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The impact of early postnatal environmental enrichment on maternal care and offspring behaviour following weaning

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Abstract

The early postnatal period is a sensitive period in rodents as behavioural systems are developing and maturing during this time. However, relatively little information is available about the impact of environmental enrichment on offspring behaviour if enrichment is implemented only during this period. Here, environmental enrichment was provided from postnatal day 1 until weaning. On postnatal day 9, maternal behaviour and nonmaternal behaviour of the dam was observed. Nursing time in the enriched group was reduced but dams showed more non-maternal appetitive behaviours. Offspring were exposed to either the open field or the elevated plus maze (EPM) after weaning. In the open field, rats from the enriched group approached the more aversive inner zone of the open field later than control rats. Offspring from the enriched group made fewer entries into the inner zone and spent less time in this part of the arena. Enrichment had no impact on behaviour in the EPM. The present study provides evidence that postnatal enrichment can interfere with maternal behaviour in rats and can possibly lead to increased anxiety in the offspring. The findings suggest that enrichment procedures can have potentially unintended effects, interfering with the development of emotional behaviours in rats.

Keywords: anxiety; elevated plus maze; enrichment; nursing; open field; rat

1. Introduction

The early postnatal period is a sensitive period in rodents as behavioural systems are developing and maturing during this period. Although many of the sensory systems are functional at birth, their functions are often not fully developed at this stage. Already on postnatal day one, pups show a ventrum-down orientation which requires a motor response. They use a quadruped stance around day ten, but walking usually does not occur before day 12 to 15 (Westerga and Gramsbergen 1990; Alberts 2005). Although the eyes open already in 14 to 15 days old pups, visual cortical functions are still immature before postnatal day 19 (Fagiolini et al. 1994). Therefore it is not surprising that sensory deprivation during this period has detrimental and long-lasting, if not permanent, effects on the nervous system and a variety of behaviours. Stressful experiences during the early postnatal period can programme an anxious phenotype (Anisman et al. 1998). It has been suggested that exposure to adverse environmental factors during the maturation of brain anxiety circuits contributes to the pathogenesis of anxiety disorders (Leonardo and Hen 2008). However, a period of exposure to an environmentally enriched environment can compensate for early stressful and detrimental effects (Escorihuela et al. 1994; Tobena et al. 1994; Fox et al. 2006; van Praag, et al. 2000).

While environmental enrichment protocols in rodents are often being started post weaning or later (Simpson and Kelly 2011), handling procedures by humans are probably the most commonly used early postnatal environmental manipulations. There is evidence that early human handling, but also environmental enrichment, reduce anxiety in rats (Ferre et al. 1995; Levine 1957; Levine et al. 1967; Meaney et al. 1996; Anisman et al. 1998; Fernandez-Teruel et al. 2002). Recent reviews of this research have discussed the choice of the appropriate control in some of those studies. In particular in studies where control rats have been kept in isolation or have not been handled at all, the handled group would rather produce 'normal' behaviour whereas the lack of stimuli in the respective control groups would increase anxiety instead. Under these conditions, the latter group should actually be regarded as the experimental group (Pryce and Feldon 2003).

Despite a general positive connotation of enrichment (Fox et al. 2006), inconsistent presentation of stimuli during the early postnatal period can be detrimental to the animal (Escorihuela et al. 1994 1994). Handling procedures include a separation from the mother making the maternal behaviour, which is essentially a mother-pup interaction, an important variable in this paradigm. In fact, it has been shown that not only nursing behaviour, but also maternal grooming (licking) reduces anxiety/stress levels in the offspring (Liu et al. 1997; Caldji et al. 1998). Although short separation from the dam will stimulate maternal behaviour, possibly by increasing demand from the pups, longer periods of maternal separation have a detrimental behavioural effect in adult age (Hofer 1996; Oitzl et al. 2000; Plotsky and Meaney 1993).

