Effects of post-weaning social isolation and environmental enrichment on exploratory behavior and anxiety in Wistar rats

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Adverse early experience is generally regarded as a risk factor for both externalizing and internalizing behavioral disorders in humans. It can be modeled in rats by a post-weaning social isolation procedure. Effects of social isolation might possibly be ameliorated by environmental enrichment. In the current study, 24 male Wistar rats were divided post-weaning into four rearing conditions: control, environmental enrichment (EE), social isolation (SI) and a combination of the two experimental conditions; (EE+SI). Two observations of the effects of rearing conditions on the rate of social and object interactions were conducted during the juvenile and post-pubertal stages of development. The SI condition led to a marked increase of social interactions during the juvenile phase, but did not affect object interactions. The EE condition increased the level of social interactions during both the juvenile and post-pubertal measurements. The effects of early rearing conditions on adult exploratory behavior were less clear, with a significant difference between the groups obtained in one of three behavioral tests. Results suggest a general robustness in the development of adult exploratory behavior and anxiety when rats were exposed to early social isolation and provided brief opportunities for social play during the juvenile period. Further studies, aimed at distinguishing play-related protective factors serving against long-term adverse effects of juvenile social isolation, are suggested.

Key words: anxiety, play-fighting, exploratory behavior, social isolation, environmental enrichment, rats

INTRODUCTION

Adverse early-life social experiences can increase vulnerability to the development of psychopathology, such as depression, anxiety disorders, PTSD and substance abuse (Heim and Nemeroff 2001). Experimental studies that address the long-term effects of variations in early social experience, due to ethical and time constraints, often require the use of animal models. There is a large body of research focused on the consequences of variation in the level of maternal care in rats. It has been shown that the quality of dam-pup interactions, co-regulation of biobehavioral (eg. sensorimotor, thermal) systems in early attachment relationships affects life-span stress-response systems development, through mechanisms regulating gene expression (Hofer 1975, Hofer 2006, Champagne 2010). High quality maternal care results in lower stress reactivity, (Liu et al. 1997) lower startle responses and higher levels of exploratory behavior (Francis et al. 1999, Menard et al. 2004), possibly leading to greater adaptation to low-risk environments (Beery and Francis 2011).

Relatively less attention has been paid to variation in early post-weaning (roughly corresponding with the period of pre-adolescence) social experience in rodents. There is some data on the life-span effects of natural variations in the level of pre-adolescence peer interactions (Branchi 2009), but the bulk of research has focused on the social isolation procedure, which produces a clearly atypical rearing environment. The aim of those studies is producing a set of symptoms similar to those observed in human psychological disorders. During the standard social isolation procedure, apart from the human handling accompanying changes in
bedding material, animals do not have any direct social interactions, but remain in the same environment as group-housed rats, with a similar level of exposure to olfactory, visual and auditory stimuli (Fone and Porkess 2008). The social isolation procedure causes long-term phenotype changes, e.g. reduction in dendritic arborization (Pascual and Bustamante 2013), especially if the procedure is initiated immediately following natural weaning, around 20–30 post-natal days (Einon and Morgan 1977).

**Effects of post-weaning isolation**

There are five commonly replicated effects of post-weaning isolation (Fone and Porkess 2008).

**Hyperactivity in a novel environment**

Motor hyperactivity (increased ambulation and rearing) is one of the first visible behavioral effects of isolation (Einon and Morgan 1977, Bakshi and Geyer 1999). Increased baseline level of activity in a novel environment may be present (Heidbreder et al. 2000) or a baseline level of activity may be similar to group housed animals, but with a diminished habituation to novelty. Because isolated rats are more apprehensive when entering a larger, open environment from an enclosed, familiar space, it is suggested that the hyperactivity is related to fear-related attempts to escape the novel environment (Einon and Morgan 1977, Arakawa 2005).

**Increased anxiety**

Isolated rats have displayed modest increases in tests associated with mild-anxiety, such as the tendency to avoid open arms in an elevated plus maze (Stanford et al. 1988, Lukkes et. al. 2009, McCool and Chappell 2009), but this effect is not always replicated (Pisula et al. 1992, Ostaszewski et al. 1992, Arakawa 2003).

