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Behavioral profiles and stress-induced corticosteroid secretion in male Wistar rats subjected to short and prolonged periods of maternal separation

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Abstract

Early life experiences are important for the development of neurobiobehavioral mechanisms and subsequent establishment of mental functions. In experimental animals, early life experiences can be studied using the maternal separation model. Maternal separation has been described to induce neurobiological changes and thus affect brain function, mental state and behavior. We have established a protocol in order to study the effects of repeated short and prolonged periods of maternal separation during the postnatal period on adult neurochemistry, voluntary ethanol intake and behavior. In the present experiment, we focus on the long-term effects of maternal separation on exploration and risk assessment behavior as well corticosteroid secretion. Rat pups were assigned to 15 min (MS15) or 360 min (MS360) of daily maternal separation and normal animal facility rearing (AFR) during postnatal days 1–21. To establish the adult behavioral profile in male rats, three tests were used: the Concentric Square Field (CSF), the Open Field (OF) and the Elevated Plus-maze (EPM). No differences between the three experimental groups were found in the traditional OF and EPM tests. The CSF test indicated that the MS360 rats were more explorative and expressed an altered risk assessment and risk-taking profile. In response to a restraint stress, MS360 rats had a blunted corticosterone release in contrast to MS15 and AFR rats. In contrast to previous results, the outcome of the present investigation does not support the notion that a prolonged period of maternal separation results in an adult phenotype characterized by an increased emotional reactivity.
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Introduction

The role of early life events in the development of neurobiobehavioral mechanisms and subsequent establishment of mental functions has been extensively studied. In humans, adverse experiences early in life such as parental loss, neglect and physical and/or sexual abuse can enhance the vulnerability to develop psychopathology in adulthood (e.g., De Bellis, 2005; Gilmer and McKinney, 2003; McEwen, 2003; Nemeroff, 2004). Furthermore, besides the fact that environmental factors can constitute risk factors, the possibility for the early life environment in providing protection against adult psychopathology has recently gained increasing support (Caspi et al., 2002, 2003; Foley et al., 2004; Nilsson et al., 2005; Sjöberg et al., 2005).

The neurobiological mechanisms underlying risk factors and protective factors for the vulnerability to develop psychopathology are not fully understood. Longitudinal studies in humans are time consuming and studies that control for environmental stimuli are almost impossible to conduct. Therefore, studies in experimental animals are favourable and the maternal separation paradigm in rats is one method of choice. During the first weeks of life, the rat pup is dependent on the mother for nursing, protection and normal development. In the course of normal mother–pup interaction, the dam shows an intense care giving behavior but also regularly leaves the pups for short and sometimes prolonged periods of time (Calhoun, 1962; Fleming and Rosenblatt, 1974; Jans and Woodside, 1990). Under experimental conditions, several different protocols for maternal separation are currently in practice, where pups are

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separated from the dam in litters or also isolated from the littermates. The maternal separation can be performed at a single occasion, at several time periods or repeatedly during the postnatal period. Many experimental protocols for maternal separation utilize both short periods (3–15 min) of maternal separation, also referred to as handling, and longer periods (often >180 min) of separation. Control groups are either non-handled or housed under normal animal facility rearing (AFR) conditions. A variety of long-term neurochemical, hormonal and behavioral changes have been observed after short and prolonged periods of maternal separation (Ladd et al., 2000; Lehmann and Feldon, 2000; Pryce and Feldon, 2003).

We have established a maternal separation protocol including short and prolonged periods of maternal separation for studies of early environmental effects on brain function, voluntary ethanol intake and behavior. Using this model, we have provided evidence for long-lasting neurochemical alterations and certain divergences in behavior (Ploj et al., 2002, 2003a,b). Furthermore, long-term effects on voluntary ethanol intake in adult male rats (Ploj et al., 2003a; Roman et al., 2003, 2005) and the absence of effects (Gustafsson et al., 2005; Roman et al., 2004) or minor effects (Roman et al., 2005) in female rats indicate sex differences in the outcome of maternal separation.

The aim of the present study was to explore what consequences maternal separation might have on adult performance that express reaction to novelty. To this end, we compared the effects of short and prolonged periods of maternal separation upon the adult animal's performance in an environment that offers a choice between more or less sheltered or non-sheltered areas. The test situation was designed for the specific aim of investigating the animal's strategy in gathering information including risk assessment and risk taking. For this purpose, the multivariate Concentric Square Field (CSF) test (Meyerson et al., 2006) was used. As reference tests, the more traditional Open Field (OF) test and the Elevated Plus-maze (EPM) test (Hogg, 1996; Lister, 1990; Pellow et al., 1985) were used in order to assess locomotor ability and aspects of emotional reactivity, respectively. In addition, we investigated the corticosterone response to a physical stressor, i.e., restraint stress, and the association between the behavior paradigm and the corticosterone response.

