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5-hydroxytryptamine 2C receptors in the basolateral amygdala are involved in the expression of anxiety after uncontrollable traumatic stress

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Abstract

Background—Exposure to uncontrollable stressors often increases anxiety-like behavior in both humans and rodents. In rat, this effect depends upon stress-induced activity within the dorsal raphe nucleus (DRN). However, the role of serotonin in DRN projection regions is largely unknown. The goals of the current study were to 1) determine if DRN activity during a post-stress anxiety test is involved in anxiety-like behavior, 2) assess the effect of uncontrollable stress on extracellular serotonin in the basolateral amygdala during the anxiety test, and 3) determine the role of the serotonin 2C receptor (5-HT_{2C}) in uncontrollable stress-induced anxiety.

Method—Rats were exposed to tailshocks that were uncontrollable. On the following day anxiety-like behavior was assessed in a JSE test. BLA extracellular serotonin concentrations were assessed during JSE by in vivo microdialysis 24 h after uncontrollable stress, controllable stress or no stress. In separate experiments drugs were administered before the JSE test to inhibit the DRN or to block 5-HT_{2C} receptors.

Results—Exposure to uncontrollable shock reduced later social exploration. Prior uncontrollable stress potentiated serotonin efflux in the BLA during social exploration, but controllable stress did not. Intra-DRN 8-OH-DPAT and systemic and intra-BLA 5-HT_{2C} receptor antagonist SB 242084 prevented the expression of potentiated anxiety in uncontrollably stressed rats. Intra-BLA injection of the 5-HT_{2C} agonist CP 809101 mimicked the effect of stress.

Conclusion—These results suggest that the anxiety-like behavior observed after uncontrollable stress is mediated by exaggerated 5-HT acting at BLA 5-HT_{2C} receptors.

Keywords

rat; learned helplessness; ptsd; serotonin; social exploration; 5-HT_{2c}

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INTRODUCTION

The pathobiologies of stress-induced anxiety disorders, such as Acute Stress Disorder (ASD) and Post Traumatic Stress Disorder (PTSD), are poorly understood, yet PTSD has an estimated lifetime prevalence of 7.8% (1) and treatment of PTSD is inadequate (2). ASD and PTSD symptoms include avoidant, anxiety-like behaviors that present just after stressor exposure or develop with time, respectively (3). Stress victims that display ASD symptoms are more likely to develop chronic PTSD (4) and early intervention can reduce the occurrence of chronic PTSD (5). Although much has been reported about the therapeutic mechanisms of PTSD pharmacotherapy, relatively little is known about the biological basis of anxiety *expression* after trauma.

The sequelae of a stressor depend on both environmental and genetic factors. In terms of environmental factors, controllable stressors tend to have less measurable impact than those that are not (6), and a lack of behavioral control over stress may be critical to the development of PTSD (7, 8). In one well-characterized paradigm, exposure to inescapable tailshock (IS) induces a number of behavioral consequences that do not follow exposure to exactly equal escapable tailshocks (ES). These outcomes include shuttle escape failure, enhanced fear conditioning, and reduced social exploration, among others collectively described as “learned helplessness effects” (for a review see (6)).

Exaggerated anxiety-like behavior is one of the most striking consequences of exposure to IS, relative to ES, as well as to acute trauma in humans. Rats exposed to IS later show greater fear conditioning (9), post-shock freezing (10), neophobia (11), and reductions in social interaction (12, 13) than do rats exposed to equal ES. The development of anxiety-like behaviors after IS depends upon an intense activation of serotonergic neurons in the dorsal raphe nucleus (DRN; for a review see (6)) during stress that is thought to “sensitize” DRN-serotonin (5-HT) neurons to respond to later stressors in exaggerated fashion (14–16). Acute activation of 5-HT neuronal systems is thought to be a critical component of anxiety expression because acute administration of selective serotonin reuptake inhibitors (SSRIs) evokes anxiety-like behaviors in both humans and rodents (17–20) and mimics the effects of IS (21). We have reported that IS, but not ES, reduces exploration of both adult (13) and juvenile (12) conspecifics, a behavior that is commonly thought to “assay” anxiety-like behavior (22). Furthermore, the effect of IS was dependent upon DRN activation at the time of stress (12).

