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Research report

The effects of early-life adversity on fear memories in adolescent rats and their persistence into adulthood

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HIGHLIGHTS

- Maternal separation (MS) decreases the expression of learned fear in adolescents.
- MS-induced impairments of fear conditioning persist into adulthood.
- MS may increase the risk for early- and late-onsets psychopathologies.

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ABSTRACT

Adolescence is a developmental period characterized by extensive morphological and functional remodeling of the brain. The processes of brain maturation during this period may unmask malfunctions that originate earlier in life as a consequence of early-life stress (ELS). This is associated with the emergence of many psychopathologies during adolescence, particularly affective spectrum disorders. In the present study, we applied a maternal separation (MS) procedure (3 h/day, on postnatal days 1–14) as a model of ELS to examine its effects on the acquisition, expression and extinction of fear memories in adolescent rats. Additionally, we studied the persistence of these memories into adulthood. We found that MS decreased the expression of both contextual (CFC) and auditory (AFC) fear conditioning in adolescent rats. Besides, MS had no impact on the acquisition of extinction learning. During the recall of extinction MS animals both, those previously subjected and not subjected to the extinction session, exhibited equally low levels of freezing. In adulthood, the MS animals (conditioned during adolescence) still displayed impairments in the expression of AFC (only in males) and CFC. Furthermore, the MS procedure had also an impact on the expression of CFC (but not AFC) after retraining in adulthood. Our findings imply that ELS may permanently affect fear learning and memory. The results also support the hypothesis that, depending on individual predispositions and further experiences, ELS may either lead to a resilience or a vulnerability to early- and late-onsets psychopathologies.

1. Introduction

Fear memory is an important acquired ability that allows an organism to predict and avoid aversive events. Acquisition of fear memory (i.e., fear conditioning: FC) is a process in which humans and animals learn that usually neutral sensory information gains a value as predictor of danger or probability of an aversive situation. In functional terms, such fear memories help to minimize exposure to threat and elicit escape and/or avoidance responses [1]. When fear memory traces are formed with inappropriate strength or duration, they may lead to malfunction of behavioral adjustment to life demands and challenges. For example, if the traces of fear memories are too weak, this condition may lead to engagement in life-threatening or risky actions. On the other hand, if the fear response is over-generalized and/or fear memories excessively strong, this state may result in anxiety disorders such as phobias, panic disorders or posttraumatic stress disorder (PTSD) [1].

The neural circuits involved in the regulation of fear responses and fear memory formation are evolutionarily conserved; thus, the mechanisms of fear learning can be successfully studied in both humans and laboratory animals using various behavioral paradigms [2,3]. One of the most frequently applied behavioral paradigms is classical (Pavlovian) FC. In classical conditioning, a

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neutral sensory stimulus (the conditioned stimulus; CS), such as a tone, is presented in close temporal contiguity with an aversive stimulus (the unconditioned stimulus; US), such as an electric shock. After several presentations of the CS + US pairing, the subjects form an association between the stimuli, and they respond to the CS with a conditioned response (CR), such as freezing, even in the absence of the aversive US [4]. However, with repeated presentation of the CS alone, the CR gradually diminishes; this process is called extinction [3,4]. In adolescents and adults, extinction is a form of learning in which subjects learn that exposure to the neutral CS is not a predictor of a potential threat [5]. The principles of fear extinction underlie exposure therapy, which is applied in PTSD treatment [1].

Recent studies have shown that fear learning changes across development in both humans and rodents, especially fear extinction learning [6–9]. It has been revealed that the process of extinction in adolescents is less effective and requires extended extinction training (increased number of the CS alone presentations) in comparison with younger subjects and adults [6,10]. This result is related to the extensive morphological and functional remodeling that occur in many brain regions during adolescence [11,12]. Generally, adolescent cortical areas are less mature than subcortical areas (e.g., the amygdala). The adolescent period is also characterized by prominent synapse pruning, a reduction in gray matter volume, dynamic changes in receptors systems and intensive myelination [11,12]. These processes occur especially at the level of the medial prefrontal cortex (mPFC), which is engaged in fear expression in addition to the expression and consolidation of extinction memory [1]. Considering the dramatic changes that occur in the adolescent brain, the emergence of psychopathologies, particularly affective spectrum disorders, in adolescence is not surprising [12,13]. Moreover, brain maturation during this period may unmask malfunctions that originated earlier in life; thus, adolescence is a period when early experiences will manifest [12,14]. Clinical studies demonstrate that early-life adversity increases the risk of mood and anxiety disorders, behavioral disorders and substance use disorders in childhood and adolescence [15].

