

Rough-and-tumble play induces resilience to stress in rats

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Positive emotions have been shown to induce resilience to stress in humans, as well as increase cognitive abilities (learning, memory, and problem solving) and improve overall health. In rats, frequency modulated 50-kHz ultrasonic vocalizations (hedonic 50 kHz) reflect a positive affective state and are best elicited by rough-and-tumble play. A well-established rat chronic unpredictable stress paradigm was used to produce a robust and long-lasting decrease in positive affect, increase in negative affect, and learned helplessness in Sprague-Dawley rats. Rough-and-tumble play (3 min every 3 days) reversed stress-induced effects of chronic unpredictable stress in the Porsolt forced swim test, novelty-induced hypophagia, sucrose preference, and ultrasonic vocalization assays compared with a light touch control group. These data demonstrate that positive affect induces resilience to stress effects in rats, and that

rough-and-tumble play can be used to explore the biological basis of resilience that may lead to the development of new therapeutics for stress-related disorders. *NeuroReport* 28:1122–1126 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Longitudinal studies show that positive affect, as measured by self-reported positive emotion, induces resilience to depression, is anxiolytic, and increases overall health with a concomitant decrease in mortality [1]. The beneficial effects of positive affective states appear to be mediated through the strengthening of diverse neuronal processes that results in increased resilience. Resilience is defined as continued global functioning despite the presence of stressors. For example, a resilient individual is less likely to develop depression or anxiety following a major life stressor [1,2]. The emergence of positive affect precedes the actual reported health benefits of positive affect in longitudinal studies, suggesting a causal relationship [3]. Activities that promote health such as regular exercise reduce depression [4]. Like exercise, only sustained positive affect induces resilience; short-term positive life events have little long-term beneficial effects [5]. In contrast, individuals who exhibit low levels of positive affect are at a greater risk for the development of anxiety disorders, depression, and global health problems [3]. Increasing positive affect by therapeutic intervention reduces levels of depression and anxiety in adults [6,7] and promotes feelings of ‘a meaningful life, optimism, and goal orientation’ in children [8].

Positive affect in rats can be modeled by frequency modulated 50-kHz ultrasonic vocalizations (USVs). A wide range of hedonic stimuli (social interaction, food, drugs of abuse), increase calling rate [9–12], whereas aversive stimuli uniformly decrease calling rate [9,12,13]. Calling rate is also positively correlated to the rewarding value of a wide range of hedonic stimuli [12,14].

In addition, the neural circuit of rat 50-kHz USVs matches that of human positive affect [9,15] and this measure readily discriminates stress-resilient and nonresilient rats [16–18].

Chronic unpredictable stress (CUS) induces a long-lasting and a robust behavioral phenotype that models clinical features of post-traumatic stress disorder (PTSD) and has been well characterized at the behavioral, physiological, cellular, hormonal, and molecular levels [19–21]. This phenotype is responsive to chronic (3 weeks) but not acute or short-term treatment with the antidepressant fluoxetine [22], and is sensitive to rapid acting therapeutics [23,24]. Thus, the CUS model provides a well-characterized paradigm for evaluating the potential therapeutic efficacy of experimental compounds for the treatment of PTSD.

The experiments in this study were designed to test whether hedonic rough-and-tumble play can prevent CUS-induced negative affect, as measured by aversive 20-kHz USVs, novelty-induced hypophagia, and CUS-induced learned helplessness using the Porsolt test. Additional studies were performed to assess the capacity of rough-and-tumble play to rescue CUS-induced deficits in positive affect that occur in response to a conditioned stimulus (CS) that predicts rough-and-tumble play by measuring sucrose preference and 50-kHz USVs.

Materials and methods

Animals

Male 2–3-months-old Sprague-Dawley rats from Harlan, Iowa, USA were used. Rats were housed in Lucite cages

with aspen wood chip bedding, maintained on a 12:12 light:dark cycle (lights on at 5 am), and given ad libitum access to Purina lab chow (Purina, Saint Louis, Missouri, USA) and tap water throughout the study. All experiments were approved by the Northwestern University Animal Care and Use Committee.

Heterospecific rough-and-tumble play

Heterospecific rough-and-tumble play was conducted as previously described [23,25]. Briefly, heterospecific rough-and-tumble play stimulation was administered by the experimenter's right hand. The experimenter was blind to the treatment condition of the animals. Rats received 3 min of heterospecific rough-and-tumble play consisting of alternating 15-s blocks of play and 15 s of no-stimulation. High frequency USVs were recorded during the 15-s no-stimulation blocks and analyzed by sonogram in a blind manner as described previously [26]. Animals in the play group were tested every 3 days during chronic unpredictable stress exposure and animals in the control group received light touch. Using this paradigm we have shown that the increase in 50-kHz USVs that occur across trial blocks reflects positive emotional learning [26].