Although the rat nervous system develops both pre- and postnatally, environmental enrichment during gestation alone has an impact on pre-pubertal behaviour in the offspring of Wistar dams. In contrast to reports of positive effects of environmental enrichment, Rosenfeld and Weller (2012) demonstrated increased anxiety-related behaviour both in the open field and the elevated plus maze. Because there were only inconsistent effects on maternal behaviour, the authors concluded that the increased anxiety in the offspring were not a direct consequence of changes in maternal behaviour. However, such an increased anxiety-related behaviour could possibly gain protective

value when offspring are exposed to stress in adult age (Cymerblit-Sabba et al. 2013). Exposure to an enriched environment during gestation and during lactation also improves performance in the Hebb-Williams maze (Kiyono et al. 1985; Venable et al. 1988).

Welberg et al. (2006) started an environmental enrichment procedure on gestational day 16 which lasted until postnatal day 23, when weaning took place. In that study, dams showed less nursing episodes on postnatal days three to five, the dam was also away from the nest more often. When measured in adult age, enrichment prevented a chronic stress-induced increase in basal corticosterone levels. However, female, but not male offspring from the enriched groups showed an increased adrenocorticotrophic hormone (ACTH) response to an acute stressor when previously exposed to chronic stress. This study reveals a sex-specific and complex effect of pre- and postnatal enrichment on endocrine response to stressors. By contrast, when dams were exposed to enrichment starting in the second half of gestation and continuing until postnatal day 12, offspring showed clear anxiolytic effects when tested in adult age. This anxiolytic effect was very similar to that observed following daily tactile stimulation of the pups until postnatal day 12 (Baldini et al. 2013).

Fewer rodent studies, however, investigated the impact of an early postnatal only exposure to inanimate objects on behaviour of the offspring. In the rat as an altricial species, this period is characterised by the impact of maternal care that correlates positively with dampened stress and anxiety responses in the offspring (Liu et al. 1997; Caldji et al. 1998). Whereas early postnatal enrichment in mice promotes maternal behaviour and development of the offspring (Cancedda et al. 2004), the impact on maternal behaviour in rats can be detrimental, but possibly dependent on the innate level of maternal care. Although restricting environmental enrichment to the lactational period, rat studies either did not investigate behaviour in the offspring or they used dams selectively bred for either high or low innate maternal licking or grooming (Mann and Gervais 2011; Champagne and Meaney 2006).

In an approach complementary to previous studies, we exposed dams to an environmental enrichment procedure during lactation only. Despite the positive effects of post-weaning environmental enrichment, we hypothesised an overall detrimental effect of a more structured, physically enriched, environment when introduced only during the early postnatal period. Both maternal and offspring behaviour have been analysed. We expected that the enrichment effect would manifest in increased anxiety-related behaviour in the offspring during the immediate post-weaning period. To this end, various objects have been introduced to the home cage during the lactational period. Shortly after weaning, offspring were exposed to either the open field test or the elevated plus maze (EPM).

2. Methods

2.1. Animals

Pregnant female Wistar rats (Harlan, UK) were housed individually with ad libitum access to a standard laboratory chow (Harlan Teklad Global 18%, UK) and water. Animals were maintained under a 12-hour light-dark cycle (lights on 08:00 hours), between 20-22°C. The dams were individually housed through the whole gestation period. Cages contained saw dust bedding, nesting material, a cardboard tunnel and a piece of wood. At birth, litters were reduced to 4 pups of each sex, and randomly allocated to the non-enriched group (control), or the environmentally enriched group. Litters of both the enriched and control groups were kept under the same ambient conditions. Cage sizes for both groups were identical (55cm x 33cm x 23cm). Pups were weaned on postnatal day (PND) 21. After weaning, offspring were kept in groups of four/cage with males and female kept in separate cages. No enrichment items were provided between weaning and behavioural testing.