Numerous studies in animal models clearly demonstrate that early-life stress (ELS) affects the acquisition and expression of FC in adults [16–22]. Nevertheless, very little is known regarding how ELS influences fear learning and extinction during adolescence. Our previous study demonstrated that ELS in the form of maternal separation (MS) strongly affected structural and functional plasticity of the mPFC in adolescent rats and increased innate anxiety-like behavior [23]. Therefore, in the present study we continued to investigate the effect of ELS on adolescent behavioral and brain plasticity. We reported the impact of MS on the acquisition, expression and extinction of FC in adolescent male and female rats. We also examined whether fear memories acquired during adolescence can persist into adulthood. Additionally, to fully control experimental conditions, a somatic pain threshold was assessed and locomotor activity monitored during all phases of the memory tests.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Committee for Laboratory Animal Welfare and the Ethics Committee of the Institute of Pharmacology, PAS, in Krakow and met the requirements of the European Council Guide for the Care and Use of Laboratory Animals (86/609/EEC).

Primiparous Wistar dams (Charles River, Germany) were mated in the animal facility at the Institute of Pharmacology, PAS, in Krakow, and their offspring were used in this study. The dams were individually housed under standard conditions with an artificial 12 h light/dark cycle (lights on from 07:00 to 19:00 h), and food and tap water were freely available. The date of birth was designated as postnatal day (PND) 0. On PND 1, the litter size was standardized to eight pups per litter (four males and four females), and the litters were assigned to one of the following rearing conditions: maternal separation (MS) or animal facility-reared (AFR). The animals remained in the assigned rearing conditions until PND 14. The animals were weaned on PND 22 and housed under standard conditions (as described above) in same-sex groups (4 animals per group) and under the same treatment protocol until PND 35 (adolescence). For the purposes of this study, both male and female rats were subjected to further behavioral analysis during adolescence and adulthood (PND 70). All experimental procedures applied in the present study and the number of animals per group are given in Table 1.

Table 1
A summary of experimental procedures.

PND	Procedure	*n*
1–14	Maternal separation (3 h/day)	10 litters
35	Paw pressure and tail-flick tests	10
35	Flinch test	6
35	Day 1: FC acquisition (Context A)	20*
36	Day 2: CFC expression (Context A) Extinction of AFC (Context B)	20* 10
37	Day 3: Recall of extinction of AFC (Context B)	10
70	CFC memory test in adulthood (Context A)	10
71	AFC memory test in adulthood (Context B)	10
77	Day 1: FC retraining in adulthood (Context A)	10
78	Day 2: CFC expression after retraining in adulthood (Context A) AFC expression after retraining in adulthood (Context B)	10

Abbreviations: AFC, auditory fear conditioning; CFC, contextual fear conditioning; FC, fear conditioning.
Note: on PND 35, separate groups of animals were used for nociceptive threshold measurements (a paw pressure test together with a tail-flick test, flinch test) and for FC tests.
n – number of subjects per experimental group.
* – before random assignment to Extinction and No Extinction subgroups.

2.1.1. Maternal separation

The MS procedure used in this study was previously described by Chocyk et al. [23–26]. Briefly, on PNDs 1–14, the dams and pups were removed from the maternity cages for 3 h each day (09:00–12:00). The mothers were placed individually in holding cages, while each litter was placed in a plastic container lined with fresh bedding material, moved into an adjacent room and placed in an incubator that was set at a constant temperature of 34 °C. After the 3 h separation, the pups and dams were returned to the maternity cages. Once per week, the maternity cages were cleaned at the time of the separation procedure. The AFR animals were left undisturbed with their mothers except during the weekly cage cleaning. The specific impact of the MS procedure on maternal and pup behaviors was described in detail in [23].

2.2. Assessment of nociceptive threshold

In order to assess the effects of different rearing conditions and sex on the nociceptive threshold, a paw pressure test, tail flick test and a flinch test were performed on PND 35 in a separate groups of animals that were not used in the FC tests.

The paw pressure test was previously described by Randall and Selitto [27]. Briefly, the rats were maintained in a normal/horizontal position in the hand of the researcher. The right hind paw was placed in an analgesiometer (Ugo-Basile, Italy). The apparatus