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Effect of nonaversive and aversive stimulations in infancy on the acoustic startle response in adult rats

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Abstract. Two groups, each consisting of 8 three-week-old rat pups, were exposed to different behavioral treatments with the aim to determine how the experimental manipulation influenced their adult emotional reactivity. Every day for two weeks the pups from the first group received 15 min of handling whereas the animals from the second group were exposed to various aversive stimuli, differing each day. Following these manipulations, after a 5-day break the acoustic startle response (ASR) was measured in all animals and the testing was repeated after another four weeks. Statistical analysis of the data revealed significant differences between groups in the ASR parameters. Surprisingly, in the test which directly followed the treatment the mean ASR amplitudes were similar in both groups. Highly significant differences, however, were observed in the ASR amplitude four weeks later. The rats from the handling group responded with greater amplitudes. The latency of the ASR was significantly shorter in the nonaversive group compared with the second group exposed to aversive stimuli. The results suggest that early exposure to aversive stimulation significantly decreases rats emotional reactivity whereas nonaversive and impoverished stimulation clearly elevates arousal levels when the animal is placed in a novel situation.

Key words: handling, stress, acoustic startle, rats

INTRODUCTION

There are some controversies about the effect of early postnatal behavioral treatment on animal emotionality. It was shown that handling of animals during the first 21 days of life tended to alter emotional reactivity as measured by defecation or exploration, and to decrease fear level in a two-way avoidance task (Eells 1961, Ader 1968, Fernández-Teruel et al. 1991, Nunez et al. 1995).

Levine (1957, 1960) described how different treatments in infancy affect an animals behavior in adulthood. He mentioned handling as a very effective manipulation that allows the reduction of fear in animals tested in an open field. Handled rats exhibit attenuated fearfulness in novel environments and show a higher level of exploratory behavior (Costela et al. 1995). Thus the handling in infancy improves behavioral adaptation to the environment, including enhanced adaptive response to stress.

Also, a hypothesis of critical periods in development has been proposed (Scott 1962). It assumed that there were certain limited periods in development during which a particular class of stimuli would have particularly profound effects on adult performance. For this reason most of the studies were limited to the postnatal period (first three weeks of life). In some studies on the critical periods the procedure of handling followed by shock have been employed (Denenberg and Smith 1963). The procedure consisted of removing the pups from the home cage and exposing them to aversive stimuli. In a series of experiments Denenberg (1964) found that the rats handled for the first ten days of life were superior with respect to avoidance learning and survival capacity to ones handled during the next ten days of life. However, the results of the study established that the critical period hypothesis is not sufficient to account for the findings. Despite the heuristic value of the critical period hypothesis it seems that the problem of changes in the adult organism's performance is more complex. We assumed that the changes may not necessarily follow a treatment during first three weeks of life as suggested by earlier studies (Denenberg 1964). Moreover, Denenberg hypothesized that stimulation in infancy reduced emotional reactivity and that this reduction might be a monotonic function of the amount of stimulus input. Therefore, in the present research we have focused on the effects of early (but after the weaning period) experience on the adult rats emotional reactivity. The acoustic startle response was selected as the best measure of this reactivity.

Although the acoustic startle response is a relatively simple behavior, its sensitivity 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 movement control. Acoustic startle reflex circuitry comprises brainstem structures and the spinal cord (Davis et al. 1982). There is however, extensive modulation of the response from the limbic system (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), making this response sensitive to the behavioral and emotional context in which the stimulus appears. It has been well documented so far that by the manipulation of an animal's emotional state, one can change the threshold of the startle reaction (Davis 1986, Bradley et al. 1990). For instance, the paradigm of a so-called "fear potentiated startle" is widely used in pharmacological studies. Thus we hypothesized that the experimental treatments should result in emotional reactivity changes that can be measured by the acoustic startle response.