Only a subset of neurons in the caudal DRN respond differentially to IS and ES (23), and precisely this subset projects to the basolateral amygdala (BLA; (24)). The BLA is a key structure in the mediation of anxiety. Environmental information about threatening stimuli is relayed to the BLA, a structure with glutamatergic projections to a wide range of limbic structures involved in the mediation of overt fear and anxiety behaviors (25, 26). Importantly, BLA output is modulated by 5-HT (25). 5-HT_{2C} receptor agonists produce anxiogenic effects (18) and lead to activation in BLA projection regions (27), while 5-HT_{2C} receptor antagonists are anxiolytic (28). Recently, Strong et al. (29) demonstrated that a systemic 5-HT_{2C} antagonist blocked, and 5-HT_{2C} agonists mimicked, the effects of IS on later freezing and shuttle escape behavior.

If IS leads to an anxiety-like state that is dependent upon DRN sensitization, then it is possible that 5-HT released in the BLA, which is increased in response to anxiogenic stimuli after IS (30), plays a critical role in the generation of IS-potentiated anxiety-like behavior. The idea here is that after IS, when the rat is presented with a stimulus such as a juvenile conspecific, 5-HT levels in the BLA rise and activate 5-HT_{2C} receptors, which may in turn

enhance BLA output and increase anxiety-like behaviors. Utilizing the juvenile social exploration (JSE) test the current set of experiments aimed to determine whether: a) prior IS exaggerates the release of 5-HT in the BLA produced by a juvenile social interaction, b) DRN 5-HT activation is required to produce IS augmented anxiety, c) BLA 5-HT_{2C} receptors are critical to the anxiety-like effect of IS, and d) 5-HT_{2C} agonism is sufficient to mimic IS effects on JSE (See supplemental materials).

MATERIALS and METHODS

Rats

Adult (60–70 days old and weighing 275–350 gm at the time of testing) and juvenile (28–32 days old and weighing 90–100 gm at the time of testing) male Sprague-Dawley (Harlan, Indianapolis, IN) rats were used in all experiments. Rats were housed with free access to food and water in groups of 2 for microinjection experiments, in groups of 4 when drugs were administered i.p., and in single cages for the microdialysis experiment. The vivarium maintained a 12 h light/dark cycle; all experimental procedures were conducted within the first 6 hours of the light phase. All procedures were conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the University of Colorado Institutional Animal Care and Use Committee.

Stress Induction Procedures

In experiments involving escapable stress (ES), 100 electric tailshocks were administered as previously described (14) (See Supplemental Materials). In brief, rats were restrained and shock was delivered through the tail on a variable interval 60 s schedule. Turning a wheel inside the restraining box terminated the shock. Shock terminated automatically if no response was made within 30 s. Rats in the yoked-inescapable shock group (IS) received exactly equal shock but were not permitted control—turning the wheel had no consequence. Animals in the homecage control (HC) remained in the vivarium. When only IS was involved, 100, 5-s shocks were delivered on a variable interval 60-s schedule.

Juvenile Social Exploration Tests

JSE testing was conducted as described previously (31). In a separate testing room, each experimental subject was allocated a single plastic tub cage with shaved wood bedding and a wire lid. To begin the test rats were placed into the test cage and after 45 min a 28 ($\pm$ 2) day-old juvenile was introduced to the cage for 3 min and an observer, blind to treatment, timed exploratory behaviors (sniffing, pinning, and allogrooming) initiated by the adult (31). In order to observe JSE during *in vivo* microdialysis the observers were not always blind to treatment and scored two tests simultaneously. In all other experiments the observers were naïve to treatment conditions and scored only one rat at a time. Juveniles were used for multiple tests but were never used for the same adult rat. Testing order was counterbalanced for stress and drug treatments.

Surgical Procedures

All surgery was performed under inhaled isoflurane anesthesia (3% in O₂). Microdialysis and microinjection guide cannulae were implanted and fixed in place with stainless steel screws and acrylic cement as previously described (14). Exact coordinates and cannulae specifications are listed in Table 1. A stylet was placed in the cannulae and each rat was inoculated with 0.25ml/kg (s.c.) penicillin (Combi-Pen, Agrilabs). At the end of the experiment, brains were collected, sliced at 40 μ m, and stained with cresyl violet for cannulae verification. Subjects were only included if tissue damage from the cannulae tips fell within the target nucleus.