**Prepulse inhibition of acoustic startle**

Prepulse inhibition is a widely used, across several species, as a measure of sensorimotor gating (Geyer et al. 2001). Impairments in prepulse inhibition are often reported in schizophrenia and other human psychological disorders (Geyer et al. 2001). The effect occurs when a weak sensory stimulus is inhibiting (“gating”) the motor response to the subsequent stronger, startle-inducing stimulus (“pulse” – e.g. loud noise). This process is disrupted in isolated rats (Heidbreder et al. 2000).

**Serotonin depletion**

Some studies show that isolated rats display features of serotonin depletion, such as increased alcohol, sucrose and saccharin consumption (Jones et al. 1990, Hall et al. 1999, Brenes and Fornaguera 2009, Hong et al. 2012).

**Atypical social behavior**

The period of pre-adolescence in rats is characterized by a peak in social play (Einon and Morgan 1977, Potegal and Einon 1989, Schrijver et al. 2002). Play deprivation during juvenile social isolation is suggested to lead to an atypical performance of complex social behaviors that require the ability to perform an action with flexibility and subtle, reciprocal adjustments in relation to the other animals’ behavior (Pellis and Pellis 2009, Bell et al. 2010). Isolation-reared animals show social withdrawal and a general quantitative decrease of adult social behavior (Hol et al. 1999, Van den Berg et al. 1999a,b) but also an increase in aggressive behavior such as increased defense in non-threatening contexts, reduced attack signaling and the targeting of opponents more vulnerable body parts (Einon and Potegal 1991, Tóth et al. 2012, Tulogany et al. 2012).

Generally, previous studies point to a set of deficits caused by post-weaning social isolation. Out of those, results on the effects of post-weaning isolation on long-term changes in emotional reactivity are relatively mixed. Furthermore, in order to verify whether the isolation-induced deficits are due to poverty of general sensory stimulation, or rather to specific deficits in social experience, socially isolated rats can be reared in a standard or sensory-rich environment. It might be expected that environmental enrichment rearing will ameliorate the effects of social isolation, by enhancing levels of exploratory behavior and lowering trait anxiety (Péfa et al. 2009, Pritchard et al. 2013). The aim of the current study is to verify the independent and combined effects of environmental enrichment and social isolation on different measures of anxiety, as well as on social and non-social exploratory behavior in rats.
METHODS

Subjects and housing

Twenty-four male outbred Wistar rats obtained from the Mossakowski Medical Research Centre (Warsaw, Poland) served as the subjects. The Wistar rat strain was chosen for this study because of its popularity in clinical research. Only male rats were used because of their higher baseline level of play-fighting behavior (Tanaś and Stryjek 2008). Animals arrived at the laboratory at 24 post-natal days and were divided into 4 equal groups: (1) Isolated from post-natal day (PND) 24 and reared in standard cage (IS24_STD; n=6), (2) isolated from post-natal day 24 and reared in an enriched cage (IS24_ENR; n=6), (3) pair-reared in a standard cage (PA_STD; n=6), and (4) pair-reared in an enriched cage (PA_ENR; n=6). Animals were housed in the same conditions throughout the experiment: transparent plastic (polycarbonate) cages (61×41×21 cm) with wood shavings, free access to food and water. Cage enrichment was achieved by placing (and replacing when needed) several objects of similar size in the cages (small metal ladder, standard 40 mm Ping-Pong ball, wooden brick, natural size plush mouse). The vivarium was controlled for temperature (22–23°C), humidity (50%) and maintained on a L:D 12/12 light cycle.

Apparatus

Social interaction test

Social interaction sessions took place in a standard laboratory cage (61×41×21 cm) equipped with a novel set of objects, similar to the ones used for environmental enrichment.

Open-field test

Open-field was a 75 cm wide square with 60 cm height plywood side walls, floor painted white and equally divided into 25 squares. The room was lit with a red light of low (100 lx) intensity.

Elevated-plus maze

Elevated-plus maze was composed of two narrow arms (length: 110 cm, width: 10 cm), one “closed” with side walls and one “opened.” The arms crossed in the middle of the maze. The maze was placed 70 cm above the room floor. The room was lit with a red light of low (100 lx) intensity.