METHODS

The research was accepted by the Ethics Committee of the Nencki Institute and was conducted according to the rules for use of laboratory animals in experimental work. Data for this study were collected from 16 Möll Wistar male rats from two litters (8 animals each) from the same colony. The animals were housed with free access to food and water throughout the experiment, and not disturbed until weaning. At the age of 21 days, one-half of the pups from each individual litter was randomly assigned to each of two treatment groups (nonaversive and aversive) and housed four rats from the same group.

The animals from the first group were exposed to a nonaversive treatment whereas the other group received aversive stimulation. Every weekday (between 09:00 and 10:00 h) for 10 days the pups in the nonaversive group were handled for 15 min, whereas the pups from the aversive group were treated with various mild stressors at the same time. The type of aversive stimulus was changed every day according to the following schedule: immobilization in a small cylindrical box, exposure to

70 dB (re: 20 µN/m2) white noise, wading in shallow water (temperature 20 °C), shaking in a linen sack, exposure to 2 kHz, 70 dB tone, exposure to a bright light of 500 W lamp, exposure to a barking dog, immobilization by fixing rat's tail to the ground, and finally sinewave tilting (10 deg amplitude) of a home cage. After a 5-day break the acoustic startle response was measured. The ASR test was repeated after four weeks. The rats remained undisturbed in their home cages during the break between ASR tests.

The startle reflex was produced by a strong acoustic pulse (frequency 6.9 kHz; 6 ms pulse duration and 2 ms the rise time; 120 dB pulse intensity) presented against the background of 70 db white noise. Testing was performed in a ventilated double-wall sound attenuating chamber (Coulbourn Instruments, USA). The method of ASR testing has been described in detail (Błaszczyk and Tajchert 1996, 1997). Briefly, the rats were tested in small cages (180 x 85 x 90 mm) constructed of plastic and aluminum rods. The cages were placed on a force platform that picked up the force of the animal's startle reaction. An adaptation period of five minutes was allowed before testing. A sequence of 30 trials with the acoustic pulses, separated by a pseudo-random (range between 6 and 40 s) intertrial interval, was presented. The signal from the platform was amplified, rectified and filtered (40 Hz low pass filter). It was then sampled at a frequency of 4 kHz. Four hundred ms long sequences of the data triggered by an acoustic stimulus were stored on a computer disc for off-line analysis. Basic startle parameters were calculated by the computer and analyzed by four-way mixed design ANOVAs (Statistica v. 5.0, StatSoft Inc.), with three independent factors (2(litter) x 2(group) x 30(trial)), and one dependent factor (2(age)). ASR peaks and latencies were analyzed independently.

RESULTS

Our main interest in the experiment was the effect of a single exposure to a shortlasting stressful stimulation of different intensities and modalities on later emotional reactivity while coping with a novel situation related to the acoustic startle testing. The startle response was observed in all rats used in the experiment. The results of this study showed that the animals which were exposed earlier to mild stressors exhibited lower emotional reactivity in a new situation compared to the handled rats. Both groups were tested in the same acoustic chamber without habituation to the test. The adult rats from the nonaversive group startled with a higher amplitude and a shorter latency compared to the animals from the second (aversive) group. Mean values of the ASR amplitudes during the first and the second session are shown in the left panel of Fig. 1. During the first ASR session, which immediately followed the training procedure, mean ASR amplitudes were almost identical in both