In Vivo Microdialysis and Quantification of 5-HT

Microdialysis probes (CMA 12, MW cut-off 20kD, 2mm) were inserted into the guide and Ringer's solution (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl) was perfused through the probes at a flow rate of 0.2 μ l/min. After 12 hr, the flow rate increased to 1.5 μ l/min. After a 90 min equilibration period, 8 samples were collected at 15 min intervals. Dialysates were immediately placed in an -80°C freezer until analysis. Samples were analyzed with high-pressure liquid chromatography (HPLC) using standard methods (14).

Drugs

The selective 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, Sigma, St. Louis, MO) was dissolved in 0.9% saline. The selective, brain-penetrant 5-HT_{2C} antagonist 6-Chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride (SB 242084, Tocris, Ellisville, MO) was dissolved in 0.9% saline.

Experimental Procedures

Overview

Except where noted all experiments were conducted in this fashion: Rats were implanted with cannulae and allowed 7–10 days to recover. JSE baseline tests were given and 24 h later stress was administered. Since the focus of the current experiments was on the expression of stress-induced anxiety, pharmacological manipulations were made before a post-stress JSE test given 24h after stress.

In vivo microdialysis and quantification of BLA 5-HT

Rats were assigned to either escapable (ES) or yoked inescapable (IS) stress or HC treatment ($n = 7/\text{group}$). After stress, microdialysis began as described above. Rats were left alone in the dialysis room with food and water for at least 12 h on a light cycle that matched the vivarium. Sample collection began 24 h after stress. Beginning 8 min into the 5th sample a juvenile was added to the dialysis bowl for 5 min. The next sample was collected 2 min after the juvenile was removed. This procedure was used to isolate in time the acute change in 5-HT that would be elicited by the JSE test and to distinguish long lasting effects of juvenile exposure to the remaining samples. Here we sought to determine whether as a result of a sensitized DRN, rats in the IS group would exhibit greater extracellular 5-HT in the BLA as a result of the social encounter than ES or HC rats.

Inhibition of DRN by 8-OH-DPAT prior to Social Exploration

Rats were assigned to either inescapable shock (IS) or home cage (HC) and 8-OH-DPAT or saline treatments in a 2×2 factorial design ($n = 10/\text{group}$). On the next day, rats were exposed to either 100 trials of IS or left in the home cage. On the day after stress rats received microinjections of 2mg/ml 8-OH-DPAT or saline (injection volume: 0.5 μ l), immediately placed in the JSE test cage and 45 min later a juvenile was added and social investigation was quantified for 3 min. This dose was based on previous efficacy in our laboratory (12). Microinjections were made in a quiet room near the testing area. Rats were gently restrained and a microinjector was inserted that extended 1mm beyond the cannulae tip (33 g). Injections were made at a rate of 1 μ l/min; injectors remained in place for 1 min to permit diffusion. DRN serotonin neurons are regulated by the somatodendritic 5-HT_{1A} autoreceptor (32) and the 5-HT_{1A} agonist 8-OH-DPAT is a potent inhibitor of 5-HT neuronal activity (33). Therefore, it was predicted that the IS-8-OH-DPAT group would have social investigation behavior comparable to HC controls.

Pharmacological blockade of the 5-HT_{2C} receptor

One set of rats was given a JSE baseline test and received either IS or HC 24 hr later. The next day they received either saline, 0.1 or 0.5mg/kg body weight SB 242084 (SB) i.p. in saline [a 2 (stress) by 3 (drug) factorial design, $n = 10/\text{group}$] followed by placement into the JSE test cage. After 45 min a juvenile was added. Systemic doses of SB were based on pilot studies and the literature (20, 34). A second set of rats, implanted with bilateral cannulae for microinjection in the BLA, was given a baseline JSE test and then 24 h later randomly assigned to either IS or HC. 24 hr after treatment they received either intra-BLA saline, 10 μM , 10mM, or 50mM SB (0.5 μl injection volume/side in saline), $n = 8/\text{group}$. Microinjection was followed by JSE testing as above. Intra-BLA concentrations of SB were chosen to capture a range of doses comparable to the only other report of microinjection of this drug (35) and a low dose. Additional groups were added as controls. First, to determine if 5-HT_{2C} signaling was involved in the *induction* of stress-induced anxiety a group of rats received either saline or 50mM SB prior to IS treatment, but no injection before JSE testing. Second, a group was implanted with guide cannulae to the CeA as a site-specificity control and these rats were treated with IS and either saline or 50mM SB before JSE testing.

