicated a significant difference between S+ and S− values by repeated ANOVA measures from the 14th to the 36th blocks for the control animals ($F_{1,18} = 5.15; P < 0.05$) except for the 15th block ($F_{1,18} = 3.31$; not significant). For the irradiated rats, a significant difference was observed from the 21st block on ($F_{1,14} = 4.70; P = 0.05$) but not on the 23rd and 30th blocks ($F_{1,14} = 4.19$; not significant). Moreover, two other blocks of trials (5th and 8th) were performed significantly differently in S+ and S− trials by the irradiated rats in the opposite way, as they responded faster in the S− than in the S+ trials.

This clear deficit for the irradiated rats may have been due either to impairment of the ability to withhold a prepotent response constantly for the S− stimuli or to a non-constant number of correct responses to the S− stimuli, as all statistical analyses performed on S+ stimuli failed to show a significant difference between the two groups of animals.

**New−previous olfactory training and reversal.** With the new−previous training (Table 1), no significant difference in latency (S+ versus S−) was found for the learning of the five new odour pairs for the control and irradiated rats (MANOVA, group × session interaction, $F_{4,72} = 2.44$, not significant; $F_{4,56} = 2.33$, not significant). In contrast, retention 24 h later of the meaning of these different odour pairs revealed significant discrimination in both groups in the S+ versus S− trials for the control group ($F_{4,72} = 5.75; P < 0.001$) and for the irradiated animals ($F_{4,56} = 3.27; P < 0.05$). Although not statistically significant since few trials were performed for the training and retention sessions, the irradiated rats continuously performed at a lower level than the control rats on retention.

To verify this observation, we decided to train all the animals consistently with an odour pair before submitting them to a reversal session 24 h later. When the rats performed on the retention of the last odour pair (i.e. pair 6) after the tenth trial, the training was pursued until the 60th trial. The latencies (S+ versus S−) performed on the odour pair are presented by blocks of ten trials (Fig. 3).

The results obtained with the irradiated and the control rats demonstrated that both groups remembered the meaning of this odour pair, learned 24 h earlier, and a significant difference appeared from the first block of ten trials (ANOVA, $F_{1,14} = 13.98; P < 0.001$ and $F_{1,18} = 29.63; P < 0.001$ respectively). During the complete session performed on this odour pair no significant difference appeared between the two groups over the six blocks of trials (MANOVA, group × block interaction, $F_{5,170} = 0.31$).

The results obtained 24 h later during the reversal session were

![Fig. 2. Mean latency (±SE) (in s) by block of 10 trials on the first odour pair, across six 60-trial sessions. (A and B) Performance of control and irradiated animals respectively.](image-url)
TABLE 1. Mean latencies (±SE) obtained by irradiated and control rats for ten trials with different odour pairs

<table>
<thead>
<tr>
<th>Area</th>
<th>Cell layer</th>
<th>-1.8</th>
<th>-2</th>
<th>-2.3</th>
<th>-3</th>
<th>-3.6</th>
<th>-4</th>
<th>-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>I</td>
<td>C</td>
<td>I</td>
<td>C</td>
<td>I</td>
<td>C</td>
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<tr>
<td>Ammonic fields</td>
<td>CA1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.175</td>
<td>0.121</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>CA3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.009</td>
<td>0.015</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.447</td>
<td>0.431</td>
<td>0.490</td>
<td>0.370</td>
<td>0.167</td>
<td>0.135</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.025</td>
<td>0.026</td>
<td>0.030</td>
<td>0.027</td>
<td>0.006</td>
<td>0.015</td>
<td>0.008</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>Dorsal blade</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.007</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Ventral blade</td>
<td>0.082</td>
<td>0.053</td>
<td>0.205</td>
<td>0.094</td>
<td>0.212</td>
<td>0.116</td>
<td>0.167</td>
</tr>
</tbody>
</table>

Mean (±SE) total surface (in mm²) of the cell layers at different rostro-caudal levels in coordinates relative to bregma.
C, control rats; I, irradiated rats.