Self-exposure chamber

This apparatus was used to measure exploratory behavior using an automated measure and for a longer time duration (60 min), than in other standard tests. Additionally need for stimulation can be assessed, as the animal can perform an action resulting in environmental feedback (light on/off switch) or an exploratory action resulting in no such change (blank hole). The chamber was a 33×30×27cm box, with side walls made of aluminum, front and back walls made of Plexiglas, floor made of parallel metal rods, ceiling made of Plexiglas and equipped with six 1.5 watt electric bulbs. Each of the side walls has a single round hole (3 cm diameter), situated 10 cm above floor level. Head entries into both holes were automatically recorded with a photocell connected with PC software and head entry to one of the openings caused a light onset (white light 27 lux) for 3 seconds.

Procedure

All experimental procedures were approved by the First Warsaw Local Ethics Committee for Animal Experimentation. All of the behavioral observations were conducted during the light portion of the cycle. Animals were not handled prior to the initiation of the experiment. The timing of the main events in the procedure: PND 0–24 Mossakowski Medical Research Centre; PND 24 weaning, transport to the lab; PND 24–39 social isolation/enrichment housing; PND 40 first social interaction procedure; PND 41–60 further social isolation/enrichment housing; PND 40 first social interaction procedure; PND 61–100 further social isolation/enrichment housing; PND 101 self-exposure chamber; PND 103 elevated-plus maze; PND 104 open-field test. This constitutes 73 full days of social isolation/enrichment, starting from PND 24.

Social interaction tests

Two social interaction measurements were conducted, during the juvenile (PND40) and post-pubertal (PND61) stages. During those measurements, the rats were placed in a novel, enriched (containing objects) cage with a random conspecific from the same experimental group (excluding the cage-mate) and provided
an opportunity for free, 15-min, social interaction. Animal behavior was recorded on a video camera. Subsequently, the following behavioral measures were scored by a human observer from VHS tapes: number of interactions with objects (chewing, pushing, biting) and number of social interactions with a conspecific (mostly wrestling and play-chase runs).

Self-exposure chamber

The adult exploratory behavior in the self-exposure chamber was measured individually in a 60-min trial at PND102. The apparatus was cleaned with diluted alcohol solution (5% ethanol-water) after each animal. The number of head-dips into both blank and light on/off holes were measured automatically with a photocell connected with PC software.

Elevated-plus maze

The animals were placed individually in the apparatus for a 5-min test on PND103. The apparatus was cleaned with diluted alcohol solution (5% ethanol-water) after each animal. Total time spent by the animals in both open and covered arms of the maze, as well as the number of entries into both arms were scored.

Open-field test

The animals were placed in the open-field for a 5-min trial on PND104. The apparatus was cleaned with diluted alcohol solution (5% ethanol-water) after each animal. Ambulatory behavior (number of squares crossed with all 4 paws), number of rearings (including leanings on walls), as well as defecation was measured.

RESULTS

A Type I error rate of $P < 0.05$ was adopted for all the statistical analysis. The descriptive statistics are presented in Table I. Whenever the assumption of homogeneity of variances (Levene’s test) was not met, the data was transformed with base-10 logarithm.