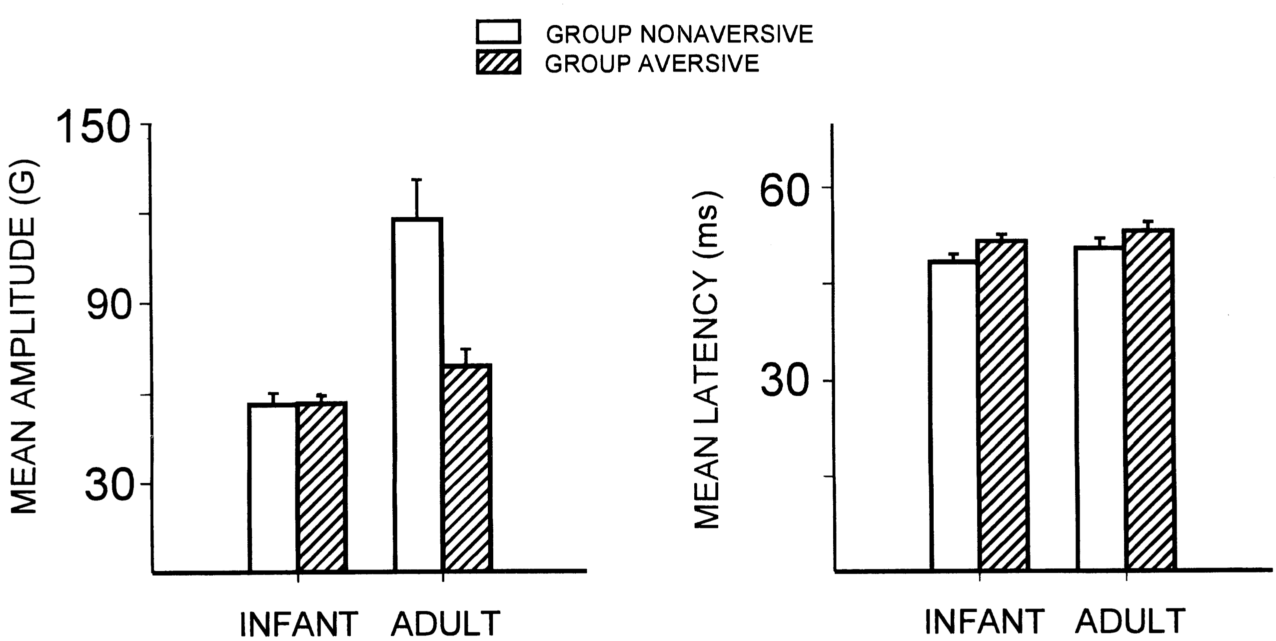

Fig. 1. Mean (± SEM) ASR amplitude and latency in nonaversive and aversive groups measured in infancy and adulthood.

groups (56.35 ± 3.9 G in the handled rats and 56.7 ± 2.6 G in the animals with the aversive treatment). Four weeks later, however, during the second ASR session the difference in the amplitude was evident (117.62 ± 13.4 G in the nonaversive group and 69.02 ± 5.6 G in rats treated with stressors). The results of a four-way ANOVA for the ASR amplitude revealed significant differences between groups ($F_{1,360}$ = 25.33, P<0.0001) and ages ($F_{1,360}$ = 56.65, P<0.0001), as well as a significant group by age interaction ($F_{1,360}$ = 25.43, P<0.0001). No litter effect was found. Moreover, statistical analysis revealed no effect of ASR habituation during each session analyzed for both groups and litters. None of the remaining double, triple or all factor interactions reached the level of significance.

The latency of the startle reaction was generally shorter in the handled group (mean value 49.94 ms) compared with the second group of animals treated with aversive stimulation (51.77 ms). The ASR latency in both groups remained, however, at almost the same level during both test sessions (see the right part of Fig. 1). This was corroborated by the results of a four-way ANOVA which showed only a significant group difference ($F_{1,360}$ = 5.51, P<0.02). No litter, trial, or age effects, nor any double, triple, or all factor interactions were revealed.

DISCUSSION

The hypothesis concerning the relationship between infantile stimulation and decreased emotional reactivity in adulthood was tested here. For this purpose we examined behavioral and physiological effects of exposure to nonaversive and aversive stimulation. We found that a set of short-term aversive stimulation is more effective in alteration of animal behavior than handling alone. The treatment proved its effectiveness even when applied after the weaning period. However, there still remains an open problem of the relative strength of the stress. Since the results from studies employing strong and chronic stressful stimulation showed their destructive effects to the nervous system (Meaney et al. 1988, Mizoguchi et al. 1992, Mohammed et al. 1993, Pham et al. 1997) we used only relatively mild and weak stimuli. Our stressors were comparable to those used by Pfan and coworkers (1997).