Statistical Analysis

Rats with pre-stress JSE lower than 60 s were excluded (31). Data were analyzed with analysis of variance (ANOVA) with stress and drug dose treated as between-subjects variables, while microdialysis samples were treated as within-subjects variables. Main effects and interactions were considered significant if $p < 0.05$ and were explored with Fisher's Protected Least Significance Difference (PLSD) post hoc tests to control the experiment-wise error to $\alpha = 0.05$.

RESULTS

Extracellular BLA 5-HT during and following social exploration

Importantly, all rats in the ES group learned to escape the tailshock, quickly reached the maximum response criterion and maintained escape behavior as previously reported (14). Only rats with dialysis probes at least 50% within the BLA were included (Figure 1); the resulting groups had $ns = 6-7$. Prior stress did not alter basal 5-HT, means(S.E.M.): ES = 0.272(0.03), IS = 0.267(0.05), HC = 0.274(0.04) pg/20 μl . Extracellular 5-HT was converted to percentage of baseline by dividing each sample by the average of the first 4 samples $\times 100$ (Figure 1). Introducing the juvenile did not raise 5-HT efflux in previously untreated subjects. In contrast, prior IS caused the 5 min juvenile encounter to increase extracellular 5-HT within the BLA while prior ES did not influence the 5-HT response to the juvenile. A 3 (Stress: ES, IS or HC) by 8 (Samples) ANOVA revealed a significant effect of Stress: $F(2, 17) = 9.982$, $p = 0.001$, Sample: $F(7, 119) = 4.744$, $p < 0.001$, and the Stress by Sample interaction: $F(14, 119) = 2.336$, $p = 0.007$. Prior IS caused significantly greater extracellular 5-HT than did either ES or HC treatment during the social exploration sample (SE) and the first post-SE sample (P1; $ps < 0.05$). Within group comparisons between baseline, SE and post-SE samples revealed a significant elevation in 5-HT from baseline only in the IS group; the SE and all post-SE means were significantly greater than the last baseline sample (BL4; $ps < 0.05$). ES and HC groups did not differ from each other or their baselines at any time.

Social exploration was quantified during the 5 min juvenile exposure. The means(SEM): ES = 99.42(5.62), IS = 59.18(8.23), and HC = 87.72(9.51) were comparable to our prior work (12, 36). A one-way ANOVA identified a significant main effect of Stress, $F(2, 17) = 7.361$, $p = 0.005$. IS significantly reduced JSE compared to ES and HC treated rats, $ps < 0.05$, which did not differ from each other.

Inhibition of DRN by 8-OH-DPAT prior to JSE testing

The mean time spent in JSE is depicted in Figure 2. Clearly, intra-DRN 8-OH-DPAT blocked the reduction in exploration produced by prior IS, but did not by itself alter social exploration. Only rats with cannulae placements within the DRN were included in the analysis; the resulting groups had $ns = 7-9$. A 2 (Stress: IS vs. HC) by 2 (Drug: saline or 8-OH-DPAT) ANOVA revealed a significant main effect of Stress: $F(1, 28) = 8.558, p < 0.01$ and a significant Stress by Drug interaction: $F(1, 28) = 5.073, p < 0.05$. Post hoc comparisons revealed a reduction in exploration in the IS-saline group, which was significantly lower than all other groups ($ps < 0.05$). Importantly, the IS-8-OH-DPAT group did not differ from either HC group.