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Fig. 3. Mean latency (±SE) (s) by block of ten trials of the retention of the last odour pair learned (fifth odour pair) and 24 h later on the reversal of the same odour pair. (A and B) Performance of control and irradiated animals respectively.

quite different. A MANOVA computed across the six blocks revealed a reinforcement x session interaction for the control group ($F_{5,90} = 2.81; P < 0.05$) but not for the irradiated group ($F_{5,70} = 2.12; not significant$).

As in the basic odour-reward associations, impairment was observed on the retention of the S- trials over the six blocks of trials ($F_{5,80} = 3.47; P < 0.1$) but not on the retention of the S+ trials ($F_{5,80} = 0.56; not significant$). Repeated ANOVA measures indicated a significant difference between S+ and S- values for the control group during the second block of trials (ANOVA, $F_{1,18} = 5.59; P < 0.5$). Another difference stressed the retention deficit of the irradiated rats as they started to learn the new meaning of the odour pair. Statistically significant progress in learning was observed during the fourth block (ANOVA, $F_{1,14} = 7.96; P = 0.01$) for the irradiated rats.
Impaired learning and memory in irradiated rats

**Experiment 1**

Neonatal γ-ray irradiation effects on olfactory bulbs, orbital prefrontal cortex, diagonal band, piriform cortex, entorhinal cortex and hippocampus (Fig. 5)

The γ-ray irradiation induced a clear reduction in the total mean area of the olfactory bulbs (Fig. 6A, C). There was a significant decrease of 35% in the mean area for the irradiated rats (eight areas) in comparison with the three control rats (six areas) (ANOVA, $F_{1,12} = 40.71; P = 0.001$). The granule cells of the olfactory bulbs were affected by neonatal irradiation: the mean ($\pm$ SE) of the total surface of the cell layer was $0.89 \pm 0.04$ and $0.61 \pm 0.03$ mm$^2$ respectively for the control and irradiated groups. This difference is statistically significant (ANOVA, $F_{1,12} = 27.5; P < 0.01$).

The total mean area of the orbito-prefrontal cortex was slightly smaller (but not always) in irradiated than in control rats, but no statistically significant difference was found (ANOVA, $F_{1,12} = 0.74$).

Neonatal irradiation did not reduce the total mean area of the diagonal band, and no significant difference between the irradiated and control groups at the 0 mm level was observed (ANOVA, $F_{1,12} = 0.65$). Likewise, the mean area of the entorhinal cortex at the -5.2 mm rostro-caudal level was not significantly different (ANOVA: $F_{1,12} = 1.43$).

The mean areas of the cell layers of the piriform cortex were not different throughout the rostro-caudal levels in irradiated animals com-

**Histological observations**

Experiment 1

**Fig. 5.** Effects of neonatal γ-ray irradiation on the size of the olfactory bulb, piriform cortex and hippocampus. Mean ($\pm$SE) of the total surface of the structure for the olfactory bulb and hippocampus, and total surface of the cell layers for the piriform cortex, in control and irradiated rats.

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Fig. 6. Effects of neonatal γ-ray irradiation on the brain from an adult control (A, B) and an irradiated (C, D) rat. Coronal sections of the olfactory bulbs (A, C) at +5.2 mm rostro-caudal to bregma, and of the hippocampus and piriform cortex (B, D) at −1.8 mm; adult control rat. Coronal sections of the same structures at the same rostro-caudal coordinates from adult rats irradiated at birth. (E) Enlargement of the hippocampus at the −3.6 rostro-caudal level in an adult control rat. The left side of this coronal section of Ammon's horn and fascia dentata of the hippocampus shows the limits of the CA1 and CA3 subfields (a, b, c) and the dorsal and ventral blades of the dentate gyrus respectively. (F) Enlargement of the hippocampus at the same rostro-caudal coordinates in an adult rat irradiated at birth. Scale bar, 1 mm.

pared with controls (Fig. 6B, D), as indicated by a MANOVA ($F_{5,60} = 0.36$). Repeated ANOVA measures on different levels did not reveal a significant difference between the two groups of animals ($F_{1,12} = 1.85$). In agreement with previous studies (Represa et al., 1991), γ-ray irradiation induced a reduction in the total mean area of the hippocampus (Fig. 6E, F). A significant difference was observed between the two groups throughout the rostro-caudal levels (MANOVA, $F_{6,72} = 3.59; P = 0.004$). Subsequent ANOVAs indicated a consistent
statistically significant difference between the two groups at each rostro-caudal level analysed ($F_{1,12} = 5.12, P = 0.04$).

