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Short communication

Short-term and long-term effects of postnatal exposure to an adult male in C57BL/6J mice

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Abstract

Rodent models provide a valuable approach to elucidating the pathophysiological mechanisms underlying the deleterious effects of childhood trauma and stress. Neonatal rats and mice emit ultrasonic vocalizations (USVs) when separated from the dam and litter. USVs are suppressed in rat pups by exposure to the putatively infanticidal threat of an adult male. In the present study, C57BL/6J mouse pups were exposed to an anaesthetized (non-sire) adult C57BL/6J male for 3-min/day from postnatal days 2–14, and subsequently tested for anxiety-related behaviors (using the novel open field, elevated plus-maze, light/dark exploration tests) and depression-related behavior (using the forced swim test) at 11 weeks of age. In a separate cohort, hypothalamic–pituitary–adrenal-axis activation was measured via plasma corticosterone levels following either a single male-exposure or separation episode. Results showed that pups exposed to an adult male emitted significantly fewer USVs than separation-only counterparts. Corticosterone levels were significantly lower following single exposure to the adult male than separation alone. Repeated neonatal male-exposure did not lead to significant alterations in anxiety- or depression-related behaviors in adulthood. Taken together, present data suggest that the form of adult male-exposure employed did not act as a significant stressor, at least in this mouse strain. Further studies will be needed to determine whether alternative mouse strains, exposure protocols or adult behavioral assays will produce a different pattern of short-term and long-term effects.

Keywords: Mouse; Stress; Ultrasonic vocalization; Early life; Predation; Child abuse; Anxiety; Depression

Mounting empirical evidence indicates that early life environmental stressors can exert a pervasive influence on emotional traits and increase risk for mood and anxiety disorders [15]. However, there remains incomplete understanding of how early life stress alters brain function to affect emotion, and how factors such as genetics may interact to mitigate or exacerbate these effects. Rodent models provide a valuable experimental approach to delineating these factors, and there are ongoing efforts to characterize neonatal 'emotion-related' behaviors and the potential long-term consequences of postnatal stress in rats and mice [19,23].

During the neonatal altricial period rodents emit ultrasonic vocalizations (USVs) when separated from the dam and litter; presumably as a means of soliciting maternal retrieval without alerting predators [4,17,26]. Separation-induced USVs in rats and mice are considered an assay of anxiety-related behavior and are reduced by anxiolytic drug treatments [27]. Various factors have been found to influence the emission of USVs, including ambient temperature and the presence of social, olfactory and tactile cues [5,16,31]. A series of studies by Shair and co-workers have shown that exposure to an unfamiliar adult male suppresses USVs in rat pups [7,30,32,33,36]. Because adult males will sometimes kill non-sired litters (possibly as a strategy to increase their own chances of reproduction [6,24]) male-induced suppression of USVs has been construed as an adaptive response to infanticidal threat. In support of this interpretation, exposure to an unfamiliar adult male activates rat brain regions mediating fear and defense such as the amygdala and periaqueductal gray [35] and produces defensive immobility in a manner that

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correlates with the postnatal age when the threat of infanticide is greatest [32–34].

Despite the potential utility of the mouse as a model system for genetic studies of early life stress [10,13], there have been relatively few studies characterizing the effects of exposure to adult males in mice [5]. In the present study, we examined the effects of exposure to an adult male on USVs and hypothalamic–pituitary–adrenal axis activation in C57BL/6J mice. While exposure to predatory stimuli (e.g., a cat or cat odor) is known to produce changes in rodent anxiety-related behaviors [1], the potential long-lasting effects of exposure to infanticidal threat have not been investigated. Therefore, a second aim was to test whether unfamiliar male-exposure would produce long-lasting effects on anxiety- and depression-related behaviors.