Systemic 5-HT_{2C} receptor antagonism

Mean JSE time is depicted in Figure 3, $ns = 9-10$. Systemic administration of the 5-HT_{2C} antagonist dose-dependently blocked the effect of prior IS on social exploration. A 2 (Stress: IS or HC) by 3 (Drug: saline, 0.1, 0.5mg/kg SB) ANOVA revealed significant main effects for Stress: $F(1, 53) = 7.211, p < 0.01$, Drug: $F(2, 53) = 4.084, p < 0.05$, and a significant Stress by Drug interaction: $F(2, 53) = 4.032, p < 0.05$. Exploration was significantly reduced in the IS-saline group compared to all HC groups, and to the IS-0.5 mg/kg SB group. The IS-0.1 mg/kg SB group was greater than the IS-saline group, $p < 0.05$, but did not differ from the IS-0.5 mg/kg SB group. These data indicate that 0.5mg/kg SB completely blocked the expression of stress-induced anxiety without affecting JSE *per se*.

Intra-BLA 5-HT_{2C} receptor antagonism

Mean JSE time is shown in Figure 4. After removing rats with misplaced cannulae $ns = 7-9$. The results indicate a clear effect of IS and a dose-dependent reversal of the effect by SB. The primary experimental groups were compared using a 2 (Stress: IS or HC) by 4 (Drug: saline, 10 μ M, 10mM, or 50mM) ANOVA that revealed significant main effects of Stress: $F(1, 63) = 45.69, p < 0.001$; Drug: $F(3, 63) = 8.153, p < 0.001$; and a Stress by Drug interaction: $F(3, 63) = 7.308, p < 0.001$. IS significantly reduced exploration in the IS-saline and IS-10 μ M SB groups compared to HC and the IS-10mM and IS-50mM SB groups ($ps < 0.05$). The IS-50mM SB group did not differ from any HC groups illustrating a complete reversal of the IS effect ($ps > 0.05$). The control groups that received drug injections to the CeA before JSE or into the BLA prior to IS were significantly different from the HC-saline group (unrestricted *t*-tests, $ps < 0.05$) but did not differ significantly from the IS-saline group ($ps > 0.05$). Although not all rats with CeA placements fell strictly within the borders of the CeA the purpose of this group was not to establish a role for the CeA but to verify specificity to the BLA. No rats were included in the CeA group if the cannulae tips were found to be in or near the BLA.

DISCUSSION

Here we explored the role of the DRN, 5-HT, and BLA 5-HT_{2C} receptors in uncontrollable stress-induced anxiety-like behavior in the JSE test. Consistent with our prior work and that of others, exposure to an uncontrollable stressor (IS) reduced the rat's tendency to investigate a juvenile conspecific (12, 13, 37), whereas equal controllable stress did not (12, 36). The hypothesis has been that IS produces an intense activation of DRN 5-HT neurons, leading to sensitization of these neurons via down-regulation of DRN 5-HT_{1A} autoreceptors. The consequence of this sensitization would be exaggerated release of 5-HT in DRN projection regions to subsequent stimuli such as those used in behavioral testing (e.g., the footshock in shuttlebox escape testing) and that excessive 5-HT is the proximate cause of the behavioral changes (6). However, there has been little evidence that uncontrollable stress

could sensitize DRN 5-HT neurons and that *activation of those neurons at the time of behavioral testing* is necessary for the occurrence of behavioral effects.

With regard to sensitization, there is only the study of Amat et al. (30) who reported that 2 footshocks was sufficient to increase 5-HT efflux in the BLA in subjects that had received IS 24 h earlier, but that no change in 5-HT to 2 footshocks was found after ES or HC treatment. Here a 5 min exposure to a juvenile conspecific had no effect on 5-HT efflux in the BLA in naïve subjects, as would be expected because DRN 5-HT neurons generally are not activated by minor stressors (38). Prior IS, but not ES, led the 5 min juvenile encounter to produce a large and sustained increase in 5-HT efflux. It should be recognized that the IS and juvenile conditions were distinct; the test environment: retraining tubes versus dialysis bowls with wood chip bedding, the lighting conditions: dark sound-attenuation chambers versus a brightly lit room, and the handling conditions: immediate handling and restraint before IS versus none during dialysis were all quite different. Thus, the sensitization that was revealed here is quite remarkable. In rats that received IS DRN 5-HT neurons responded to a seemingly innocuous event as if it were a potent stressor.

