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# Changes of the acoustic startle reflex in rats with radiation-induced hippocampal lesion

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**Abstract**. The main question of the study was: to what extent does a neonatal radiation-induced hippocampal lesion lead to emotional changes in adulthood? Acoustic startle response (ASR) was studied in two groups of adult rats. The rats from the first group (14 animals) were exposed to neonatal x-ray irradiation. Their ASR were compared with those from the 10 intact rats that formed a control group. The ASR was tested during two sessions with different illumination of the acoustic chamber. During the first session the rats were tested in the darkness while during the second test the acoustic chamber was illuminated with a 15 W bulb. Irradiation resulted in a significant reduction of granule cells of the hippocampus (about 55%). The lesion resulted in emotional and behavioral changes evidenced by modification of the ASR. The irradiated rats exhibited a significantly increased amplitude of the startle response. In contrast to the light condition, the darkness context caused a decline of the ASR amplitude in the control group and failed to elicit significant changes in the lesioned animals. The results support the hypothesis that hippocampal lesions disrupt motor inhibition.

**Key words:** hippocampus, irradiation, acoustic startle, rat

## INTRODUCTION

Sensitivity of the acoustic startle response to a variety of experimental treatments has made it an important contemporary research tool in studies of brain mechanisms of learning, memory, and emotions as well as motor control. The acoustic startle reflex is mediated by the brainstem structures and the spinal cord. There is, however, extensive modulation of the response by the limbic system. For instance, the amygdala plays a crucial modulatory role in the fear potentiation of the startle response (Yeomans and Pollard 1993, Decker et al. 1995, Wan and Swerdlow 1997). The ASR circuitry receives modulatory inputs *via* the amygdalofugal pathways that pass through the midbrain (Hitchcock and Davis 1987, Hitchcock et al. 1989, Yeomans and Pollard 1993, Koch and Schnitzler 1997). It is also established that the septohippocampal system influences the startle response (Mickley and Ferguson 1989, Melia et al. 1991, Lee and Davis 1997a). The exact role and mechanism of the hippocampal influence is not clear. One of the theories of hippocampal function suggests that it may play a role in response inhibition (for review see: Isaacson and Pribram 1986).

It is commonly believed that fear conditioning during spatial learning depends on the hippocampus. Lesions of this structure disrupt conditioned freezing to the context but not to the explicit cues (McNish et al. 1997). Damage of the CA3 hippocampal area produces hyper-reactivity to sensory stimulation (Handelmann and Olton 1981). Locomotor hyperactivity following hippocampal granule cell removal has also been reported (Mickley and Ferguson 1989, Mickley et al. 1989, Czurko et al. 1997). These data support the hypothesis proposed by Douglas (1967) that hippocampal lesions produce a deficit in behavioral inhibition. Hippocampal lesions may disrupt polymodal associations required for representation of context or may differentially disrupt weak and strong memories (for review see McNish et al. 1997).

Previously described effects of hippocampal lesions on the startle reaction are inconsistent. Some authors (Groves et al. 1974, Leaton 1981) have reported that lesions of the hippocampus do not significantly alter the startle response, while others (Coover and Levine 1972, Mickley et al. 1989) have found increased acoustic startle after surgical lesioning of the hippocampus. In rats with colchicine-induced selective lesions of hippocampal granule cells, the ASR results were also divergent. The acoustic startle amplitude was either significantly reduced (Walsh et al. 1986) or enhanced (Tilson et al. 1987). To clarify this discrepancy the following experiments were performed. The ASR was examined in adult rats with hippocampal lesions caused by neonatal x-ray irradiation. This method results in a selective lesion of the granule cells of the dentate gyrus accompanied by a smaller decrease of the number of hilar neurons but no cell damage in Ammons horn (Czeh et al. 1998).

## METHODS

The research project was approved by the Ethics Committee of the Nencki Institute and was conducted according to the rules for use of laboratory animals in experimental work. Startle response was tested on 24 male, five month old, Long Evans rats divided into two groups: 14 animals with x-ray irradiation induced hippocampal damage and 10 control rats. The rats from the first group were exposed to neonatal x-ray irradiation (600 rads) 6 - 18 h after birth. The irradiation procedures were performed in the University Medical School in Pecs and were described in detail elsewhere (Czurko et al. 1997).