A review of the literature leads to the conclusion that in rats stimulation administered between birth and weaning results in a reduction of emotional reactivity. Denenberg (1964) suggested that emotional reactivity might be reduced as a monotonic function of amount of stimulation in infancy. Rats handled during the first three weeks of life exhibited reduced emotional reactivity as measured by exploratory behavior (DeNelsky and Denenberg 1967), they also coped better with a stressful situation and they were superior in learning in shuttle-box avoidance (Ecorihuela et al. 1994, Nunez et al. 1995). On the other hand, the common assumption that every traumatic experience in infancy must inevitably have deleterious effects in later life is clearly not consonant with this study. Our results document that short-period exposure to stress or, to a stressor results in a decrease of emotional reactivity compared with animals which were treated with nonaversive stimulation. This finding is in agreement with results of previous studies by Pham and coworkers (1997). They evidenced that heterotypic mild stressors are very effective in enhancing spatial learning and altering the level of neurotrophic growth factor (NGF) in the hippocampus. Their rats treated with stressors such as exchanging wood shavings between cages, moving the home cages, tail marking, transportation or placing the animal into a strange cage were found to be better in spatial learning in the Morris water maze. To compare with this set our stressors are much stronger but variable and short lasting. Despite the fact that they were presented to older animals the stressors remained very effective in shaping the animals emotional responsiveness. These rats coped very well with a new stressful situation, exhibiting lower arousal.

Thus, the general outcome of this study is that aversive stimulation, even in infancy, results in a decrease of emotional reactivity in adulthood. In the previous research the emotional reactivity of nonaversive stimulation was measured by such divergent procedures as open field testing, consumatory behavior, learning, etc. All these studies showed that different stimulation in infancy have similar consequences in adulthood. The different operational measures of emotional reactivity yield consistent results. The rats handled daily between birth and weaning were significantly more active than nonhandled rats (Denenberg and Morton 1962). A more intriguing fact is that even animals receiving 0.25 mA of current for 3 min daily were more active than nondisturbed controls (Dennenberg and Smith 1963). Schulkin and associates (1994) hypothesized that any change in physical treatment during early stages of development permanently modifies allostasis which is tied to anticipation of needs. Thus the animals behavior is driven by anticipatory and not just reactive responses (Schulkin et al. 1994).

In summary, our results suggest that the early (but post-weaning) exposure to a mild aversive stimulation relatively decreases rats emotional reactivity or arousal compared with the reactivity of animals which were exposed to handling only. Our data clearly support a hypothesis that stimulation (even a mild aversive one) in infancy permanently reduces the rat's emotional reactivity and arousal evoked by new experimental stimuli (Levine 1957, 1960, Barbazanges et al. 1996, Pham et al. 1997). The mechanisms of these changes seem to be well established (Meaney et al. 1988, Schulkin et al. 1994). It has been proven in rats that an early manipulation during the first 3 weeks of an animal's life resulted not only in behavioral alterations as mentioned above but also modified the anatomy and the neuroendocrine function of the brain. For example, Meaney et al. (1988) documented that postnatal handling in rats altered their adrenocortical response to stress and the handling was associated with a permanent increase of glucocorticoid receptor concentration in the hippocampus. Such elevated receptor concentration leads to greater hippocampal sensitivity to glucocorticoids and enhanced negative feedback in the adrenocortical axis. The nonhandled rats, in contrast, showed at all ages elevated glucocorticoid levels in response to stress, which accelerates hippocampal neuron loss and cognitive impairments in aging.

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