By preventing DRN 5-HT neuronal activity with 8-OH-DPAT, we demonstrate that DRN activity at the time of JSE testing is critical to the expression of a stress-induced anxiety-like effect. The role of the DRN here is consistent with observations from other research. Ethanol withdrawal decreases social interaction (39) which is prevented by intra-DRN buspirone a, 5-HT_{1A} partial agonist (28). Social defeat activates DRN neurons, downregulates 5-HT_{1A} receptor mRNA (40) and increases later fear-like behavior which can be blocked by DRN inhibition (41). Finally, humans with social anxiety disorder have significantly less *in vivo* DRN 5-HT_{1A} binding compared to controls (42). In each of these examples, DRN hyperactivity is a correlate of the expression of anxiety.

The DRN is not viewed to be part of the circuit that mediates “normal” fear or anxiety behaviors, but BLA-projecting 5-HT neurons are situated to be potent *modulators* of anxiety (43). That only IS would affect BLA 5-HT levels suggests that 5-HT activity in the BLA is involved in the divergent anxiety outcomes observed after uncontrollable versus controllable stress. 5-HT receptors in the BLA have received attention as a site-of-action for numerous compounds with potential as anxiolytic therapeutics. We focused on the 5-HT_{2C} receptor for several reasons. First, 5-HT_{2C} receptors are involved in the potentiation of fear produced by 5-HT. The increase in the expression of conditioned fear by acute SSRI treatment is blocked by systemic administration of SB (20, 21). Second, 5-HT_{2C} knock-out mice display an anxiolytic phenotype and reduced neuronal activation in BLA projection regions in response to anxiogenic stimuli (44). Third, non-specific systemic 5-HT₂ agonists activate the amygdala and limbic structures associated with anxiety-like behaviors (45). Lastly, systemic 5-HT_{2C} antagonists block the shuttlebox escape deficit produced by IS (34).

Using the selective and brain-penetrant 5-HT_{2C} antagonist SB 242084 (SB), we observed a dose-dependent and site-specific effect on the expression of anxiety. That is, SB administered before JSE testing, either systemically or intra-BLA, blocked the effects of IS. When injected during IS, SB was without effect indicating that the 5-HT_{2C} receptor in the BLA is not involved in the induction or acquisition of stress effects. The BLA appears to be the critical site of action because injections to the central amygdala before testing were without effect. Furthermore, BLA 5-HT_{2C} binding is sufficient to reduce social exploration (See supplemental materials). The involvement of the 5-HT_{2C} receptor in the expression of anxiety is consistent with the expression of ethanol-withdrawal induced anxiety which is also prevented by intra-BLA 5-HT_{2C} antagonism (28). Although we have reported that systemic 5-HT_{2C} antagonism prevents the expression of learned helplessness in the shuttle box (34), future work is required to determine if, in fact, the BLA is critical to that effect.

Anxiety- and fear-like behaviors are correlated with an increase in output of the BLA complex to limbic structures that mediate specific behaviors (25, 26, 43, 46). The anxiogenic effect of IS in later JSE may be mediated by an enhancement of BLA output by 5-HT action at the 5-HT_{2C} receptor. Unfortunately, the mechanism by which 5-HT_{2C} receptors modulate BLA neurotransmission is poorly understood and there are conflicting reports. For example, activation of the 5-HT₂ receptor with nonselective agonists increases BLA -aminobutyric acid (GABA) release (47, 48), an effect that would be anxiolytic in the JSE test (22). However, 5-HT₂ agonists exert a dose-dependent effect, with low doses promoting, and higher doses reducing, net GABAergic activity (48). In contrast, 5-HT_{2C} receptors co-localized with GABA_A receptors are able to reduce GABA_A inhibitory currents (49) and may account for the dose-dependent effect of 5-HT₂ agonists. Since 5-HT_{2C} agonists enhance excitability in a majority of BLA neurons (50) one would predict that high levels of 5-HT_{2C} stimulation, akin to those observed after IS, would cause a net excitation of BLA neurons including projection neurons. Clearly, the precise mechanism(s) by which 5-HT_{2C} activation in the BLA potentiates anxiety-like behavior awaits further study.