In addition, γ-ray irradiation had different effects on the subfields of the ammonic fields and the dorsal and ventral blades of the dentate gyrus; these subfields and blades were measured separately (Table 2). Nevertheless, as these different areas were absent or not sharply distinguishable for CA1, CA3a, CA3c and the dorsal blade of the dentate gyrus, only the last five rostro-caudal levels were considered in the analysis of these subdivisions.

Taking the CA1 field globally, the mean area did not differ significantly throughout the five rostro-caudal levels, as revealed by a MANOVA (group × rostro-caudal level interaction: $F_{5,48} = 0.7$). However, subsequent ANOVAs on different levels revealed a significant difference between the irradiated and control groups at the -2.3,-3 and -3.6 mm levels ($F_{1,12} = 7.80, P = 0.02$). Whatever the rostro-caudal level of the CA3 fields considered, the values for the irradiated rats were not significantly different from those of the control rats for subfields CA3a (MANOVA, $F_{4,48} = 1.5$), CA3b (MANOVA, $F_{5,57} = 1.57$) and CA3c (MANOVA, $F_{4,48} = 1.71$). Subsequent ANOVAs for these intra-field subdivisions were not statistically significant.

Both dorsal and ventral blades of the dentate gyrus were strongly affected by the neonatal γ-ray irradiation. MANOVA performed on the mean areas at different levels of both blades revealed a highly significant difference (group × rostro-caudal interaction: $F_{4,48} = 10.78, P < 0.001$ and $F_{6,72} = 13.26, P < 0.001$).

Moreover, separate ANOVAs revealed a significant difference, whatever the rostro-caudal level considered, for both the dorsal blade ($F_{1,12} = 7.90, P < 0.02$) and the ventral blade ($F_{1,12} = 16.30, P < 0.002$). However, the ventral blade of the dentate area was always much more affected by irradiation than the dorsal blade, and the molecular layer shrank considerably on the irradiated side.

**Experiment 2**

As suspected, the statistical analysis of the irradiated rats revealed the same specific cellular losses observed for the irradiated rats in the first experiment.

The mean area of the olfactory bulbs of the irradiated rats (six areas) in comparison with the three control rats (six areas) was significantly smaller (ANOVA, $F_{1,10} = 32.92, P < 0.01$) and no significant difference was observed between the irradiated rats (Ir1) used in the first experiment and the irradiated rats (Ir2) used in the second experiment (ANOVA, $F_{1,12} = 3.66$).

No significant differences were observed either for the total mean area of the orbito-prefrontal cortex and diagonal band (ANOVA, $F_{1,10} = 1.37$ and $F_{1,10} = 0.007$ respectively) and between Ir1 and Ir2 ($F_{1,12} = 0.68$ and $F_{1,12} = 0.40$).

Likewise, the total mean area of the cell layers was not significantly affected in the piriform cortex at the six rostro-caudal levels considered (MANOVA, $F_{5,50} = 0.52$) and in the entorhinal cortex at the -5.2 mm rostro-caudal level (ANOVA, $F_{1,10} = 0.15$), and no significant differences were observed between Ir1 and Ir2 (MANOVA, $F_{5,60} = 0.54$; ANOVA, $F_{1,12} = 0.58$).

