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Effects of social rearing conditions on conditioned suppression in rats

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Abstract. Twenty-two rats were reared in standard conditions during the first two months of their life. Then the animals were divided into two groups exposed to different rearing conditions. Twelve animals (Group SO) were housed socially, six animals per cage, and for three weeks they were subjected to sensory stimulation in an enriched environment. The other ten subjects were kept individually (Group IN); one rat per mesh cage, in conditions of relatively impoverished sensory stimulation. In both groups the training of the conditioned emotional response (CER) was performed when animals were three months old. In contrast to IN subjects, the rats subjected to permanent social contacts and reared in the enriched environment (Group SO) revealed almost equally low instrumental response rates in trials with the conditioned stimulus (CS) paired with nociceptive foot-shock (US), and in periods when no CS and/or US were applied. The results suggested that early exposure to an enriched environment caused a later decrease of the animals' capability to differentiate between the aversive CS and cues of the experimental context. This cognitive impairment was probably a secondary effect of fear generalized to the entire experimental situation.

Key words: enriched environment, isolation, CER procedure, rats

INTRODUCTION

Early postnatal environmental conditions in pre- and post-weaning periods exert complex and long-lasting effects on emotional reactivity and learning capabilities in adulthood. The amount of sensory and social stimulation during infancy seems to be a particularly significant factor that provokes several functional and anatomic changes (Denenberg 1964, Mohammed et al. 1993, Rosenzweig and Bennett 1996). For example, it was found that the forebrain of animals that were placed at weaning in an enriched environment was consistently heavier than that of the isolated subjects. According to Cummins et al. (1977) the main factor that determined these developmental changes was arousal caused by exploration and social interactions. The cerebral effects of enriched or impoverished sensory stimulations can be induced not only in the pre- and post-weaning periods but also during adulthood (Bennett et al. 1964, Rozenzweig and Bennett 1996).

Rats reared in an enriched environment performed better in object recognition and object exploration tests than the standard group (Escorihuela et al. 1995). Moreover, these animals habituated faster to an open field, but showed an increased exploratory activity when a new object was placed in that situation (Levine et al. 1967, Mohammed et al. 1990, Mohammed et al. 1993, Zimmermann et al. 2001). Improved learning performance in the Morris maze was also observed (Mohammed et al. 1990). In the Hebb-Williams maze rats that were exposed to pre-weaning enrichment presented a shorter latency and a shorter running time in comparison with littermates exposed to post-weaning enrichment (Smith 1972, Venable et al. 1988). Animals reared in an enriched environment were superior in swimming to a platform in the Morris maze in comparison to animals reared in a standard environment (Kempermann et al. 1997).

Rosen and Schulkin (1998) postulated that early exposure to aversive sensory stimulation was able to establish and to sensitize specific neural fear circuits that were later reactivated in fear-inducing situations in adulthood. It was shown (Woodcock and Richardson 2000) that animals kept in an enriched environment and later exposed to a limited pre-shock period revealed better discrimination of contextual stimuli and had more freezing reactions than rats reared in standard conditions. The early life experience influenced acquisition of defensive instrumental responses. Subjects reared in enriched conditions improved in two-way avoidance performance faster than animals housed in standard conditions (Escorihuela et al. 1994). On the other hand, learning of the two-way avoidance responding was clearly slower in subjects reared in impoverished conditions (Lovely et al. 1972).

Surprisingly, no experimental procedures have been carried out to examine more complex emotional and cognitive mechanisms implicated in defensive conditioning. In our opinion the CER procedure is one of the most appropriate methods to explore both animals' learning capabilities and subtle changes in behavioral strategy. The essence of the CER procedure is an experimental situation that involves two entirely opposite motivational states. One evokes instrumental alimentary responding, a relatively stable level of bar pressing for food in response to contextual stimuli. Fear conditioned to the aversive CS and US is the second motivational state. It evokes suppression of bar presses during the CS action (Walasek et al. 1995). The aim of our study was to test whether social contacts and enriched stimulation of young adult rats influenced the acquisition of the conditioned emotional responses in such a stressful and complex experimental situation.

METHODS

Subjects and group treatment

The data for this study were collected from 22 experimentally naive male hooded rats from the Nencki Institute colony. In the pre-weaning period they were housed with their mothers. At the age of 21 days rats were removed to Plexiglas opaque white home cages with a wire cover (48 x 40 x 25 cm), five or six subjects *per* cage. The rules established by Ethical Committee on Animal Research of the Nencki Institute were strictly followed.