Social interaction tests

Two repeated measures ANOVA were conducted, separately for the number of social interactions and the number of object interactions, with time of measurement (2: PND40, PND60) as the within subject factor and enrichment (2: standard cage, enriched cage) and isolation (2: isolation rearing, social rearing) as between subject factors. For the number of social interactions, a significant interaction between time of measurement and isolation rearing was obtained, $F_{1,20}=14.91, P<0.001, \eta^2=0.43$, see Figure 1. Number of social interactions in isolated rats dropped between the first and second measurement, $M_{PND40}=41.66, 95\% CI [36.05, 48.13]$, $M_{PND60}=29.40, 95\% CI [25.32, 34.14]$, but stayed on the same, generally lower level in socially reared rats, $M_{PND40}=13.70, 95\% CI [11.86, 15.83]$, $M_{PND60}=12.90, 95\% CI [11.02, 14.87]$. Fig. 1. Interaction between time of measurement and rearing conditions in the amount of social play; (A) PND40; (B) PND61.
Interaction between the time of measurement and environmental enrichment was not significant, $F_{1,20}=1.04, P=0.32$, but a between subjects main effect of enrichment was present, $F_{1,20}=5.75, P<0.05, \eta^2=0.22$. Enrichment reared rats had a greater number of social interactions, $M=24.58$, 95% CI [21.68, 27.86], than rats reared in standard cages, $M=20.05$, 95% CI [17.69, 22.72]. For the number of object interactions, only the main effect of time of measurement was found, $F_{1,20}=36.32, P<0.001, \eta^2=0.65$, with no other effects present. Rats interacted with objects more frequently during the first, $M_{\text{PND40}}=47.12$, 95% CI [42.56, 52.16] rather than second, $M_{\text{PND60}}=29.79$, 95% CI [25.62, 34.64], measurement.

**Self-exposure chamber**

Due to the fact that the chamber is a relatively uncommon measure of exploratory behavior, results from behavioral tests were correlated with both open field and elevated-plus maze. The “need for stimulation” ratio, that is ratio of light on/off head-dips to the total number of head-dips, was positively correlated with the elevated-plus anxiety measures: time spent in closed arms, $r=0.48, n=24, P<0.01$, time spend in open arms, $r=-0.4, n=24, P<0.05$. Total number of head-dips was not correlated with any of the main open-field and elevated-plus measures. Separate ANOVAs for each of the dependent variables were conducted, with enrichment (2: standard cage, enriched cage) and isolation (2: isolation rearing, social rearing) as independent factors. The main effect of isolation rearing was found for the total number of head-dips, $F_{1,20}=7.73, P<0.05, \eta^2=0.28$, with isolated rats being more active than paired reared rats (see: Fig. 2). The main effect of enrichment failed to reach significance, $F_{1,20}=3.53, P=0.075$. No significant effects were found for the “need for stimulation ratio”, that is number of light on/off dips vs. total number of head-dips.

**Elevated-plus maze**

ANOVA for each of the dependent variables were conducted, with enrichment (2: standard cage, enriched cage) and isolation (2: isolation rearing, social rearing) as independent factors. No significant effects were found for any of the variables.

Ratio of total to open-arm entry (typical anxiety measure) was not significantly affected by the isolation rearing, $F_{1,20}=0.09, P>0.05$, nor enrichment, $F_{1,20}=0.37, P>0.05$. Isolation rearing did not affect the number of entries to the open arms, $F_{1,20}=0.15, P>0.05$, number of entries to the covered arms, $F_{1,20}=0.23, P>0.05$, total time spent in the open arms, $F_{1,20}=0.01, P>0.05$, nor the total time spent in closed arms, $F_{1,20}=1.58, P>0.05$. Environmental enrichment did not affect the number of entries to the open arms, $F_{1,20}=0.34, P>0.05$, number of entries to the closed arms, $F_{1,20}=0.92, P>0.05$, total time spent in open arms, $F_{1,20}=0.27, P>0.05$, nor the total time spent in closed arms, $F_{1,20}=0.71, P>0.05$.

**Open-field test**

ANOVA for each of the dependent variables were conducted, with enrichment (2: standard cage, enriched cage) and isolation (2: isolation rearing, social rearing) as independent factors. No significant effects were found for any of the variables. Isolation rearing did not affect ambulatory behavior, $F_{1,20}=0.13, P>0.05$, number of rearings, $F_{1,20}=2.01, P>0.05$, nor defecation, $F_{1,20}=1.24, P>0.05$. Environmental enrichment did not affect ambulatory behavior, $F_{1,20}=2.41, P>0.05$, number of rearings, $F_{1,20}=1.98, P>0.05$, nor defecation, $F_{1,20}=0.09, P>0.05$.