As for Ir1, the hippocampus was substantially affected by neonatal γ-ray irradiation and a significant difference was observed between the irradiated rats and their respective controls at all the rostro-caudal levels (MANOVA, $F_{5,60} = 12.15; P < 0.01$). Subsequent ANOVAs indicated a consistent decrease in the total mean area at each rostro-caudal level analysed ($F_{1,10} = 6.91, P < 0.025$). The comparison between the Ir1 and Ir2 groups did not reveal a significant difference at any of the rostro-caudal levels (MANOVA, $F_{5,72} = 0.54$).

Moreover, the effects of irradiation on the subfields of the ammonic fields and dorsal and ventral blades of the dentate gyrus were similar to those observed in the Ir1 rats.

The mean area of the CA1 field at all five rostro-caudal levels did not differ significantly from the mean control area (MANOVA $F_{4,49} = 0.8$), but, as with Ir1, a significant difference was found at the -2.3, -3 and -3.6 levels ($F_{1,12} = 6.91, P < 0.02$), and no significant difference was observed between the Ir1 and Ir2 groups (MANOVA, $F_{4,48} = 0.67$).

The subdivisions of the CA3 fields, whatever the rostro-caudal level considered, were not affected by the irradiation, and the corresponding analysis did not reveal a statistically significant difference (MANOVA, CA3a, $F_{4,40} = 1.13$; CA3b, $F_{6,60} = 1.42$; CA3c, $F_{4,40} = 1.49$). The subsequent ANOVAs performed between Ir2 and their respective controls and between Ir1 and Ir2 were not statistically significant.

The irradiation had similar effects on the dorsal and ventral blades of the dentate gyrus in the Ir1 and Ir2 groups. Both MANOVAs were statistically significant (group × rostro-caudal interaction, $F_{4,40} = 10.59, P < 0.001$; $F_{6,60} = 10.22, P < 0.001$).

Again, the cellular loss was greater for the ventral blade of the dentate area than for the dorsal blade, and separate ANOVAs were statistically significant whatever the rostro-caudal level considered, for both the ventral blade ($F_{1,10} = 22.64; P < 0.001$) and the dorsal blade ($F_{1,10} = 6.15; P < 0.03$). No significant differences were observed between the Ir1 and Ir2 groups on either the dorsal or the ventral blade (MANOVA, $F_{4,48} = 0.19$ and $F_{6,72} = 0.15$ respectively).

**Table 2. Effects of neonatal γ-ray irradiation on the surface of the ammonic fields of the hippocampus and blades of the dentate gyrus**

<table>
<thead>
<tr>
<th>Odour pair</th>
<th>Learning session</th>
<th>Retention session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S^+$</td>
<td>$S^-$</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
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<tr>
<td>C</td>
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<tr>
<td>C</td>
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<td>I</td>
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</tbody>
</table>

Each odour pair was used for one learning session and 24 h later, for one retention session.

C, control; I, irradiated.

$S^+$, latency for the positive odours; $S^-$, latency for negative odours.
learning. When they were submitted to a new−previous paradigm only a small non−significant deficit was observed, while substantial impairment was found in retention during reversal training. In experiment 2, when the intertrial interval was decreased from 15 to 5 s, the irradiated rats exhibited similar performance to the control rats.