At the age of 60 days rats were divided into two groups: SO (12 subjects) and IN (10 subjects). In Group SO housing conditions were not changed. These rats were reared in two Plexiglas, opaque white cages with a wire cover, six rats *per* cage. During the period of three weeks, once every day, rats were exposed for 45 min to the enriched environment: a big wire mesh cage (85 x 58 x 43 cm) with tunnels, hiding places, toys, attractive food and water in different places of this cage. Group IN was housed individually from the age of 60 days. Each subject was reared in small wire mesh cage (40 x 16 x 25 cm). IN animals were not handled or disturbed with the exception of feeding, weighing and cleaning of cages. Groups SO and IN had free access to water and food in their home cages.

When the animals were three months old 10 days of food deprivation began. The daily portion of food was slightly limited, and the rat body weight was gradually reduced to 85%. Immediately after the food deprivation procedure training of the instrumental alimentary responses was performed. A natural light/dark cycle from external illumination was maintained.

Apparatus

The behavioral training was conducted in eight identical sound- and light-insulated operant chambers (Skinner boxes, 30 x 20 x 26 cm), each with an electrifiable grid floor, a single response bar on one of the walls and a food receptacle under the bar. A pilot light centered at the top of the Skinner box provided illumination in the vicinity of the bar equal to 205 ± 5 lx. A permanent magnetic speaker located below the food receptacle on the same wall as the bar served as the source of an auditory stimulus (wide-band noise of 70 dB, re: 20 $\mu N/m^2$). The subjects' behavior was observed on a TV monitor. The equipment for automatic programming and data recording was located in the adjoining room.

Training procedure

The training performed in the operant chambers was composed of 6 daily sessions of preliminary training, 1 pretest session, and 5 CER training sessions, for both groups of rats. During the preliminary training subjects learned to press the bar for food reinforcement. The first day of this training consisted of initial presentation of 40 "free" pellets on a 1 min variable interval (VI) schedule of reinforcement, followed immediately by a period with a continuous reinforcement of bar presses until 120 food pellets were delivered in a single session. Five subsequent daily sessions lasted 2 h during which the bar presses were reinforced with food according to a 2.5 min VI schedule. The stability of the bar press response was controlled during the last (sixth) session of the preliminary training (Dummy day, D-day).

To examine an unconditioned effect of the auditory stimulus 1 min of white-band noise (70 dB) was presented four times during the following pretest day (P-day). The CER training started the next day. Four 1 min auditory conditioned stimuli (CSi) were given, and each presentation of the CS was terminated with 1 s of scrambled inescapable unconditioned stimulus (US), foot-shock of 2 mA intensity.

Behavioral measures

For each subject during the D-day, P-day and five daily sessions of the CER training the number of bar presses emitted in consecutive 30 s periods were collected and served as the main measure of behavior. The rats' performance was characterized by mean rates of bar presses in 1 min periods: immediately before the CS onset (index A), during the CS action (index B), and immediately after termination of the CS (index C). For each subject and session the numbers of responses in each of those periods were summed. Then mean daily rates of responses were calculated and subjected to statistical tests. A two-factor ANOVA (Statistica v.5.0, StatSoft Inc.) for one repeated measure of the baseline responding (in period A) observed in all training session was used to analyze between-group differences. Further three independent three-factor mixed design ANOVA (type III, Lindquist 1953) were applied to compare mean rate of responses observed in D-day, P-day (two groups x session x A, B, C periods), and the CER training sessions (two groups x five sessions x A, B, C periods).

RESULTS

Rats housed socially and later exposed to the enriched environment (Group SO), when compared with the rats kept in single home cages (Group IN), generally exhibited a lower level of the bar press response during the pre-stimulus intervals (period A), a stronger response suppression to the conditioned stimulus action (period B) and marked response suppression after the stimulus termination with the shock (period C) during the training sessions (Fig. 1). The comparison of the baseline responding (period A) in the subsequent sessions revealed clear between-group differences. A 2 x 7 ANOVA for repeated measures of the bar press rate observed in all consecutive D-day, P-day, and five CER training sessions was done. It yielded a significant group effect ($F_{1,20}$=68.71, P<0.001). The day effect was not significant ($F_{6,120}$=1.57) but a significant groups and days interaction ($F_{6,120}$=2.35, P<0.05) showed that the dynamics of baseline response observed in the subse-