Materials and methods

Animals and housing

Forty-two time-mated pregnant Wistar rats (Scanbur BK AB, Sollentuna, Sweden) arrived at the animal facility on gestational days 13–15. Upon arrival, the dams were singly housed in standard macrolon cages (59×38×20 cm) containing wood chip bedding material and nesting material. The dams were maintained on standard pellet food (R36 Labfor; Lactamin, Vadstena, Sweden) and water ad libitum in a room used for this experiment only. The animals were housed in a temperature (22±1°C)- and humidity (50±10%)-controlled environment on a 12-h light/dark cycle with lights on at 6:00 a.m. At the time for behavioral testing in adulthood, the animals were housed under a reversed light/dark cycle regimen, see Section Experimental design of the behavioral testing procedures. Gloves were used in all contact with the animals. All animal experiments were performed under a protocol approved by the Uppsala animal ethical committee and in accordance with the Swedish Animal Protection Legislation.

Maternal separation procedures

The pups were sexed by measurement of ano-genital distance and culled on the day of birth (postnatal day (PND) 0). The litters were cross-fostered and arranged to contain 10 pups with a homogenous distribution of males and females. The litters were thereafter randomly assigned to one of three rearing conditions: maternal separation for 15 min (MS15) or 360 min (MS360) or housed under normal AFR conditions (n=8 litters/experimental group). The maternal separation procedures occurred once daily during PND 1–21, starting at 9:00 a.m. for the MS15 groups and at 9:30 a.m. for the MS360 groups. First the dam and then the whole litter were removed from the home cage. The pups were kept close together at all times in the respective litter. Each litter was placed in macrolon cages (26×20×14 cm) containing wood chip bedding material and moved to an adjacent room (27±1°C). The cages in which the litters were placed during the separation were changed every day. In the MS360 group, the dams were returned to their home cages during the separation procedure, but taken out prior to the return of the litters. In the MS15 group, the dams were transferred to another cage during the separation and the litters were returned to the home cages before the dams. Separation sessions were always performed in the same room. Home cages were changed, with old bedding material mixed with clean bedding material, three times during the period from the arrival of the dams until PND 22 for all experimental groups. During the separation period, the same three persons performed the separation procedures and the cage changes. The MS15 and MS360 litters were weighed before the separation on PND 1, 4, 7, 10, 13, 16 and 19, and the AFR litters were weighed when the cages were changed on PND 7 and 16. On PND 22, all litters were weighed and the pups were weaned. Thereafter, the animals were housed in same-sex and same-experimental groups of five animals per cage in standard macrolon cages (59×38×20 cm). The rats were left undisturbed except for cage changes two times a week, done by one of the experimenters who also carried out the separations. Seven weeks after weaning (PND 59–62), 1–2 males per litter were randomly selected and used for behavioral testing. The rest of the animals were used for other analyses.

Experimental design of the behavioral testing procedures

The males (n=15 rats/group) were transferred to an animal room (temperature 22±1°C and humidity 50±10%) with a reversed 12-h light/dark cycle (lights off at 9:00 a.m.) and allowed to adapt for 2 weeks before the start of the behavioral testing, on PND 73–76. Three methods were used: the Concentric Square Field (CSF) test, the Open Field (OF) test and Elevated Plus-maze (EPM) test. The tests were chosen to cover measures of general activity, exploration, and approach and avoidance performance in open versus sheltered areas. The purpose of using the multivariate CSF test was to gather information that taken together would illustrate the influence of short and prolonged periods of maternal separation on adult strategies for risk assessment and risk taking. The OF and EPM tests were used as reference tests for assessment of locomotor ability and emotional reactivity, respectively. Each animal was run in the three behavioral tests during 3 consecutive days for a period of 2 weeks starting with the CSF, then the OF and finally the EPM test. The sequential order of the three behavioral tests was based on a pilot study in mice using the same battery of tests. By alternating the order of the different tests, it was found that parameters recorded in the CSF test, but not in the other two tests, were affected. It was therefore concluded that the CSF test was most sensitive to previous experience and should be performed as the first test in the battery of tests in order to reduce the likelihood of any carry over effects (Augustsson, 2004). The rats in each experimental group were tested using a running schedule in order to avoid time and order bias. All testing was performed in an adjacent room with similar temperature and humidity conditions as in the animal room. Observations were carried out during the dark period of the light/dark cycle, starting at 11:30 a.m. The animals were weighed at the end of the first day of testing.

Behavioral tests

The multivariate CSF test

The multivariate CSF test has been described in detail elsewhere (Meyerson et al., 2006). The technique has been used to measure risk/benefit assessment in rats after experimental brain lesions (Clausen et al., 2001; Roos et al., 2003) and

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