As observed in behavioural phase 1 of experiment 1, irradiated rats started to make the odour−reward associations on the fourth session, while the control animals made them by the third session. This impairment can be explained by the higher number of errors made by the irradiated rats over the sessions. Moreover, when the irradiated rats performed as well as the controls in the learning of an odour pair at the end of phase 2 of behavioural experiment 1, the beginning of reversal facilitation of the original cue associations or facilitation of the discrimination learning was observed for these animals 24 h later. Similar results with olfactory tasks have already been reported in studies that used rats with hippocampal damage (Staubli et al., 1984; Eichenbaum et al., 1986). Nevertheless, as the olfactory bulbs were sensitive to neonatal irradiation, the impairment observed in our experiments could be due to dysfunctioning of the olfactory system itself, and a deficit in the coding of the olfactory stimulus could not be excluded. In this case, it would not be surprising to find that the irradiated animals were deeply impaired in their ability to discriminate the odours. Four sets of data refute this hypothesis. First, all of the irradiated rats were able to discriminate in the learning of the first odour pair in phase 1 of experiment 1. Second, as demonstrated by the decline in the cumulative times (Fig. 1C), the irradiated rats learned even faster than the control animals to respond only when the odours were distributed. Third, when both groups performed at the same level during the last session of the new−previous olfactory training, 24 h later during a reversal session, only the irradiated group began to forget the associations, which cannot be explained by olfactory impairment. Fourth, when the hippocampal system is not required to solve the olfactory discrimination problem, as demonstrated by Eichenbaum et al. (1986) using a task similar to ours, no deficit in comparison with control rats was observed. This last observation indicated that, when using simple, non−complex odours, the efficacy of the olfactory system is sufficient to perform the discrimination like the control rats. Thus, even if a quantitative anatomical difference exists in the olfactory system between the irradiated animals and the control rats, no deficit due to an olfactory dysfunction could be observed using the simple paradigms described above. Finally, as neonatal irradiation affected the development of the hippocampus (including the dentate gyrus), it appears legitimate to account for the deficit observed in the irradiated rats as dysfunctioning of hippocampal circuitry.

Among the subdivisions of the entorhinal areas, our correlation studies indicate that the rostral part of the ventral blade of the dentate gyrus might be particularly involved in olfactory tasks. Recent anatomical data (for review see Witter et al., 1989; Witter, 1993) have shown that the olfactory input of the telencephalon, in particular the olfactory bulb, the anterior olfactory nucleus and the piriform cortex, terminates throughout most of the rostro−caudal extent of both lateral and medial entorhinal areas (Heimer, 1968; Haberly and Price, 1978; Kosel et al., 1981; Room et al., 1984). In addition, electrophysiological data demonstrate that olfactory responses recorded from the hippocampal formation are probably relayed selectively through the lateral entorhinal area, since destruction of this area abolishes the responses in the hippocampal formation (Wilson and Steward, 1978). Finally, the main efference of the medial part of the lateral entorhinal area targets the infrapyramidal blade of the dentate gyrus (Witter, 1993). Thus the synaptic connections between the lateral entorhinal cortex, in which olfactory information is relayed, and the hippocampus cannot be made as the cells of the ventral blade of the olfactory bulb granular cell layer and the different cellular loss. These correlations pertained to the olfactory bulb, CA1 and the dorsal and ventral blades of the dentate gyrus. As indicated in Figure 7, no correlation was found for the CA3.

**Correlation between anatomical and behavioural data**

To examine the relationship between the effect of the irradiation on the cellular loss and the impairment of behavioural performance observed in experiment 1, individual data from the eight irradiated and the three control rats were analysed. As a clear deficit appeared in session 6 of the basic odour−reward training, the correlations were tested between this session and the olfactory bulb granular cell layer and the different ammonic and dentate gyrus cell layers. Only three significant correlations were found between the percentage of correct responses and cellular loss. These correlations pertained to the olfactory bulb, CA1 and the dorsal and ventral blades of the dentate gyrus. As indicated in Figure 7, no correlation was found for the CA3.

**Discussion**

Using a basic odour−reward paradigm, we showed in experiment 1 that irradiated rats were deeply disturbed in odour−reward association
dentate gyrus are destroyed by the neonatal irradiation. Nevertheless, since olfactory information can partially reach the hippocampus via the medial entorhinal cortex and via residual parts of the ventral blade of the dentate gyrus, irradiated rats were deeply impaired but still able to perform this olfactory task.