The startle reaction was measured in these rats in darkness and in light. The method of ASR testing was described in detail in our previous papers (Błaszczyk and Tajchert 1996, 1997). Briefly, an acoustic startle test was performed in a ventilated, double-walled sound-attenuating chamber (Coulbourn Instruments, USA.). The rats were placed in small cages (180 x 85 x 90 mm) consisting of a plastic box covered with an aluminum grid. The cages were placed on force recording platforms sensitive to the vertical reaction force of the animal's startle response. The signal from the platform was amplified, rectified and filtered (40 Hz low pass filter) and then sampled at a frequency of 4 kHz. Four hundred millisecond sequences of data, triggered by an acoustic stimulus, were stored on a computer for off-line analysis. The startle parameters of the ASR latency-to-peak and the peak amplitude were determined for each trial by the computer.

Four animals were placed simultaneously into the test chamber and the door was shut. An adaptation period of five minutes was allowed before testing. A sequence of acoustic pulses, separated by a pseudo-random interstimulus interval (ranging between 5 and 60 s), was presented. The acoustic stimuli were wide-band noise pulses, 10-ms in duration with 2 ms rise/fall times and intensity of 110 db SPL. Two experimental sessions

were performed on two successive days between 9 and 11 a.m. During the first session the acoustic pulses were presented in darkness (darkness session) while in the second session the test chamber was illuminated with a weak 15 W lamp located inside (light session). Each animal received a total of 20 stimuli during each session. Parameters of the startle response, amplitude and latency to peak, in two groups of tested rats were analyzed using a three-way ANOVA (Statistica v. 5.0, StatSoft, Inc.) with lesion as an independent measure, and illumination conditions and trials as repeated measures. Between-cell differences were estimated with planned contrasts.

Next, the control and irradiated rats were deeply anesthetized with sodium pentobarbital (Nembutal 8 mg/100 g body weight) and perfused intracardially with 0.1 M phosphate buffer followed by a solution of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were cut on Vibratome at 50 m thick coronal sections. All sets were stained according to the standard Nissl method with Cresyl Violet. Sections were examined with the Microcomputer Imaging Device System to assess the volume of the dentate gyrus of the dorsal hippocampus.

## RESULTS

Neonatal x-ray irradiation resulted in a 55% reduction of the volume of the dentate gyrus (Table I). These results are in good agreement with previously reported data in a similar experimental model (Czeh et al. 1998).

The experiments showed that the ASR was affected by the hippocampal lesion. Startle was elicited in nine out of ten rats in the control group. A freezing tendency was positively classified based upon mean amplitude from the twenty repetitions, namely, when the mean normalized ASR amplitude (amplitude divided by rats body weight) was lesser than 0.3 or when the rat did not respond in 15 out of 20 trials during each session. In the hippocampal group good startle responses were recorded in nine rats in the light condition and in ten rats in the darkness. Statistical analysis was performed on data from 18 subjects (9 subjects for each group). The results of the experiment showed that there was a tendency for an increase of the ASR in subjects with radiation-induced hippocampal damage. Figure 1 shows that the amplitude was significantly higher in lesioned than in control rats ($F_{1,16} = 4.5$, $P<0.05$), and it was lower in darkness than in light ($F_{1,16} = 7.89$, $P<0.02$). Light x lesion interaction was nonsignificant ($F_{1,16} = 0.89$), however, between-cell comparisons with planned contrasts revealed that the lesioned rats startled more vigorously in the illuminated chamber ($P<0.05$), but not while they were tested in darkness. Mean ASR amplitudes (± SD) while the rats were tested in the presence of light was 279.69 ± 108.85 G in the hippocampal, and 186.02 ± 117.25 G in the control groups, respectively. In darkness the lesioned rats responded with a greater amplitude (251.81 ± 120.98 G) in contrast to intact animals which startled with a lower mean amplitude (129.83 ± 101.31 G). A significant effect of test ($F_{19,304} = 3.99$, $P<0.001$) indicates fluctuations of the animals' responding within each trial, rather then a habituation to repeated presentation of the stimulus.