Chronic unpredictable stress

Rats were exposed to a CUS protocol previously shown to elicit long-lasting behavioral impairments in rats [25,27]. Animals received 21 days of CUS before behavioral testing and continued to receive CUS until the animals were sacrificed 1 day after the last behavioral test (total of 26 days of CUS). A total of nine different stressors were used (two stressors/day). The stressors (days) included rotation on a shaker for 1 h (days 3, 9, 13, 19, 24), placement in a 4°C ambient for 1 h (days 1, 5, 12, 14, 18, 22, 26), lights off for 3 h from 10:00 am to 1:00 pm (days 2, 10, 17, 23), lights on overnight (days 1, 5, 8, 13, 16, 22), strobe light overnight (days 3, 6, 9, 14, 17, 20, 23), 45° tilted cages overnight (days 4, 7, 11, 15, 18, 21, 25), food and water deprivation overnight (days 2, 6, 10, 15, 19), crowded housing overnight (days 4, 7, 11, 16, 21, 25), and isolation housing overnight (days 8, 12, 20, 24). Animals in the no-CUS group ($n = 9$) were weighed every 4 days and received behavioral testing without additional stressors.

Porsolt forced swim test

Testing was conducted as described in Burgdorf *et al.* [23,25]. Rats were placed in a 46 cm tall × 20 cm in diameter clear glass cylinder filled to 30 cm with tap water ($23 \pm 1^\circ\text{C}$) for 15 min. Porsolt testing occurred 1 day after the last rough-and-tumble play session and 3 h after USV testing. Water was changed after every other animal. Experiments were conducted in a blind manner and time spent floating during the last 5 min of the 15 min session was scored offline by an experimenter with high inter-rater reliability (Pearson's $r > 0.9$). Floating was defined as the minimal amount of effort required to keep the animal's head above water.

Sucrose preference test

Testing was conducted as described in Burgdorf *et al.* [25, 27], and testing occurred 3 days after the last rough-and-tumble play session. Rats were exposed to a palatable sucrose solution (1%; Sigma, Saint Louis, Missouri, USA) for 48 h, followed by 4 h of water deprivation and a 1 h exposure to two identical bottles, one filled with sucrose solution and the other with tap water. Sucrose preference was defined as the percentage of the volume of sucrose versus total volume of sucrose and water consumed during the 1-h test.

Novelty-induced hypophagia

Testing was conducted as described in Burgdorf *et al.* [23,25] and occurred 2 days after the last rough-and-tumble play session. Rats were food deprived overnight before testing. Rats were placed in an open field ($40 \times 40 \times 20$ cm) with lab chow in the center for 10 min under dim-red lighting. Between animals, feces and urine were removed from the apparatus. Immediately after testing, the latency to eat in the animal's homecage was determined as a control. Animals were videotaped and latency (s) for the animal to take the first bite of food, as well as locomotor activity (line crosses) was scored offline by an experimenter blinded to the treatment condition.

Experimental timeline

Animals received 26 days of CUS exposure. Heterospecific play exposure or light petting control exposure occurred on days 1, 4, 7, 10, 13, 16, 19, and 22. Heterospecific play testing occurred on day 23, and Porsolt testing occurred 3 h after play testing on day 23. NIH testing occurred on day 24. Sucrose preference testing occurred on day 25. Animals were euthanized on day 26.