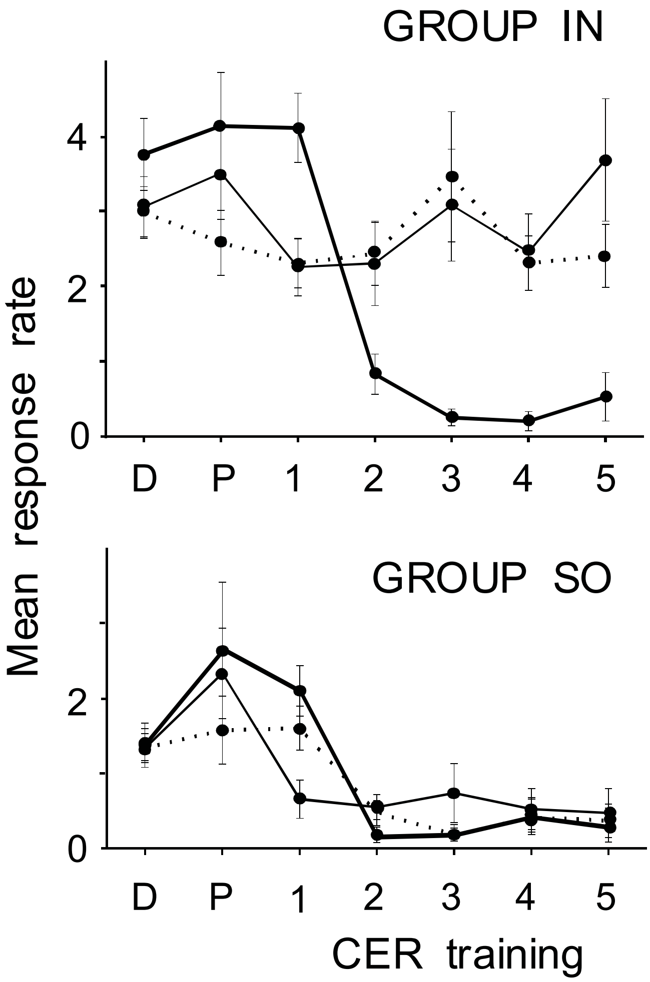

Fig. 1. The mean response rate per minute (± SEM) before (index A, dotted line), during (index B, solid line), and following (index C, thin line) stimulus presentation in D-day, P-day and subsequent days of CER training. The upper panel for Group IN, the lower panel for Group SO.

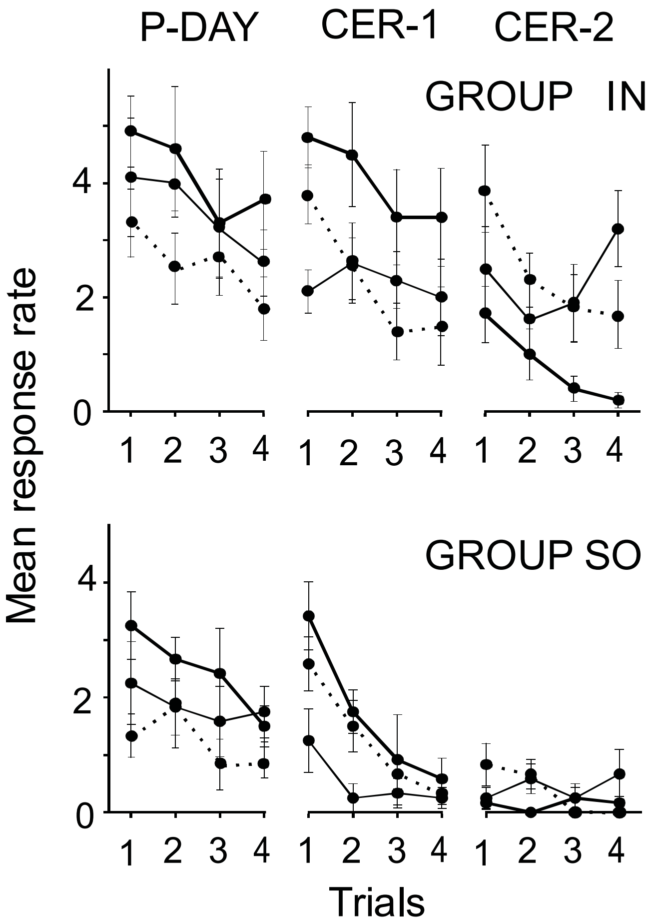

Fig. 2. The mean response rate per minute (± SEM) before (index A, dotted line), during (index B, solid line), and following (index C, thin line) stimulus presentation in the subsequent trials of the P-day, CER-1 and CER-2 sessions. The upper panel for Group IN, the lower panel for Group SO.

quent sessions was different in Groups SO and IN. Further *post hoc* Newman-Keuls tests showed that the level of the bar press responding in Group SO was lower than in Group IN ($P<0.001$), from the second session of the CER training.