2.2. Enrichment procedure

Enrichment items were introduced on PND 1. On that day, the cardboard tunnel and the wood were removed from all cages. In both the control and enriched group bedding and nesting material remained. Five different enrichment items have been used. A seesaw of plastic was always present. Three of the four remaining objects were placed in the cage, and one of the three was exchanged every third day. A total of four objects were present at a time. The enrichment objects included a plastic tunnel, wall, platform, seesaw and a wooden block. The manipulations associated with the swapping of objects have been mimicked in the control group, i.e. the cages were briefly opened and the experimenter moved his hand swiftly in and out.

General husbandry routines (e.g., feeding, drinking, changing cages) were identical between groups, but were never performed directly before any behavioural testing or assessment.

2.3. Behavioural assessments and testing

All behavioural tests with the exception of activity monitoring, were conducted between 0900 and 1200 h. The activity monitoring routinely started at 0900 and lasted for 24 hours.

Home cage locomotor activity

24-hour home cage locomotor activity was measured twice during the lactational period. The first measure was taken after 7 days of exposure to environmental enrichment (PND 8 to PND 9) when the pups did not show locomotor activity themselves. The second measure following 19 days of exposure (PND 20-PND 21). This was shortly before weaning and the pups were already roaming the cage. To register locomotor activity, a data logger (Mouse-E-Motion, INFRA-E-MOTION GmbH, Hamburg, Germany) was mounted above each cage. The logger was connected to an infrared sensor (resolution: 1 motion detection/second; recording interval 60 minutes) to cover the complete space of the cage underneath. The data measured by each Mouse-E-Motion device was downloaded onto a personal computer and processed with Microsoft Excel. Data is expressed as arbitrary units.

Maternal behaviour and dam behaviour in the home cage

On PND 9, maternal and non-maternal behaviours of the dam were observed for 45 minutes. This took place in a separate observation room, and the home cage was placed in the observation room 20 minutes prior to the beginning of the observation period to allow the dam to habituate. Behaviours were recorded manually by the same person each time, as well as being digitally recorded in case there was need to revisit the material at a later date. In total, 8 dams per housing condition were observed.

The duration of the following four nursing behaviours was recorded: 1. Hovering: Dam is positioned over all or most of the pups. 2. Low crouch: Dam's back is slightly arched. 3. High crouch: Dam's back is highly arched with significant limb extensions. 4. Supine: Dam is lying on her side. A total of nursing time was calculated from the individual nursing positions. In addition licking and grooming of the pup's body or anogenital region was monitored. Nesting behaviour, i.e. moving nesting material around the cage and/or manipulating the original nest was also recorded (Rees 2005).

The nest was scored at the end of the 45 minute maternal observation period. A scale from 0-5 was used; 0 = no nest, 1 = poor nest with very little nesting material used, 2 = flat nest with nesting material arranged in a single location, 3 = low walls of nest present, 4 = high walls of nest are distinct, but do not enclose all pups, 5 = high walls of nest conceals pups (Marino et al. 2002).

Furthermore, the duration of the non-maternal behaviours feeding, drinking, self-grooming and the frequency of rearing (vertical exploration with both front feet off the ground) were recorded.

Offspring behaviour after weaning

Offspring behaviour was tested for anxiety-related behaviour on PND 23, i.e. on the second day after weaning. A total of 80 offspring (40 each sex) was tested. All animals have only been tested once to avoid interactions between tests. To avoid any litter effect, they were randomly selected from litters. All behavioural testing took place between 0900 and 1200. The pups were moved from the holding room to the adjacent behavioural suite and left to habituate for 20 minutes before commencing the tests. Behaviours were recorded using Ethovision XT (version 7.0, Noldus, Netherlands). Testing arenas were cleaned with 70% ethanol between each animal to remove olfactory cues.

Open field test

The open field was made out of dark grey plastic and its dimensions were 100cm x 100cm x 50cm. An inner zone was defined as a square, 20 cm in from the walls of the arena. Light intensity in the centre of the open field was 70lx.

