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Deep brain stimulation, histone deacetylase inhibitors and glutamatergic drugs rescue resistance to fear extinction in a genetic mouse model

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ABSTRACT

Anxiety disorders are characterized by persistent, excessive fear. Therapeutic interventions that reverse deficits in fear extinction represent a tractable approach to treating these disorders. We previously reported that 129S1/SvImJ (S1) mice show no extinction learning following normal fear conditioning. We now demonstrate that weak fear conditioning does permit fear reduction during massed extinction training in S1 mice, but reveals specific deficiency in extinction memory consolidation/retrieval. Rescue of this impaired extinction consolidation/retrieval was achieved with D-cycloserine (N-methly-D-aspartate partial agonist) or MS-275 (histone deacetylase (HDAC) inhibitor), applied after extinction training. We next examined the ability of different drugs and non-pharmacological manipulations to rescue the extreme fear extinction deficit in S1 following normal fear conditioning with the ultimate aim to produce low fear levels in extinction retrieval tests. Results showed that deep brain stimulation (DBS) by applying high frequency stimulation to the nucleus accumbens (ventral striatum) during extinction training, indeed significantly reduced fear during extinction retrieval compared to sham stimulation controls. Rescue of both impaired extinction acquisition and deficient extinction consolidation/retrieval was achieved with prior extinction training administration of valproic acid (a GABAergic enhancer and HDAC inhibitor) or AMN082 [metabotropic glutamate receptor 7 (mGlu7) agonist], while MS-275 or PEPA (AMPA receptor potentiator) failed to affect extinction acquisition in S1 mice. Collectively, these data identify potential beneficial effects of DBS and various drug treatments, including those with HDAC inhibiting or mGlu7 agonism properties, as adjuncts to overcome treatment resistance in exposure-based therapies.

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1. Introduction

Anxiety disorders, such as post-traumatic stress disorder, social phobia, specific phobias, panic and obsessive-compulsive disorders, are among the most common mental disorders in humans (Olesen et al., 2012). These disorders are associated with problems to extinguish learned fear responses and also to consolidate extinction memories (Myers and Davis, 2007; Milad et al., 2009). To date, the most effective strategies in the treatment of anxiety disorders include use of pharmacological and cognitive strategies (Nutt, 2005). However, significant drawbacks to current therapy exist including lack of treatment response and also a lack of long-term beneficial effect of combining available drugs with exposure-based therapy (Barlow et al., 2000; Davidson et al., 2004; Foa et al., 2005; Yehuda and LeDoux, 2007; Norberg et al., 2008).

Clinical interventions which rescue deficits in fear extinction acquisition and consolidation would constitute important treatment options in these disorders. Along these lines, pharmacological and genetic silencing studies, predominantly in normally behaving rodents and using massed extinction training paradigms, have identified a number of neurobiological mechanisms that govern acquisition of fear extinction learning and extinction consolidation.

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Acquisition of extinction learning requires N-methyl-D-aspartate (NMDA) receptor 2B (Sotres-Bayon et al., 2007), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Zushida et al., 2007; Yamada et al., 2009) and metabotropic glutamate 7 (mGlu7) (Fendt et al., 2008) receptor activities in addition to GABAergic signalling [for example, see (Harris and Westbrook, 1998; Chhatwal et al., 2005; Akirav et al., 2006; Lin et al., 2009; Dalton et al., 2012)], amongst others. Following acquisition of extinction, there is a consolidation phase which lasts up to several hours which serves to stabilize plastic events into long-term memory (McGaugh, 2000). Activation of NMDA receptors (Davis, 2011), brain-derived neurotrophic factor (BDNF) signalling (Chhatwal et al., 2006; Soliman et al., 2010) and inhibition of histone deacetylases (HDAC) (Guan et al., 2009; McQuown et al., 2011), amongst others, are important for consolidation of extinction [for detailed reviews, see (Myers and Davis, 2007; Quirk and Mueller, 2008; Herry et al., 2010; Pape and Pare, 2010; Steckler and Risbrough, 2011; Orsini and Maren, 2012; Tronson et al., 2012)].