A comparison of the number of bar presses observed in groups SO and IN during A, B, C periods in D-day also revealed between-group differences. That was confirmed by a 2 x 1 x 3 ANOVA in which a significant group effect ($F_{1,20}=35.59$, $P<0.001$) was shown. The introduction of the auditory cue in the pretest day (P-day) clearly increased the value of index B in both groups of subjects. A 2 x 1 x 3 ANOVA yielded significant effects of group ($F_{1,20}=6.76$, $P<0.02$) and periods ($F_{2,40}=9.34$, $P<0.001$). Further *post hoc* Newman-Keuls test showed a higher level ($P<0.001$) of bar presses during the auditory stimulus action (B) than before (A) and after its presentation (C), in Groups SO and IN.

During the CER training, each presentation of the auditory CS was paired with a brief electric foot shock. This procedure provoked a strong and long-lasting decrease of the bar presses in Group SO from the beginning of the second training session. In contrast to the individually housed rats (Group IN), dramatic impairment of the differentiation capabilities was shown in SO rats. The number of instrumental responses in Group SO was decreased not only during CS action, but also during the entire experimental situation (periods A and C). A statistical evaluation confirmed these observations. A 2 x 5 x 3 ANOVA for repeated measures of the instrumental responding level in five sessions showed significant effects of group ($F_{1,20}$=34.56, P<0.001), sessions ($F_{4,80}$=11.38, P<0.001) and A, B, C periods ($F_{2,40}$=8.84, P<0.001). Interactions of groups and periods ($F_{2,40}$=9.92, P<0.001), days and periods ($F_{8,160}$=15.11, P<0.001), as well as interaction of all main effects ($F_{8,160}$=11.19, P<0.001) were also significant. In Group IN a low bar press rate was seen only during presentation of the conditioned stimulus (period B). On the other hand, in this group a relatively high baseline level (period A) was revealed, and it gradually increased from the second session toward the end of the CER training (*post hoc* Newman-Keuls test, P<0.001).

In contrast, a strong response suppression was observed in Group SO, especially after the shock termination from the first to the last day of the CER training. Further analysis of the early sessions of CER training (Fig. 2) showed that Group SO clearly differed from Group IN not only in the amount of suppression during the second CS presentation (period B). Between group differences were also seen when index C was compared. A 2 x 4 x 3 ANOVA for repeated measures of the instrumental responding level observed in the first CER training session, during four CS presentations (trials), and in A, B, C periods yielded significant effects of group ($F_{1,20}$=22.40, P<0.001), trials ($F_{3,60}$=12.38, P<0.001), and periods ($F_{2,40}$=26.37, P<0.001). Also significant were groups and trials ($F_{2,40}$=5.40, P<0.01), as well as trials and periods interactions ($F_{6,120}$=2.53, P<0.05). Analogous analysis of the bar presses performed in period C after the second trial of the first CER session revealed a significant group effect ($F_{1,20}$=11.44, P<0.01).

DISCUSSION

The main results of the study can be summarized as follows. In contrast to IN rats, the animals subjected to permanent social contacts and a short-lasting housing in the enriched environment (Group SO) revealed an almost equally low instrumental response rate in trials with CS paired with the nociceptive US (period B), as well as in both A and C periods when no CS and/or US were applied. Moreover, in this group baseline instrumental responding was clearly decreased even in preliminary training before introduction of any conditioned and/or nociceptive stimuli.

The discussion of our results requires consideration of several hypotheses. The first concerns the effect of housing conditions on the stimulus processing mechanisms. In fact, SO rats seemed to be oversensitive to the signaling and emotional properties of the CS and stimuli of the experimental context, in comparison to the IN subjects. It might be supposed that the experimental conditions that were employed in Group SO before the training caused dramatic impairment of the subjects' differentiation capabilities. The conditions of permanent social contacts and housing in the enriched environment constitute an intense sensory stimulation. This could enhance habituation mechanisms and cause a decline in several measures of motivated behavior (see McSweeney and Swindell 1999 for review). For example, many authors showed that the amount of exploration and locomotion (Adlerstein and Fehrer 1955), aggressiveness (Peeke et al. 1979), and touching or manipulating objects (Welker 1956) tended to decrease over time in contact with the goal object. Moreover, nonreinforced preexposure to a stimulus retarded the acquisition and performance when this stimulus was subsequently paired with a reinforcer (Lubow and Moore 1959, Killcross and Balleine 1996). It might be supposed that longer exposure to the exceptionally high amount of sensory information caused a relatively persistent decrease of subjects' attention to salient and/or subtle features of the environmental stimuli. As a consequence, the SO rats failed to process the context and sporadic (CS) warning signals in a manner permitting efficient differentiation mechanisms.