**DISCUSSION**

The full set of behavioral changes associated with social isolation was not reproduced in the current
experiment. It was expected that isolated rats would show increased activity in a novel environment, a decreased number of social interactions, and increased anxiety. Instead, socially isolated rats showed higher levels of social interactions and relatively minor effects of exploratory behavior and anxiety were observed. The first interpretation of this result is that the study might have been underpowered to detect a significant effect of isolation rearing. Studies which used twice as many animals per experimental did show the expected result (Hellemans et al. 2004). Significant results in this study might be interpreted as showing the rebound effect of juvenile social play after short-term post-weaning social isolation (Niesink and van Ree 1982, Vanderschuren et al. 1995). In these studies, rats showed a strong increase in the amount of play after short-term social deprivation and this is especially pronounced in juvenility. This increase in play-fighting after short term isolation can be interpreted as “attachment promoting” and related to social bonding. Similar effect occurs with brief separations of rat pups from dams (Levine et al. 1967) as it enhances dam-pup interactions upon reunion (Liu et al. 1997) and has positive long-term consequences for emotional regulation.

One possible alternative interpretation might be mentioned. In the current experiment, the typical procedure for measurement of play-fighting was not used (e.g. Pellis and Pellis 1987). In this typical procedure, two, previously cohabitating animals are separated for a brief period of time before measurement. In the current procedure, the play partner was unfamiliar during the first juvenile, social interaction test. This change in procedure requires a careful distinction between playful fighting and serious fighting, which is generally likely to occur between two unfamiliar, isolated animals. A more precise way to distinguish playful fighting from aggression might be necessary in subsequent studies. None the less, a high level of interactions between the isolated animals, when considering the general lack of features of increased emotionality in isolated rats, should rather not be interpreted as due to enhanced aggression. Furthermore, at least during the peak of juvenility (~40 PND), encountering an familiar as well as unfamiliar peer tends to result in a typical play behavior pattern in rats (Himmler et al. 2014).

Surprisingly, since both the isolation procedure and environmental enrichment used in the current study were relatively typical (Fone and Porkess 2008), experimental manipulation resulted in minor effects on the measures of anxiety and exploratory behavior used. Social isolation caused an increase in the total number of head-dips in an automated measure of exploratory behavior. This could be expected as hyperactivity in a novel environment is the most commonly replicated effect of isolation. Isolated rats generally show greater locomotor activity in novel environments (Pritchard et al. 2013). On the other hand isolation did not show any significant effect on the ambulatory behavior and rearing in the open-field test.

A relatively simple interpretation of this pattern of results might be offered. The self-exposure chamber was the first test used and the order of the tests was not randomized. Therefore, the effect might be due to the novelty of the testing procedure, not due to any particular aspect of the apparatus. In subsequent studies handling should be provided as well as habituation of rats to the home-cage transport procedure.

What is more surprising is the relative lack of effects of environmental enrichment on exploratory behavior (Belz et al. 2003). We could speculate that this was due to the enrichment procedure used. Rats in the enriched condition had access to a set of novel objects, but the general area of their home cage was not changed, nor did they have access to a larger number of conspecifics. It is possible that the spatial and social aspects of “enriched” environment are required to produce long-term behavioral changes, not so much an opportunity for increased object exploration.

**CONCLUSIONS**

The fact that relatively long-term post-weaning social isolation (73 full days in total) did not result in long-term changes in anxiety and exploratory behavior might be due to the timing of the first social interaction session. This was a brief session (15 min), but it provided isolated animals with the opportunity for social play. Social feedback offered by both of the social interaction sessions (PND 40 and 61) may have dampened the impact of social isolation This raises the question on the timing/amount of social play, during the sensitive period of juvenility, which would be sufficient as a protective factor against the adverse effects of social isolation. Another study could be conducted with exactly the same procedure, but in which one of experimental groups would not have the possibility of social play during juvenility, but would be socially isolated up to the pre-pubertal stage. If this
group were to show reductions in adult exploration and increase in anxiety, then we would have an interesting experimental setting to verify what play-related experiences serve as the protective factor during the juvenile period. Furthermore, it could be argued that pair-housing cannot yet be considered as group housing. Variability in the amount of early social stimulation (e.g. 2 vs. 4–6 cage mates) could have a long term effect on brain and behavior development (Branchi 2009).

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REFERENCES

Rearing effect on exploratory behavior and anxiety