The latency of the ASR in the light condition was 58.28 ± 1.62 ms in normal subjects and 52.73 ± 1.03 ms in the hippocampal rats, respectively. Neither group nor illumination effects were statistically significant. How-

TABLE I

Mean volume (± SD, $n = 9$, $P<0.05$) of the granule cell layers in dorsal part of the dentate gyrus in control and x-ray

| | Dentate gyrus | Control | Irradiated |
|---|---|---|---|
| Total volume in $mm^3$ | left | 0.71 ± 0.06 | 0.37 ± 0.11 |
| | right | 0.75 ± 0.09 | 0.3 ± 0.09 |

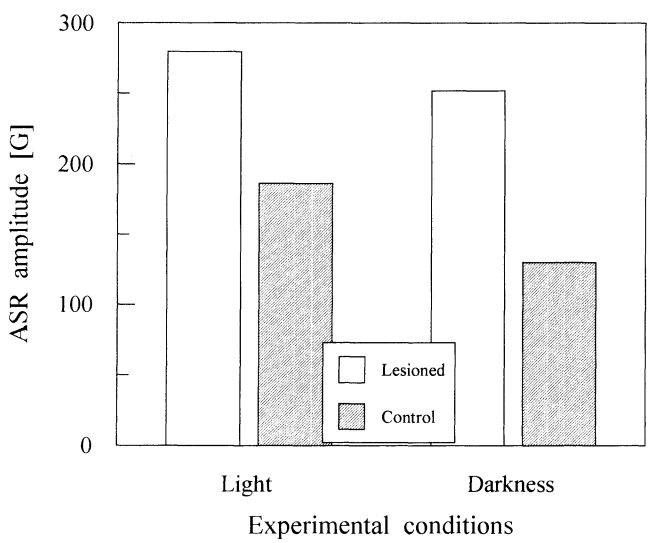


Fig. 1. Mean acoustic startle amplitudes measured in the light and darkness conditions in normal (control) and x-ray irradiated (lesioned) rats.

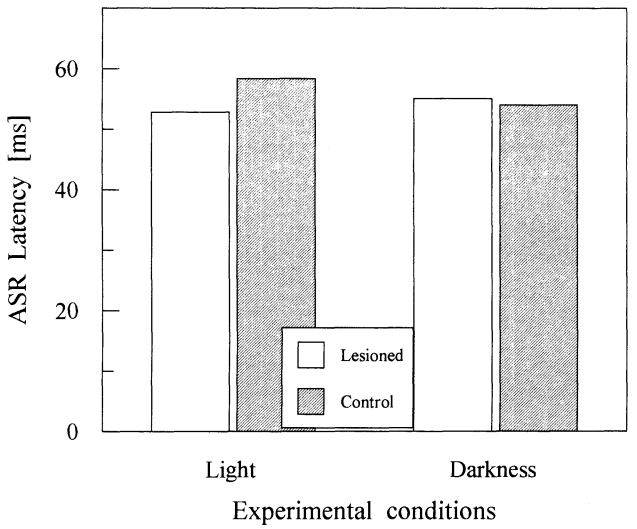


Fig. 2. Mean ASR latencies in control and lesioned rats.

ever, there was a significant group x illumination interaction $F_{1,16} = 4.96$, $P<0.05$. Post hoc analysis (LSD test) revealed significant differences in the ASR latency between hippocampal and control groups in the light condition ($P<0.015$). The results of the analysis are summarized in Fig. 2.

## DISCUSSION

The main results of the present study are the following: firstly, the hippocampal lesion elevates and modifies the acoustic startle response, and secondly, light as opposed to dark conditions increases the ASR in each group.