Subjects were C57BL/6J mice bred in-house from parents obtained from The Jackson Laboratory (Bar Harbor, ME). This strain was chosen on the basis of its widespread use in behavioral neuroscience and behavior genetics research [18]. Nine litters, each containing six or more pups at birth (designated as postnatal day 0 (PD0)), were divided into three experimental conditions (no more than three mice per condition from a given litter): separated/exposed (S/E), separated/not exposed (S/NE) and not separated/not exposed (NE/NS) ($n = 20$–21 condition). *S/E condition*: between 1100 and 1330 h pups were individually removed from the home cage and placed on a clean paper towel with an anaesthetized (via treatment with 4 g/kg ethanol), sexually intact adult male C57BL/6J mouse that had not sired the pup (or any progeny) for 3 min in an empty holding cage housed within a sound-attenuating chamber (ambient temperature ∼22 °C) (based on method previously described for rats [7]). A novel adult male was used for each daily test session. The stimulus male was anaesthetized in order to control for variability in behavioral interactions with pups that might have occurred across males and across sessions. At the end of the session, the pup was marked and returned to the litter. Test sessions were conducted from PD2 through PD14 inclusive. *S/NE condition*: procedure was the same as for S/E except that pups were separated but not male-exposed. *NS/NE condition*: pups remained with the litter in the home cage. Cages were changed once weekly, avoiding the first few days post-partum. All experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee and followed the National Institute of Health guidelines outlined in 'Using Animals in Intramural Research'.

Ultrasonic vocalizations were recorded during each test session using an ultrasonic detector with built-in microphone (Mini-3 bat detector, Ultra Sound Advice, London, UK) suspended above the pup. Auditory input was amplified and filtered to produce a flat range and converted from analog-to-digital using UltraVox 2.0 software (Noldus Information Technology, Leesburg, VA). Inputs were recorded as USVs if they were between 30 and 80 kHz, lasted longer than 10 ms, and separated from the previous input by at least 20 ms (as previously described for mice [11]).

Subjects remained with the dam and sire until weaning at PD21, at which time they were co-housed with same-sex littermates (2–4 cage) in a temperature- and humidity-controlled vivarium under a 12-h light:12-h dark cycle (lights on 0600 h). Testing for adult behaviors began at 11 weeks of age with 1 week between tests and the putatively more stressful tests occurring later in the sequence, as follows: novel open field, elevated plus-maze, light/dark exploration test, forced swim test. Mice were acclimated to the test room for 1 h prior to each experiment. Where appropriate, apparatuses were cleaned with 70% ethanol solution and dried between subjects.

The novel open field test for locomotor exploration and anxiety-like behavior was conducted as previously described [14]. The apparatus was a 40 × 40 × 35 cm square arena (illuminated to 50 lx) constructed of white Plexiglas. The mouse was placed in the perimeter and allowed to explore the apparatus for 15 min under 65 dB white noise (Sound Screen, Marpac Corporation, Rocky Point, NC) to minimize external noise disturbance. Total distance traveled in the whole arena and time spent in the center (20 × 20 cm) was measured by the Ethovision videotracking system (Noldus Information Technology Inc., Leesburg, VA).

The elevated plus-maze test for anxiety-like behavior was conducted as previously described [14,22]. The apparatus consisted of two open arms (30 × 5 cm; 90 lx) and two closed arms (30 × 15 cm; 20 lx) extending from a 5 × 5 cm central area and elevated 20 cm from the ground (San Diego Instruments, San Diego, CA). The walls were made from black ABS plastic and the floor from white ABS plastic. A 0.5 cm raised lip around the perimeter of the open arms prevented mice from falling off. The mouse was placed in the center facing an open arm and allowed to explore the apparatus for 5 min under 65 dB white noise to minimize external noise disturbance. Time spent in the open arms [(open arm time/total session duration) × 100] and entries into the open and closed arms was measured by the Ethovision videotracking system.

The light–dark exploration test was conducted as previously described [9,14]. The apparatus was housed in sound-attenuating chamber and consisted of two compartments (each 17 cm × 13 cm × 13 cm), one with white Plexiglas walls and clear Plexiglas floor (40 lx) ('light' compartment) and the other with black Plexiglas walls and clear Plexiglas floor (∼1 lx) ('dark' compartment) that were connected by a partition at floor level with a small opening (5 cm) (Med Associates, Georgia, VT, Model ENV-3013). The mouse was placed into the dark compartment facing away from the aperture and allowed to explore the apparatus for 10 min. Time spent in the light compartment and whole-body transitions between the light and dark compartments was measured by photocells connected to Med Associates software.

The forced swim test was conducted as previously described [14,29]. The apparatus was a transparent Plexiglas cylinder 25 cm high, 20 cm diameter) filled halfway with water (24 ± 1 °C). The mouse was gently lowered into the water and manually observed for the presence/absence of immobility (cessation of limb movements except minor involuntary movements of the hind limbs) every 5 s during the last 4 min of a 6-min test session.