Statistical analysis

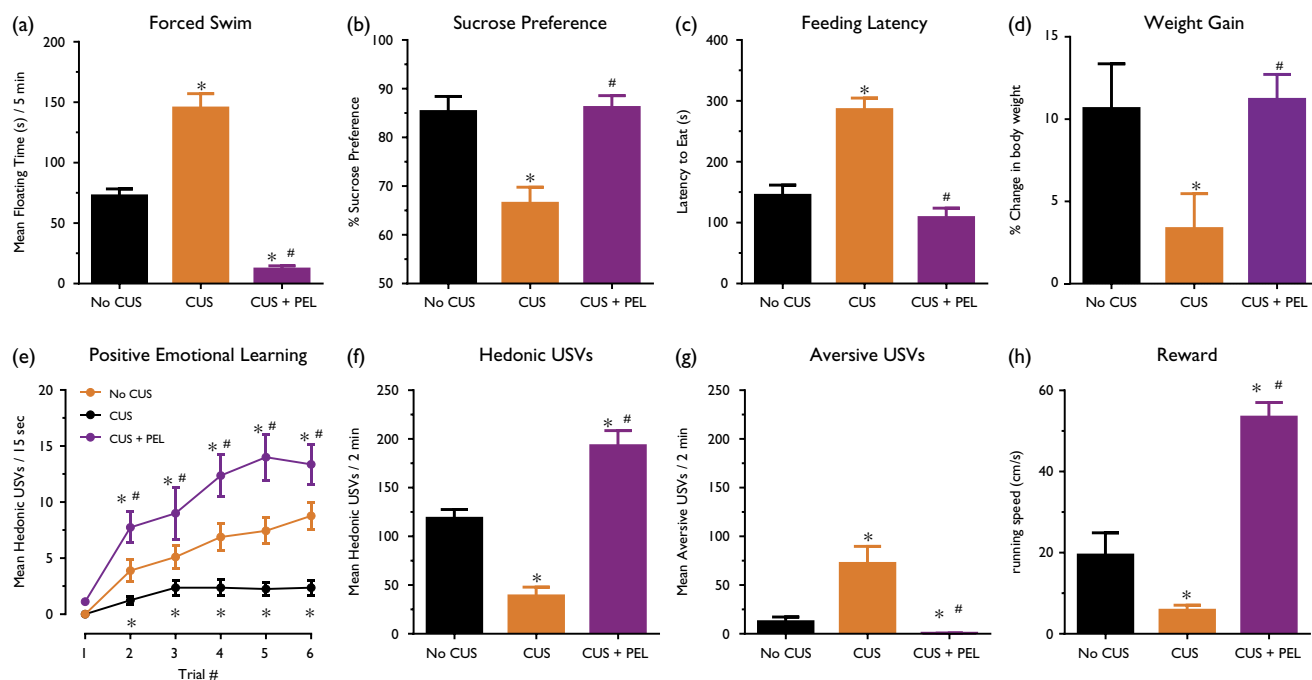
Data were analyzed by analysis of variance, followed by Fisher's protected least significant difference (PLSD) post-hoc test Statview (SAS, Cary, North Carolina, USA). The level of statistical significance was set at P less than 0.05.

Results

Rough-and-tumble play prevented chronic unpredictable stress-induced behavioral deficits

Rats exposed to repeated heterospecific play (one trial every 3 days for 21 days, for a total of seven trials) and to CUS had significantly reduced floating time in the Porsolt test compared with CUS-only rats [Fig. 1a; $F(2, 22) = 73.68$, $P < 0.0001$; Fisher's PLSD post-hoc CUS plus play or CUS-only vs. no-CUS control, $P < 0.0001$], indicating that play could be antidepressant. As shown in Fig. 1b, exposure to play increased sucrose preference scores in CUS-treated rats, whereas CUS-only rats showed significantly decreased sucrose preference compared with no-CUS-treated rats [$F(2, 22) = 13.61$, $P < 0.001$; Fisher's PLSD post-hoc CUS plus play vs. CUS-only, CUS-only vs. no-CUS-treated rats, $P < 0.001$].

Fig. 1



Hedonic heterospecific rough-and-tumble play prevents chronic unpredictable stress (CUS)-induced deficits in post-traumatic stress disorder (PTSD)-like behavior. Rats received either 3 min of heterospecific rough-and-tumble play or light touch every 3 days for the 21 days of CUS exposure. (a) Floating time in the Porsolt forced swim test in naive, CUS-exposed, or play + CUS-exposed rats. (b) Sucrose preference in the sucrose preference test. (c) Latency to eat in the novelty-induced hypophagia test. (d) Change in body weight (as compared with CUS day 0). (e) Hedonic ultrasonic vocalizations (USVs) in response to conditioned stimuli that predicts heterospecific play. Hedonic (f) and aversive (g) USVs in response to heterospecific play. (h) Running speed to self-administer heterospecific play. $N=8-9/\text{group}$. * $P < 0.05$ Fisher's protected least significant difference (PLSD) post-hoc versus no-CUS; # $P < 0.05$ Fisher's PLSD post-hoc versus CUS group. Data are reported as mean \pm SEM. PEL, positive emotional learning.