Each pup spent 5 minutes in the open field and the total distance travelled and latency to first entry into the inner zone, as well as number of entries and time spent in the inner zone were automatically recorded by the software. The frequency and duration of rearing and grooming was recorded manually using Ethovision.

Elevated plus maze

The apparatus consisted of two opposite open arms (50cm x 15cm) and two opposite closed arms (50cm x 15cm) arranged in a cross position elevated approximately 60cm from the ground. All arms extended from a central zone (15cm x 15cm). Two standing lights providing 50lx (in closed arms) to 70 lx (open arms) were used to illuminate the maze. All animals were exposed for 5 min to the EPM. Rats were initially placed on the centre platform facing a corner, allowing equal choice of entering an open or a closed arm. The time spent on the open and closed arms, the entries into the arms and the total distance travelled were monitored. In addition, rearing and time spent self-grooming were analysed and the ratio of open arm entries to total arm entries was calculated.

2.4. Data analysis

The statistical unit for maternal behaviour was the dam. This part of the study was powered to detect a difference of 30 % in total time (s) nursing, based upon $\sigma = 500$ (determined from previous studies) and an α value of 0.5 at 80 % power. The statistical unit for exploratory and anxiety-related behaviour was the offspring.

All data was analysed and all graphs were created using GraphPad Prism Version 6.00 for Windows (GraphPad Software, USA). Significance was accepted at a P-value < 0.05 and tendencies were accepted with a P-value <0.1 but >0.05. All graphs are shown as mean + SEM.

Dam behaviours, nest scores, and home cage locomotor activity were compared by Student's *t*-test. Offspring behavioural data were analysed by Two-way ANOVA (sex, enrichment).

All experimental procedures were approved by the institutional Ethical Committee.

3. Results

3. 1. Behaviour of the dam

Following 8 days of exposure of the dam and litter to the enrichment procedure, changes in maternal behaviour have been observed. Although some individual nursing positions showed tendencies ($P < 0.10$) towards a decrease in the enriched group, only the combined total time spent nursing reached significance. Dams of the enriched group spent overall less time nursing ($t=2.64$ $df=14$, $P < 0.05$) (Fig.1). This goes along with increased number of non-maternal behaviours in these dams, as they spend more time feeding ($t=3.47$ $df=14$, $P < 0.01$) and drinking ($t=2.148$ $df=14$, $P < 0.05$) outside the nest. Although a slightly increased ($P=0.07$) nesting frequency was observed in the enriched group, neither the duration of nesting behaviour or the overall nest score were affected by enrichment (data not shown). Dams from the enriched group tended to show more self-directed behaviour as expressed by the increased duration of self-grooming ($P=0.07$) (Fig.1). Despite the

increased non-maternal behaviour during the observational period, overall 24 hour home cage locomotor activity was not affected by exposure to objects (control: 9784 ± 670 units; enriched: 9188 ± 1053 units; $n=8/\text{group}$). When locomotor activity was measured shortly before weaning, i.e. when activity of the whole litter was measured, no significant difference between enriched and control litters could be observed either (control: 18255 ± 1190 units; enriched: 20913 ± 799 units; $n=8/\text{group}$).

3. 2. Offspring behaviour

Two days after weaning, offspring of both sexes were either exposed to an open field test or the elevated plus maze. In the open field, offspring from both groups travelled the same distance. Rearing was reduced in offspring from an enriched environment ($F_{1,26}=4.5$, $P<0.05$) and there was a tendency ($P=0.09$) in females for showing less rearing when compared to males. Control rats approached the more aversive inner zone of the open field sooner than offspring from the enriched group ($F_{1,26}=4.8$, $P<0.05$), although there was an interaction with sex ($F_{1,26}=6.6$, $P<0.05$), suggesting that the enrichment effect on this parameter occurred largely in male rats. Offspring exposed to enrichment during lactation made fewer entries into the inner zone ($F_{1,26}=7.1$, $P<0.05$) and spent less time in this part of the arena ($F_{1,25}=10.1$, $P<0.01$). No direct effect of sex on these parameters has been observed. Enrichment had no effect on grooming in the offspring during exposure to the open field (Fig. 2).

