Physiology & Behavior 131 (2014) 57–61

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Effects of juvenile exposure to predator odor on adolescent and adult anxiety and pain nociception

Ryan J. Post, Kaitlyn M. Dahlborg, Lauren E. O'Loughlin, Christopher M. Bloom *

Providence College, 1 Cunningham Sq., Providence, RI 02918, USA

HIGHLIGHTS

- Animals exposed to stress in juvenility had increased anxiety in adolescence.
- A secondary adolescent stressor negated anxiety differences prior to the stressor.
- Animals stressed in juvenility demonstrated analgesia in adulthood.

ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form 11 March 2014
Accepted 4 April 2014
Available online 13 April 2014

Keywords:
Trimethylthiazoline
Predator odor
Juvenile stress
Analgesia
Anxiety
Animal models

ABSTRACT

Clinical researchers have tracked patients with early life trauma and noted generalized anxiety disorder, unipolar depression, and risk-taking behaviors developing in late adolescence and into early adulthood. Animal models provide an opportunity to investigate the neural and developmental processes that underlie the relationship between early stress and later abnormal behavior. The present model used repeated exposure to 2,3,5-trimethyl-3-thiazoline (TMT), a component of fox feces, as an unconditioned fear-eliciting stimulus in order to induce stress in juvenile rats aged postnatal day (PND) 23 through 27. After further physical maturation characteristic of the adolescent stage (PND 42), animals were tested using an elevated plus maze (EPM) for anxiety and plantar test (Hargreaves method) for pain to assess any lingering effects of the juvenile stress. To assess how an additional stress later in life affects anxiety and pain nociception, PND 43 rats were exposed to inescapable shock (0.8 mA) and again tested on EPM and plantar test. A final testing period was conducted in the adult (PND 63) rats to assess resulting changes in adult behaviors. TMT-exposed rats were significantly more anxious in adolescence than controls, but this difference disappeared after exposure to the secondary stressor. In adulthood, but not in adolescence, TMT-exposed rats demonstrated lower pain sensitivity than controls. These results suggest that early life stress can play a significant role in later anxiety and pain nociception, and offer insight into the development and manifestation of anxiety- and trauma-related disorders.

1. Introduction

It is widely accepted that stress and trauma in early life can predispose an individual to stress and anxiety disorders later in life [11]. In their meta-analysis Kessler et al. [10] reported lifetime prevalence of anxiety and mood disorders to be 28.8% and 20.8%, respectively and found that these disorders typically emerge in adolescence. Among these disorders are post-traumatic stress disorder (PTSD) and non-suicidal self-injury (NSSI), both of which are linked to stress [3,11].

A large body of work has been published regarding the importance of developmental time periods when considering the anxiogenic and cognitive effects of stress exposure. In their study of the effect of a time lapse between two stressors and the consequent behavioral outcomes, Avital and Richter-Levin [2] concluded that the developmental period at which stressors occur is more predictive of later emotional and cognitive dysfunction than the length of time between two stressors. When exposed to a stressor first in juvenility and then again in adulthood, rats had lower anxiety-like behaviors and poorer spatial learning than rats exposed to both stressors in adulthood but with the same intervening amount of time between stressors. These results suggest a dynamic effect of early stress insofar as it correlates with lower anxiety-like behaviors but at the cost of poorer cognitive learning. Although it is well agreed upon that stress affects anxiety-like behaviors, the direction of that effect is a matter of some controversy. Tsoory and Richter-Levin [18] reported that stress-coping behaviors exhibited in adulthood are significantly influenced by the period of early development at which stress was experienced. Rats stressed in juvenility (postnatal day 28; PND) and rats stressed in adolescence (PND 34) showed equal deficits in avoidance learning compared to controls, but only juvenile-stressed rats *also* demonstrated significant learned

* Corresponding author at: Department of Psychology, Providence College, 1 Cunningham Sq., Providence, RI, USA. Tel.: +1 401 865 2613.
E-mail address: cbloom@providence.edu (C.M. Bloom).

http://dx.doi.org/10.1016/j.physbeh.2014.04.009
0031-9384/

helplessness-like behavior in adulthood. These findings suggest that juvenility is a more impactful stress-sensitive period than adolescence and that stress during juvenility has severe anxiogenic, rather than anxiolytic, effects in adulthood [18]. Regardless of the direction of effect, the most prevalent hypothesis is that stress during development significantly impacts the immature neuroanatomy.

Neuroanatomical research has found that the hypothalamic–pituitary–adrenal (HPA) axis is an essential structure in mediating stress responses. McCormick et al. [12] have shown that stress during adolescence has longer lasting behavioral effects than similar stress experienced in adulthood, perhaps because of the HPA's sensitivity to glucocorticoids and the general abundance of hormones present during adolescence. The medial prefrontal cortex (mPFC), which undergoes significant development from juvenility to adolescence, may also be important as it is suggested that it could be responsible for the relationship between the developmental period at which stress is endured and the subsequent anxiogenic effect [4].

In an effort to further elucidate which developmental time periods are more susceptible than others to stressors and how they relate to later anxiety, the current experiment further investigates the role of early life stress on later emotional functioning but includes aspects of pain nociception to study whether stress-induced analgesia has similar developmental contingencies. Animals in the experimental group were exposed to 2,3,5-trimethyl-3-thiazoline (TMT) odor, a derivative of fox-feces, repeatedly during juvenility. During adolescence, anxiety and pain sensitivity levels were measured before and after exposure to an inescapable shock (IS). Animals were tested again in adulthood to assess any long-term changes as a result of stressors experienced earlier in their development.

