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Research report

Bred to breed?! Implications of continuous mating on the emotional status of mouse offspring

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HIGHLIGHTS

- Continuous mating affects the emotional behaviour of mouse offspring.
- Females are more sensitive to disturbances in the perinatal environment.
- Effects of the breeding environment should be included in the experimental design.

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ABSTRACT

Working with mice represents a smart method to study pathophysiological mechanisms in vivo. However, using animals as model organisms also bears immense caveats. While many aspects in animal research are meanwhile standardized (e.g. nutrition, housing, health) the breeding environment remains unaddressed.

Moreover, since the “production” of mice is mostly performed pragmatically, continuous mating (CM) represents a common method to boost the amount of offspring. This condition implies simultaneous pregnancy and lactation in presence of the male, which is associated with increased costs for the breeding dam.

Facing the widely-accepted impact of perinatal conditions, our aim was to elucidate how CM affects emotional behaviour of mouse offspring. We therefore compared pregnant mice in CM with mice raising their pups without potentially disturbing influences. According to our hypothesis CM-deriving offspring should demonstrate increased anxiety and depression-like behaviour shaped by pre- and postnatal stress of the mother.

Maternal care, i.e. nest building and pup retrieval, was analysed around delivery. To assess the emotional state of the offspring, males and females of either condition were exposed to a behavioural test battery for exploration, anxiety and fear, social and despair behaviour. In addition we analysed corticosterone as stressphysiological correlate.

Our study demonstrates that CM affects the emotional phenotype regarding nearly all parameters addressed. These findings emphasize (i) the impact of the perinatal environment on stress-associated behaviour such as depression, and (ii) the need to imply perinatal conditions in the experimental design to decrease the risk of artefacts and increase the overall validity of animal studies.

1. Introduction

Without any doubt the mouse has become the most prominent model organism over the last decades [1,2]. This is due to the relatively easy handling, a great variability of in- and outbred strains with distinct properties and of course the high amount of offspring which can be generated within a conceivable time window [3]. Other arguments to work with mice include the great number of publications that are accessible to look up experimental protocols, to review and cite recent findings in this species.

Abbreviations: NC, Novel cage; NT, Nest test; OF, Openfield; NO, Novel object; DLB, Dark-Light Box; SR, Social Recognition Test; FST, Forced Swim test; h, hour; PND, postnatal day; m, male; f, female; CM, continuous mating; CON, control; g, gram.

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Working with mice nowadays is highly standardized. There are advices and guidelines for nearly all conditions, which could affect the experimental outcome. Recommendations of this kind imply the consideration of health state, e.g. SPF, housing conditions, nutrition, genetic background etc. While this is definitely essential to consider and in extension needs to be added to any data published, the breeding strategies of mice remain unaddressed.

The reproduction of mice in the wild varies seasonally and – as in most mammals – long daily light phases in the circadian cycle support robust reproduction. Thus laboratory mice are permanently kept on an artificially long (12 h of light 12 h of dark) light cycle. Gestation in mice takes around 21 days. When the litter is delivered maternal care behaviour comprises nestbuilding, grooming and lactation, which may vary in different strains of mice [4–8].

What is known and described by recent literature is that the perinatal environment, including prenatal as well as neonatal stress evokes long-lasting effects, especially with regard to stress-sensitive aspects [4,7,9–19]. A fortiori it is surprising that no official statements concerning breeding procedures exist are made by scientific societies. Moreover, in fact one has to realize that breeding of experimental animals is mostly motivated by the need of a great amount of offspring, be it on side of the commercial breeders or of scientists. Whether or not this is fruitful for the overall validity of the results is not questioned anywhere.

One example of breeding strategies represents the continuous mating (CM) of mice. The intention of this method is to benefit from the postpartum oestrus as female mice present it in natural but also laboratory settings. Thus this strategy requires the permanent presence of a male counterpart that mating can take place directly after delivery of the litter. It also demands from the female to bear the costs—in the truest sense of the word [20,21]. While simultaneously nursing the actual litter with all energy available, the female is stressed by the upcoming pregnancy with all physical challenges that are associated with such a state. Additionally there is no way to cope, e.g. avoid the contact with the male, which could also be potentially dangerous for the youngborn pups [22].