In contrast to the open field, no enrichment effects have been observed upon exposure to the EPM. Offspring from the control group stayed most of the time in the protected closed arms of the EPM, but only 13 % in the open arms (Fig. 3). Environmental enrichment during lactation had no impact on this anxiety-related parameter, nor had it an effect on the relative number of entries into the aversive open arms (Fig.3). Offspring from both groups travelled, similarly to the open-field, the same distance upon exposure to the test device (Fig. 3) and did this with similar velocity (data not shown). Sex differences in rearing did not reach significance and there was no effect on enrichment on this parameter either (Fig.3).

4. Discussion

The present study revealed an anxious phenotype in offspring from rats that have been exposed to environmental enrichment during the lactation period. This effect was seen upon exposure to the open field, but not to the EPM. However, a false positive effect in the open field, due to confounding locomotor effects, can be excluded, as the total distance travelled was not different between groups. Various behavioural measures have been used to measure anxiety in the open field in the past, and among them the latency to and time spent in the aversive inner zones proved to be reliable indicators of the emotional state of the rat (Prut and Belzung 2003; Voigt et al. 2005).

A closer inspection of the EPM data shows that offspring from the control group spent most of the time in the protected closed arms, suggesting an already high baseline level of anxiety (Pellow et al. 1985). This high baseline anxiety could possibly account for a ceiling effect, where no further increase in anxiety could be induced. As of to date, there is little knowledge about anxiety-related

behaviour in rats during the immediate post-weaning period, and the underlying neurobiological mechanisms are most likely not fully matured yet (Ganella and Kim 2014). The discrepancies between open field and plus maze results warrant further research as aspects of anxiety related behaviours could possibly mature at different times during the postnatal period.

The lack of significant and direct sex difference in EPM and open field behaviour is in accordance with the literature where such differences had not been reported before PND 60 (Masur, Schutz, and Boerngen 1980; Imhof et al. 1993). Nevertheless there was a trend in that females showed more vertical exploration.

Taken together, the behavioural effects of the enrichment procedure are rather anxiogenic in nature. This is in contrast to some (Fox et al 2006; Baldini et al. 2013), but not all studies (Magalhaes et al. 2008; Magalhaes et al. 2007). However, previous studies applied enrichment not only during lactation, but mostly in combination with gestational exposure.

Two factors could have potentially contributed to the observed anxious behaviour in the offspring; direct effects induced by exposure of the pups to the varying objects or indirect effects mediated by changes in maternal behaviour as a result of this exposure.

As rats are an altricial species, rat pups are heavily dependent on the dam during the early postnatal period (Wood et al. 2003). Therefore maternal care provided by the dam determines the early postnatal environment of the pups (Hellstrom et al. 2012). The quantity of pup licking/grooming has been suggested as a reliable measure to assess maternal care (Champagne et al. 2003; Hellstrom et al. 2012); increased licking/grooming of pups is associated with a reduction in neuroendocrine responses to stress in rat offspring (Liu et al. 1997), and this altered response may persist throughout life (Caldji et al. 1998). Previous studies have shown inconsistent results as to how environmental enrichment impacts on pup licking/grooming of dams. While some (Cancedda et al. 2004) found significantly more licking/grooming for female mice living in an enriched environment, others (Welberg et al. 2006) did not report any enrichment-induced changes in the amount of pup licking/grooming in rats. The latter would be in line with the present results, although the overall reduced nursing time, alongside the increased time spent eating and drinking, would rather suggest a decreased maternal care. The latter could possibly contribute to an anxious phenotype in the offspring (Macri et al. 2008). Although Welberg et al. (2006) started enrichment during gestation and analysed maternal behaviour more frequently, but earlier in the postnatal period, they also observed less overall nursing and more absence of the dam from the nest. Despite a different design, results from this study are in keeping with their findings, suggesting a robust behavioural effect of early enrichment on the dam. The relative contributions of pre- and early postnatal enrichment periods are still to be identified though.