Play exposure also significantly decreased feeding latency in a novel environment compared with CUS-only rats, whereas CUS-only rats showed greater feeding latencies compared with no-CUS rats [Fig. 1c; $F(2, 22) = 29.21$, $P < 0.0001$; Fisher's PLSD post-hoc CUS plus play vs. CUS-only, CUS-only vs. no-CUS control, $P < 0.0001$]. No differences in locomotor activity as measured by line crosses or feeding latencies were seen in the homecage immediately following testing (Burgdorf J, unpublished data).

Exposure to play increased weight gain in CUS-treated rats compared with CUS-only rats, whereas CUS-only rats showed decreased weight gain compared with no-CUS rats [Fig. 1d; $F(2, 22) = 3.76$, $P < 0.05$; Fisher's PLSD post-hoc CUS plus play vs. CUS-only, CUS-only vs. no-CUS, $P < 0.05$].

Play in CUS-treated rats increased rates of hedonic USVs in response to a temporal CS that predicted heterospecific play compared with CUS-only rats, and CUS-only rats showed decreased CS-elicited hedonic USVs compared with no-CUS rats [Fig. 1e; $F(2, 22) = 15.14$, $P < 0.0001$; Fisher's PLSD post-hoc CUS plus play vs. CUS-only, $P < 0.0001$, CUS-only vs. no-CUS, $P < 0.05$, CUS plus

play vs. no-CUS control, $P < 0.01$]. Repeated experience with rough-and-tumble play also increased rates of hedonic USVs (Fig. 1f) and reduced rates of aversive 20-kHz USVs (Fig. 1g) compared with CUS-only rats, whereas CUS-only rats showed decreased hedonic USVs and increased aversive USVs compared with no-CUS rats [hedonic USVs $F(2, 22) = 43.46$, $P < 0.0001$; Fisher's PLSD post-hoc CUS plus play vs. CUS-only, CUS-only vs. no-CUS, $P < 0.0001$, CUS plus play vs. no-CUS, $P < 0.001$; aversive USVs $F(2, 22) = 13.96$, $P < 0.0001$ Fisher's PLSD post-hoc CUS plus play vs. CUS-only, $P < 0.0001$, CUS-only vs. no-CUS, $P < 0.001$]. Experience with play in CUS-treated rats significantly increased running speed to self-administer heterospecific play compared with CUS-only rats, whereas CUS-only rats showed decreased running speed compared with no-CUS rats [Fig. 1h; $F(2, 22) = 35.84$, $P < 0.0001$; Fisher's PLSD post-hoc CUS plus play vs. CUS-only, CUS plus play vs. no-CUS, $P < 0.0001$, CUS-only vs. no-CUS, $P < 0.05$].

Discussion

The data presented here demonstrate that hedonic rough-and-tumble play induces resilience to stress in multiple behavioral tests using a well-established CUS

rat model. Play was able to reduce CUS-induced deficits in positive affect and learning, in addition to reducing negative affect and learned helplessness. As CUS has been shown to suppress the induction of long-term potentiation in the medial prefrontal cortex [19], the rescue of CUS-induced impairments in positive emotional learning suggests that play can reverse the suppression of synaptic plasticity observed following CUS.

The neuronal circuit underlying hedonic 50-kHz USVs is suppressed by CUS [15]. Electrical stimulation of the medial prefrontal cortex (MPFC) elicits hedonic 50-kHz USVs with concomitant play behavior, by a descending connection to the ventral periaqueductal gray and subsequent motor neurons responsible for producing vocalizations [15,28]. CUS robustly suppresses long-term potentiation in the MPFC [19], and play increases cortical protein levels of *N*-methyl-D-aspartate receptors (NMDAR) and growth factors [26,29] that are critical for synaptic plasticity. Thus, it is likely that play restores plasticity in the MPFC circuit responsible for hedonic 50-kHz USVs that is suppressed by CUS.

Measuring rat 50-kHz USVs has been used to model positive affect and quantify resilience to depression. Behavioral, biochemical, and molecular studies using this model have identified NMDAR-dependent synaptic plasticity in the MPFC as critical for positive affect-induced resilience to depression [15]. This form of NMDAR-dependent plasticity in the MPFC appears to mediate positive emotional learning that is critical for the development of resilience to depression [19,26].

Conclusion

Positive affect in rats prevents behavioral deficits induced by chronic stress by suppressing plasticity in the MPFC. Behavioral or pharmacological interventions that target MPFC plasticity may therefore be a useful therapeutic strategy for the treatment or prevention of stress-related disorders, such as depression or PTSD.

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This manuscript is dedicated to the memory of Jaak Panksepp (1943–2017).

Conflicts of interest

There are no conflicts of interest.

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