Facing the unsatisfactory awareness that such perinatal conditions, we were interested in elucidating the following hypothesis: CM represents a sustainable form of perinatal stress that is induced by several challenging factors, (i) stress of the dam which is induced by an increase of costs and permanent presence of the male and (ii) stress of the offspring due to the limited energy the mother can raise when taking care of two generations at the same time.

To follow up these thoughts our experimental design comprised two parts of investigations. First, maternal behaviour, i.e. nestbuilding, maternal exploration and pup retrieval were analysed. The second set of experiments assessed emotional behaviour of male and female offspring, which were analysed in a behavioural test battery. Emotional behaviour was examined by exploring changes in activity, anxiety, social behaviour as well as fear and depressive-linked aspects.

All CM-induced behaviours were consecutively compared with those of dams maintained with one single litter and the behaviour of the offspring.

2. Materials and methods

2.1. Housing and breeding conditions

Female mice at an age of 3–4 month were derived from Charles River Germany (Charles River, Sulzfeld) and were experienced in bringing up litters.

All mice were housed in Macrolon cages type III (Tecniplast, Italy), which contained wooden chips (ABEDD LTE-001, Lab & Vet Service, Vienna, Austria), tissue nesting material made of cellulose and food and water ad libitum (Rod16A, Lasvendi, Soest, Germany). Housing of the animals was standardized by 12:12 h dark–light cycle (dark phase: 9.00 a.m.–9.00 p.m.) at a room temperature of $22 \pm 2\,^{\circ}$C. Humidity was set at 50%. The hygienic status was SPF according to recent FELASA recommendations [23]. Before first mating female mice were allowed to acclimate to the new housing room for 14 days.

We examined two groups of mice: *Group I (n = 7)*: control group (CON): mating was performed in Macrolon cages type III with one male (2–3 months) and two female mice (3–4 months). Females were separated from the male when pregnancy was ascertained by positive vaginal plug check and indicative weight changes. Females from then on were housed individually under same conditions outlined above. After delivery dams were left undisturbed till pup retrieval testing at postnatal day (PND) 7. Weaning took place at PND21, from then on brothers and sisters were group-housed in type III cages equipped with wooden bedding and tissue as nesting material and food and water ad libitum (Rod16A, Lasvendi, Soest, Germany). *Group II (n = 5):* continuous mating group (CM): mating was performed in Macrolon cages type III with one male (2–3 months) and one female mice (3–4 months). Males remained in breeding cages during pregnancy and lactation to generate the CM condition. All other housing conditions were comparable to those of the CON animals. The comparably smaller number of dams in Group CM was caused by cannibalism and disrupted pregnancy.

2.1.1. Maternal behaviour

The assessment of maternal behaviour comprised: (i) pup retrieval behaviour (ii) maternal exploration and (iii) nest building.

Maternal exploration and nest building performance was studied once a week (exceptionally during light phase between 8.00 and 9.00 a.m.) during pregnancy and during lactation. Pup retrieval testing was conducted unique at PND 7.

If indicative weight changes could be observed one week after mating the first maternal care tests were conducted during pregnancy. Thus the maternal care assessment comprised two timeframes during pregnancy and during breeding.

To avoid interference with test procedures with accompanying disturbance of dams and litter, tests were conducted within cage change procedures.

2.1.1.1. Pup retrieval behaviour: Pup retrieval test. On postnatal day (PND) 7 the pup retrieval behaviour was tested. Two pups were put into the far corners of the nest in the homecage. The latency until the dam retrieved her pups back into the nest was recorded [24]. The dams had a maximum of 300 s to perform the retrieval.

2.1.1.2. Maternal exploration: Novel cage test. In the context of cage changes once a week, the animals were placed into a new macrolon cage type III containing a layer of new bedding material and the latency as well as total number of rearings were measured for 5 min at the end of the light phase (during breeding period the pups are placed into new cage with the dam before measuring behaviour), (CM group: males were placed after novel cage testing into new cage).

2.1.1.3. Maternal nest building performance: Nest test. After novel cage test the dam with her pups (and for the CM group also the male) remained in the new cage. A nestlet (PLEXX, Arnheim) was introduced into new cage and nest building was analysed by scores of Deacon [25] after 5 and 24 h. Score 1: nestlet untouched, Score 2: nestlet partly picked to pieces, Score 3: nestlet completely picked to pieces, Score 4: recognizable nest, Score 5: complete covered nest.