The slightly increased self-grooming in dams from the enriched group is difficult to interpret on its own and warrants further investigation. However, self-grooming can be seen as a de-arousing activity which is increased in anxious rats (Spruijt et al. 1992; Kalueff and Tuohimaa 2005; Voigt et al. 2005). Although the present design did not allow for analysing anxiety-related behaviour in dams, earlier studies demonstrated that offspring from dams previously exposed to a stress-inducing environment will show stronger responses to anxiogenic stimuli (Plotsky and Meaney 1993; Ladd et al. 2000; Wei et al. 2010; Maccari et al. 2014; Barzegar et al. 2015). Alternatively, grooming could

also be associated with the significantly increased feeding as observed in the dams in the present study.

There is ample evidence that a high intensity of licking and grooming of the pups is associated with a reduced anxiety response in adult age (Liu et al. 1997; Caldji et al. 1998). However, a linear relationship between maternal behaviour and emotional behaviour of the offspring has been questioned. Instead, an U-shaped inverse relationship between environmental adversity and maternal care has been suggested. Above a certain level of adversity, environmental stress could affect the pups independently of maternal care (Macri and Wurbel 2006; Wei et al. 2010). For example, it remains unclear from the present study, if, and to what extent, the enrichment procedure has a direct effect on the offspring. Around PND day 16, pups started to explore the environment and roam around the cage. Therefore they contribute to home cage total locomotor activity reading at the time which was not different between groups though. However, measuring gross locomotor activity, although non-invasive, does not detect subtle locomotor effects, or provides qualitative insights, e.g., if activity in the pups was more directed towards to or away from objects. Effects primarily on sensory processing (Cancedda et al. 2004) cannot be excluded either.

One may argue that pups are not prepared to cope with a changing environment during lactation and hence question the validity of the present findings. Although the dam will encounter changing environments when outside the burrow during this period, these encounters are results of her active behaviours, at least in the wild. However, one should assume that in the immediate vicinity of the nest, the environment would be rather stable. By contrast, changing the environment close to the nest could be of more relevance under laboratory conditions, where various enrichments procedures are being employed. To gain some more insight into potentially aversive effects of the enrichment procedure, a choice paradigm would be useful. Giving the dam a choice where to build the nest, e.g. by using a two compartment cage where only one compartment is enriched, would help to analyse maternal and non-maternal behaviour of the dam in more detail. As the dam could possibly avoid the enriched compartment, one could find evidence for adverse effects of environmental enrichment. It has been suggested that rats prefer, when given a choice, nesting material and nesting boxes, but less so other enrichment objects, over living in a standard cage (Patterson-Kane et al. 2001).

Environmental enrichment during the perinatal period has been shown to have either positive or detrimental developmental or behavioural effects (Mychasiuk et al. 2012; Zhu et al. 2006; Mann and Gervais 2011; Anisman et al. 1998) and various variables could account for discrepancies between enrichment studies. For example, variability in maternal behaviour could depend on rat strain, in particular as rat strains differ in anxiety-related behaviour (Rosenfeld and Weller 2012; Rex et al. 1996). Interestingly, postnatal enrichment had more pronounced anxiolytic effects in rats with higher trait anxiety, i.e., in rats selectively bred to show more anxiety-related behaviour on the EPM (Ravenelle et al. 2014). Furthermore, protocols for environmental enrichment vary across studies. Although our control dams and dams from the enriched group have been exposed to a similar intensity of handling and husbandry procedures, many enrichment studies leave control groups unhandled, generating an artificial environment for these rats (Pryce and Feldon 2003). It could be argued that the present analysis of maternal behaviour is limited, because, and in contrast to other studies (Champagne et al. 2003), it is based on a single observation at a single time point during lactation. However, pups are heavily dependent on the mother around that time (Pachon, McGuire,