2. Methods

All procedures remained in compliance with the animal safety regulations and guidelines set forth by the Providence College IACUC.

2.1. Animals

Twenty male Sprague–Dawley rats were delivered from Charles River Laboratories (Wilmington, MA) at age 22 days and individually housed in 25 × 25 × 35 cm Plexiglas cages maintained in temperature controlled (21.6 °C) quarters with a 12-h light–dark schedule (lights on 8:00–20:00 h). Ten rats were randomly assigned to each of the control and experimental conditions. Animals were allowed 24-h acclimation before the start of the experimental procedure, and had ad libitum access to standard solid-pellet rat food (LabDiet ProLab® RMH 3000, Wausau, WI) and water throughout the experiment.

2.2. Behavioral procedures

2.2.1. Scent exposure in metabolic cage

On PND 23 through 26, rats were individually placed in small (48 × 28 × 36 cm) metabolic cages (Harvard Apparatus, Holliston, MA) and exposed to 0.5 mL of either 10% TMT (Contech Enterprises, Delta, B.C., Canada) or water vapor, which was pipetted into a cotton ball and placed below the metal grid floor of the cage. The control group ($n = 10$) was exposed first on all 4 days to ensure that their testing environment was not affected by any TMT odor that may have lingered in the room. Rats remained in the exposure chamber for 30 min, after which boli were counted as a measure of fear and anxiety. The cages were then thoroughly cleaned and the experimental group ($n = 10$) was exposed to TMT in the same manner.

2.2.2. Scent exposure in open field

On PND 27, rats were exposed in an open field apparatus in order to analyze motion as a measure of fear and anxiety. Contained in a sound-attenuating chamber, the open field consisted of an open-top Plexiglas box (43.2 × 43.2 × 30.5 cm) with infrared motion sensors lining the walls (Med Associates Inc., St. Albans, VT). Activity Monitor MDB software recorded the amount of time the rat spent in the center of the field (13.2 × 13.2 cm; Med Associates Inc., St. Albans, VT). Animals were individually exposed and the control rats were run first, again to prevent any lingering scent of TMT. A cotton ball with 0.5 mL of either 10% TMT or water vapor was placed on a small glass directly outside of the open field, but within the chamber in which the field was contained. Rats were exposed for 30 min and motion was detected with the Activity Monitor software within the open field hardware (Med Associates Inc., St. Albans, VT).

2.2.3. Plantar test and EPM

At age PND 42, pain sensitivity and anxiety-like behavior were measured with the plantar test (Hargreaves Method; IITC Life Science, Inc., Woodland Hills, CA) and an elevated plus maze (EPM; custom-built, Providence, RI), respectively.

In a protocol adapted from Hargreaves et al. [8], the glass surface of the plantar test apparatus was allowed to reach a uniform 29 °C, at which point rats were placed five at a time into plastic cubicles (9 × 22 × 25 cm) atop the surface. After a 10 min habituation period, a focused, high-intensity projector lamp beam was shone below onto the mid-plantar surface of the left hind paw of the first rat. Initially at an idle intensity of 10%, the lamp was then activated, at which point it gradually increased to a maximum of 50% active intensity. When the paw was withdrawn from the surface, or after a maximum of 25 s, the lamp was switched off and latency from the start point was recorded. In identical fashion, latency for withdrawal of the left hind paw was recorded for the remaining rats, followed by a 2 min inter-trial interval, and then latency for each rat's right hind paw was recorded. For every rat, three trials of each hind foot were recorded, the median latency for each paw was kept, and the two median values were averaged.

The rats were then transferred back to home cages for a period ranging from 10 to 30 min acclimation period before undergoing the EPM test, during which time the next five rats began the plantar test. Following the acclimation period, subjects were tested on EPM. The custom-built maze consisted of four 50 cm arms made of polished particle board mounted on a medium-density fiberboard frame. Two of the arms were surrounded by 30 cm-high walls, and the entire maze sat 50 cm above the floor. Rats were placed in the center of the maze and allowed to freely explore the enclosed and open arms. Suspended from the ceiling was an EthoVision camera which recorded the amount of time spent on open and closed arms (Noldus Information Technology, Wageningen, Netherlands). After 5 min, the rat was removed from the maze, returned to its home cage, and the process was then repeated for the next rat.

2.2.4. Inescapable shock

On PND 43, 24 h after the first set of plantar and EPM testing, all rats were subjected to IS to examine the effect of a stressor in adolescence—both alone and in conjunction with juvenile stress—on pain tolerance and anxiety-like behavior. Rats were placed five at a time in individual operant chambers (30.5 × 24.1 × 29.2 cm) with metal grid flooring connected to an electric shocker controlled by MED-PC IV (Med Associates Inc., St. Albans, VT) software. Subjects were shocked at 0.8 mA intensity for 1 s every minute for 30 min. After the 30 min exposure period, the five rats were moved to the plantar test and EPM where they were tested for pain sensitivity and anxiety-like behavior under the same protocols as described in Section 2.2.3.

2.2.5. Adulthood

On PND 64, all rats were tested again on plantar and EPM under the same protocols as previously described in Section 2.2.3 to examine long-term effects of early life stressor on pain tolerance and anxiety-like behavior. A summary timeline of rat development and experimental manipulations is shown in Fig. 1.