Chronologically, the first significant difference from control animals was observed by analysing the cumulative time, which was shorter mainly during the first session of experiment 1. However, no difference was observed between the latencies of the two groups during the first session, and these shorter cumulative times did not lead to a greater number of errors. One explanation may be found in the fact that, at least during the first session, the irradiated rats were more active than the controls. Thus, the occurrence of trials was less delayed in irradiated rats than in controls since they did not stay in the corner where the rewards were distributed. Similar observations were made in rats with ibotenate hippocampal lesions, but in their home cage (Jarraud, 1993). However, the irradiated group performed similarly on S+ in all the experiments in comparison with control animals, and shorter latencies on S+ were never exhibited by the irradiated animals over the six training sessions. In contrast, using the concepts and experimental procedures of scalar timing theory, Meck et al. (1987) found that lesions of the basal forebrain affected timing ability in the performance of a variety of temporal discriminations, although nearly complete recovery was observed with extended training. In our free operant task, although there was no demand for timing per se, the ability to regulate temporal criteria was dependent on the behaviour of the rat. The animals had to await a trial onset signal; if they did not, responding during the intertrial interval delayed the next trial by a cumulative amount of time. Thus, even if irradiated rats were affected in their timing ability, similar performance was not reached in experiment 1 once they had recovered normal timing abilities at the end of the basic behavioural training. Moreover, as the total intertrial interval (intertrial interval + cumulative time) was shorter for the irradiated rats in experiment 1, they should have exhibited greater ease in making consistent discriminations, as in experiment 2, which would have been in agreement with Winocur’s (1984, 1985) observations. In this study, a timing dysfunction of the hippocampal circuitry cannot comprehensively explain the observed deficit.

The second difference, which was a clear deficit in learning and remembering the cue–reward associations, was observed in phase 1 of behavioural experiment 1 in the irradiated rats. This deficit seemed to be due to a larger number of errors made by the irradiated animals in comparison with the control animals when both groups started to make significant discriminations on S+ and S− trials.

These results are consistent with the theory of Rawlins (1985), in which the hippocampus allowed the formation of temporally discontinuous associations. According to this hypothesis the hippocampus functions as a high-capacity, intermediate-term memory buffer, and is critically involved during the intermediate stages of learning when associations are formed between temporally discontinuous events. The length of the critical interval is thought to vary depending on factors such as task complexity and the amount of within-task interference, or the cumulative times in our experiments.

Other experiments that used olfactory tasks have demonstrated facilitation of discriminative learning after hippocampal damage during reversal of an already learned discrimination (Staubli et al., 1984; Eichenbaum et al., 1986). In our study, although no facilitation was observed in phase 2 of behavioural experiment 1, irradiated rats exhibited a beginning of reversal facilitation of the original cue associations, or a facilitation of discrimination learning during the reversal session. Thus, our data support the findings that the hippocampal system participates in associations between olfactory cues and reward and facilitates the long-term storage of associations after learning has been accomplished (Lynch, 1986; Eichenbaum et al., 1988).

These functions of the hippocampal circuitry in our olfactory task can be considered in the light of recent proposals. After observing monkeys and men with hippocampal lesions, Squire (1986) made the distinction between procedural and declarative memory, and argued that procedural memory is spared whereas declarative memory is deeply impaired. Yet Eichenbaum et al. (1986), using a successive odour, go–no go task, indicated that sequential olfactory discrimination problems can be solved either procedurally by an approach versus no approach response or declaratively when a positive odour means that water was forthcoming. Thus, no impairment should be observed in irradiated rats if our task is carried out using a procedural strategy. Because substantial impairment was observed with the protocol used in this olfactory task, modifications of the hippocampal circuitry impaired declarative memory. Thus, in several important respects the deficit observed here in the irradiated rats resembles that found in humans with hippocampal damage (Cohen and Squire, 1980; Squire and Zola-Morgan, 1988).

The involvement of the hippocampus in this odour reward association task will allow us to establish correlations between electrophysiological investigations and behavioural performance. Indeed, single pulses distributed in this tract make it possible to record evoked potentials in the dentate gyrus (Wilson and Steward, 1978). Thus, it would be interesting to use this successive cue olfactory paradigm with electrical mimetic odours as previously described (Roman et al., 1986, 1993a), and then follow the electrophysiological responses of the dentate gyrus in relation to behavioural performance over learning sessions.

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Abbreviations

Ir1 irradiated rats used in experiment 1
Ir2 irradiated rats used in experiment 2

References

Impaired learning and memory in irradiated rats