and Rasmussen 1995) and dams show a particular high intensity of maternal behaviour during the light phase (Leon et al. 1984; Grota and Ader 1969; Ader and Grota 1970). In addition, some exposure time to enrichment would be required to exert any effect at all. A single observation period has also been used successfully in the past (Levay et al. 2008). Although the present data on maternal behaviour are by and large in line with a previous study, that study looked only into the neuroendocrine but not behavioural effects of enrichment (Welberg, Thirivikraman, and Plotsky 2006). A study that included also behaviour (Champagne and Meaney 2006) found that exposure to stress during the last week of gestation decreased licking/grooming in the dam and increased anxiety in the offspring, but only in dams and their offspring with an innate high licking/grooming frequency. Mann and Gervais (2011) exposed virgin rats to environmental enrichment to reduce stress before inducing maternal behaviour. Although using a model of fostering to express maternal behaviour, which differs from the present approach, they found a delayed onset of maternal behaviour in their model. Following one week of exposure, a weak tendency of increased anxiety was observed in the dams upon exposure to the EPM, suggesting some adversity of the enrichment procedure. However, enrichment had no impact on basal corticosterone or EPM exposure-induced increase in corticosterone levels in the dam, and offspring have not been tested. Nevertheless, one would expect a possible increase of anxiety in the offspring as found in the present study. Although future studies of postnatal enrichment should combine behavioural and neuroendocrine analyses, one cannot always expect a direct relationship between the two (Lehmann et al. 1999; Slotten et al. 2006).

Weaning under laboratory conditions is rather a traumatic experience, other than under natural conditions, where it is a more gradual process. The sudden loss of maternal care and protection in the laboratory upon weaning generates an aversive situation per se where increased anxiety could possibly have some protective value. Nevertheless further studies should also include behavioural models of trait anxiety which would be based on free exploratory behaviour (Bert et al. 2013) because the effects of environmental enrichment can be different in animal that differ in state and trait anxiety (Chapillon et al. 1999; Goes et al. 2015).

Another important question is if the anxious phenotype will continue to exist into adult age, as early stressful experiences including lack of maternal care can “programme” anxiety (Weaver et al. 2006; Seckl and Meaney 2006; Maccari et al. 2014). This has not been addressed in this study though. Several earlier studies (Bodnoff et al. 1987; Ferre et al. 1995; Nunez et al. 1995) demonstrated long lasting anxiolytic effects of early postnatal handling into adult age. In these studies, handling was paralleled by brief dam pup separations. By contrast, longer periods of separation caused long lasting, anxiogenic, i.e. detrimental effects in the offspring (Huot et al. 2001; Kalinichev et al. 2002; Daniels et al. 2004).

In conclusion, the present study provides evidence that early postnatal environmental enrichment can interfere with maternal behaviour in rats and behavioural changes in the offspring. The latter finding warrants further explorations as to what extent the open-field data reflect a robust anxiogenic effect.

The findings suggest that enrichment procedures can have unintended effects, potentially interfering with the normal development of emotional behaviours in rats.

Declaration of interest

The authors report no conflict of interest

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Legends

Fig. 1

Duration of maternal (A) and non-maternal behaviours (B,C,D) of the dam during the 45 minutes observation period. Dam from the enriched group (black columns) show less nursing, but more non-maternal behaviours when compared to control dams (white columns). Mean + SEM. N = 8/group *P<0.05; **P<0.01. Student's *t*-test.

Fig.2

Offspring open field behaviour. Offspring exposed to an enriched environment during lactation (black columns) show behavioural signs of increased anxiety when compared to offspring from control group (white columns). M=male, F=female. Mean + SEM. n = 8/group *P<0.05; **P<0.01. Significant effect of enrichment. Two-way-ANOVA.

Fig.3

Offspring plus maze behaviour. Offspring exposed to an enriched environment during lactation (black columns) show no behavioural change when compared to offspring from control group. M=male, F=female. Mean + SEM. n = 12/group