To conclude, early treatment of ASD is an important step in the prevention of PTSD (5). Here the expression of anxiety after stress was correlated with BLA 5-HT during a JSE test, was prevented by 5-HT_{2C} antagonism, and was mimicked by 5-HT_{2C} agonism. Hyperactivity at BLA 5-HT_{2C} receptors may be central to anxiety expression in ASD and PTSD and 5-HT_{2C} antagonism may be a relevant early intervention for victims exhibiting ASD after trauma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Mean($\pm$ S.E.M.) extracellular 5-HT in the BLA before, during and after a 5 m juvenile social exploration (SE). Rats received prior escapable tailshock (ES, $n = 7$), inescapable tailshock (IS, $n = 7$), or home cage control (HC, $n = 6$) treatment. 5-HT is expressed as a percentage of the average of 4 baseline samples (B1–B4). A juvenile conspecific was added to the dialysis bowl 8 m after B4 was collected, remained in the cage for 5 m and was removed 2 m before the SE sample was collected (depicted as a solid black box). Prior IS significantly increased BLA 5-HT during (SE) and after (P1) compared to ES and HC groups, $*ps < 0.05$. (B) Graphic reconstruction of 2mm dialysis probe placements within the BLA. Illustrations were adapted from the atlas of Paxinos and Watson (51) and are listed in relationship to bregma in mm.

Figure 2.

(A) Mean(+ S.E.M.) time spent in exploration during a 3 m juvenile social encounter. Prior IS resulted in a significant reduction in exploration compared to a HC control treatment, $*p < 0.05$. The effect of IS was completely blocked by intra-DRN injection of the 5-HT_{1A} agonist 8-OH-DPAT 45 m before the encounter. (B) Graphic reconstruction of microinjector tip placements within the DRN. Illustrations were adapted from the atlas of Paxinos and Watson (51) and are listed in relationship to bregma in mm.

Figure 3.

Mean(+ S.E.M.) time spent in exploration during a 3 m juvenile social encounter. Prior IS resulted in a significant reduction in exploration compared to HC treated controls, $p < 0.05$. Systemic administration of the 5-HT_{2C} antagonist SB 242084 45 m before the encounter dose-dependently blocked the effect of IS with 0.5 mg/kg resulting in complete reversal compared to IS-saline, $p < 0.05$. SB 242084 was without effect in the HC treated groups. * $p < 0.05$ compared to HC-Saline.

Figure 4.

(A) Mean (+ S.E.M.) time spent in exploration during a 3 m juvenile social encounter. Prior IS resulted in a significant reduction in exploration compared to HC controls, $p < 0.05$. Intra-BLA injection of the 5-HT_{2C} antagonist SB 242084 45 m before the social encounter dose-dependently blocked the effect of prior IS. 10 μ M was without effect, 10mM exhibited a partial effect, and 50mM completely blocked the effect of prior IS, p s < 0.05 when compared to IS-Saline. Intra-BLA administration of SB prior to IS had no effect [SB 50mM (IS)]. Furthermore, intra-central amygdala (CeA) injection of SB prior to JSE had no effect suggesting a site-specific effect of SB in the BLA. * $p < 0.05$ compared to HC-Saline, IS-Saline and IS-SB 242084 50mM. ** $p < 0.05$ compared to HC-Saline. (B) Graphic

reconstruction of microinjector tip placements within the BLA or (C) CeA. Illustrations were adapted from the atlas of Paxinos and Watson (51) and are listed in relationship to bregma in mm.

Table 1

Type and location coordinates of microdialysis and microinjection cannulae.

Target	Cannula Type	AP	LM	DV
BLA *	CMA 12 (Carnegie Medicine, Sweden)	-3.0	+4.8	-6.2
DRN	26g, 15.5mm length (Plastics One, Roanoke, VA)	-8.1	0	-5.1
BLA	26g, 7.5mm length (Plastics One)	-3.0	±4.8	-6.2
CeA	26g, 7.5mm length (Plastics One)	-2.0	±4.0	-6.2

Coordinates are in mm and were measured from bregma and dura.

* Unilateral microdialysis cannulae. AP = anterior-posterior, LM = lateral-medial, DV = dorsal-ventral, BLA = basolateral amygdala, DRN = dorsal raphé nucleus, CeA = central nucleus of the amygdala.