We report here a potentiation of the ASR in rats with radiation-induced hypoplasia of hippocampal dentate gyrus. This can be explained by an hypothesized inhibitory role of the hippocampus in this behavior. Irradiation of the neonatal rat hippocampus results in reduced proliferation of granule cells in the dentate gyrus (Mickley and Ferguson 1989, Czurko et al. 1997). Our own histological evaluation of the brains of hippocampal rats revealed that postnatal irradiation caused 60% reduction of the volume of dentate gyrus and a 57% decrease of the number of granule cells. Such damage of the hippocampus may additionally cause secondary anatomical changes (Zimmer et al. 1986, Mickley and Ferguson 1989, Czeh et al. 1998). For example, the brain compensates for radiation-induced lesion by stimulating dendritic growth. The most massive changes, however, can be found within the granule cell layer of the dentate gyrus (Mickley and Ferguson 1989).

The anatomical position of the hippocampus makes it a key structure of the limbic system closely involved in such functions as endocrine control, the expression of emotional states as well as in memory and learning. Thus it should be expected that the anatomical changes in the dentate gyrus must elicit a multitude of behavioral symptoms such as behavioral perseveration, locomotor hyperactivity (Teitelbaum and Millner 1963, Means et al. 1971, Mickley et al. 1989), impairment of spatial orientation (Czurko et al. 1997) and deficits in learning. Classical symptoms of hippocampal damage were described as impaired passive avoidance learning (Isaacson and Wickelgren 1962, Blanchard and Fial 1968), facilitated acquisition of active avoidance (Isaacson et al. 1961) and reduced spontaneous alternation in a T-maze (Means et al. 1971). The same effects were observed in rats with radiation-induced lesions of the hippocampal dentate gyrus (Bayer et al. 1973).

Activity of granule cells of the hippocampus inhibits the primary acoustic startle circuitry in the brainstem (Mickley and Fergusson 1989). After impairment of this inhibition the ASR is greater and less likely to habituate. This is in accordance with our results. The irradiated rats were more likely to exhibit a startle reaction and responded with higher amplitude than controls. These animals also failed to habituate to the acoustic stimulus. Rapid habituation of startle in rats with hippocampal damage has been reported (Leaton 1981). However, results of another study (Mickley and Ferguson 1989) suggest that the habituation was not so evident in irradiated rats and the animals exhibited a trend toward potentiation of their startle response. In the present study the effect of habituation was observed only in the control group.

The amplitudes of the startle recorded in the light condition were significantly higher. It is well established that prolonged illumination acts as an unconditioned anxiogenic stimulus which increases startle amplitude in the rats (cf. Walker and Davis 1997a,b).

It appears that corticotropin-releasing hormone (CRH) may play a crucial role in these changes. Hippocampus contains a moderate number of CRH receptors (Chalmers et al. 1995, Yan et al. 1998) which are essential for modulating the startle reflex (Lee and Davis 1997a,b). In particular the dorsal hippocampus is involved in mediating locomotor facilitation effects (Lee and Tsai 1989). The CRH system in the dorsal hippocampus is involved in consolidation and retention of aversive memory in the rats (Lee and Davis 1997a,b).

The ventral hippocampal CRH system could also modulate startle through its projection to the bed nucleus of the stria terminalis in addition to an indirect projection *via* the amygdala (Lee and Davis 1997a,b).

The Coulborns setup measures the latency from the onset of the acoustic pulse to the peak of motor reaction. Thus, the measure includes both the neural delay and mechanical delay. Whereas the former latency subcomponent is relatively short (about 10 ms, Ison et al. 1973) the latter effect (i.e., the time from the onset of muscle activation to the development of the maximum mechanical response measured by the force platform) is mostly responsible for the latency changes. In such defined latency an increase of the ASR amplitude is, theoretically, positively correlated with an increase of the latency. However, additional emotional effects cannot be excluded. In the light condition, elevated fear resulted in agonist-antagonist muscle contraction and thus in the lengthening of the ASR latency. These effects are masked by the mechanical delays and to reveal them the latency must be recalculated. Contributions of the other effects of fear such as postural changes cannot be excluded and require further research.

In conclusion, our results support a hypothesis that the granule cells of the hippocampal dentate gyrus exert an inhibitory influence on the acoustic startle response that results in an increase of the ASR amplitude.

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