On principle, in the CER procedure fear is classically conditioned to the CS, and it provokes suppression of ongoing instrumental alimentary responding only during the CS action (see Zieliński and Walasek 1977, Węsierska and Zieliński 1985). In contrast to IN rats, SO subjects showed suppression of the bar press rate not only during the presentation of the CS and US, but also after termination of the nociceptive stimulus. Moreover, SO rats revealed a low response rate during the preliminary training, when no aversive stimuli were presented. These results can be explained by the hypothesis that assumes that the permanent social contacts and a short-lasting housing in the enriched environment tended to evoke high generalization of fear to all the stimuli that occurred in the experimental situation. This interpretation is compatible with some earlier findings of Woodcock and Richardson (2000). They found that rats exposed to a variety of sensory stimulation in the pre-weaning period, and an enriched environment in the post-weaning period had more freezing reactions than animals housed in standard conditions.

The present results are not sufficient to settle whether the behavioral effects observed in Group SO were caused mostly by the cognitive or emotional changes. Additional research is needed, the more so as results of many other studies suggested improvement of most behavioral measures after earlier exposure to enriched environmental conditions (see for review Mohammed et al. 1990, 1993). We did not apply any specific measurements of rats' emotional reactivity during the period of housing in Groups SO and IN. However, the rearing conditions in Group SO seemed to be generally not aversive. Further procedures of food deprivation and CER training were more traumatic for SO than for IN rats. As a consequence, these animals become emotionally hyperreactive. Various innocuous and warning signals of a new experimental situation evoked similarly a high level of fear. In fact, Błaszczyk et al. (1999) found that amplitudes of the acoustic startle response (ASR), used as behavioral measure of arousal, were increased in rats subjected to early exposure to a nonaversive experimental situation. It has been well documented (see Swets et al. 1961) that the actual state of emotion significantly influences information processing mechanisms. The decreased attention of SO subjects to salient and subtle features of a new aversive experimental situation was probably caused mostly by fear generalization. Therefore, it is postulated that in this group the cognitive impairment was the secondary effect of emotional changes.

Several authors (Denenberg 1964, Escorihuela et al. 1994) found that animals kept in the enriched environment were able to cope better with a stressful situation and acquired two-way avoidance responses faster than animals subjected to impoverished and/or standard conditions. However, it has been well documented that a relatively high level of fear is able to improve avoidance learning (Werka et al. 1978, Zieliński 1981) and exerts an effect on the escape performance (Zieliński and Savonenko 2000). Moreover, the acquisition and performance of the defensive classical and instrumental reactions significantly depend on the intensity of US applied in the particular conditioning procedure. In the studies of Denenberg (1964) and Escorihuela et al. (1994) relatively weak foot-shock (0.2 to 0.8 mA intensity) was used. It should be stressed, however, that in these experiments a weak nociceptive US was effective to evoke fast acquisition of the two-way avoidance responses only in animals previously housed in the enriched social condition, but it appeared to be not effective to provoke a similar effect in the isolated group of subjects. Therefore, probably just as in our study, the enriched housing condition enhanced fear sensitivity, more than rearing in isolation. Consequently, the much stronger nociceptive US of 2 mA intensity used in our study was able to provoke in Group SO not only fast classical conditioning to the CS (suppression of the ongoing alimentary responses), but also generalized suppression to the entire training situation.

It is unreasonable to ignore another possible hypothesis that early social interactions are the main factor, which totally determines the defensive conditioning in adulthood. Especially the social dominance (Blanchard et al. 1993) and various stress correlates may form complex physiological mechanisms, which influence further emotional coping strategies and learning capabilities (for review see Benus et al. 1991, Blanchard et al. 2001). However, the methods used in our experiment did not allow solving this problem, since neither social dominance nor stress markers were measured in our study.

CONCLUSION

In contrast to early impoverished rearing conditions, the enriched environment provoked generalization of fear during later exposure to a new aversive experimental situation. It resulted in handicapped differentiation between the sporadic and context stimuli presented during the CER training.

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