The present study used isogenic 129S1/SvImJ (S1) mice which display deficient extinction acquisition, poor recovery of fear-induced suppression of heart-rate variability, enlarged dendritic arbours in basolateral amygdala neurons and functional abnormalities in cortico-amygdala circuitry mediating fear extinction (Hefner et al., 2008; Camp et al., 2009, 2012; Whittle et al., 2010). In the current study we reduced the intensity of fear conditioning and observed that "weak" fear conditioning allowed for extinction acquisition in S1 mice but revealed deficient extinction retrieval. Recent findings show that impaired extinction acquisition and dysfunctional cortico-amygdala circuitry can be rescued by a novel zinc restricted diet (Whittle et al., 2010), which interestingly did not affect either fear learning or fear expression. Also, recent findings reveal that the S1 fear extinction phenotype can be rescued by fluoxetine (a serotonin-selective reuptake inhibitor) (Camp et al., 2012) which is of clinical relevance as serotonin-selective reuptake inhibitors are first-line treatment for anxiety disorders (Nutt, 2005). Considering the well-known disadvantages of long-term SSRI treatments, we screened for novel treatments which rescue both deficient extinction acquisition and extinction consolidation/retrieval in S1 mice.

Evidence suggests that deep brain stimulation (DBS) of the nucleus accumbens (AcbC) can enhance extinction-like behaviour in intractable obsessive-compulsive disorder patients, which is characterised by avoidance behaviours that fail to extinguish (Lipsman et al., 2007; Burdick et al., 2009; Goodman et al., 2010; Greenberg et al., 2010; Grant et al., 2011). Furthermore, a small clinical study involving patients suffering from treatment-resistant depression has shown that DBS of the AcbC can enhance various measures of cognition independent of mood status (Bewernick et al., 2012). However, there are also reports of no effect of AcbC-DBS in mood disorder patients (Grubert et al., 2011). Very recently, DBS of the AcbC during extinction training reduced fear expression and strengthened extinction memory in normally behaving rats (Rodriguez-Romaguera et al., 2012) potentially showing the utility of DBS of the AcbC as a novel fear reducing adjunct during exposure-based therapy. Here, we went beyond using normally behaving rodents and assessed whether DBS of the AcbC during extinction training can rescue deficient extinction acquisition and extinction consolidation/retrieval in S1 mice.

Pharmacological compounds which enhance glutamatergic signalling, including DCS (NMDA receptor partial agonist), PEPA (AMPA receptor potentiator) or AMN082 (mGlu7 receptor agonist), can facilitate fear extinction in normally extinguishing animal models and in clinical studies (Myers et al., 2011). We have previously shown that prior extinction training administration of DCS is ineffective in rescuing deficient extinction learning in S1 mice (Hefner et al., 2008). However, it remains unknown whether DCS can rescue deficient extinction consolidation/retrieval once at least some extinction learning is induced, which we revealed in "weak" fear conditioned S1 mice. Furthermore, it is not known whether PEPA or AMN082 can reduce fear in an animal model of impaired extinction.

Drugs inhibiting HDAC proteins to prevent the removal of acetyl groups on histone tails is a promising approach in central nervous system disorders associated with disturbed learning and memory (Kazantsev and Thompson, 2008). Interestingly, inhibiting HDACs has been shown to enhance fear learning (Guan et al., 2009) and rescue deficits in fear learning in mouse models of anxiety or neurodegeneration (Li et al., 2006; Dash et al., 2009; Kilgore et al., 2010). Facilitation of fear extinction by HDAC inhibition has been reported in normally extinguishing mice (Bredy et al., 2007; Lattal et al., 2007; Bredy and Barad, 2008; Guan et al., 2009; Monsey et al., 2011). However, it is not known whether inhibiting HDACs can improve extinction learning in animal models of impaired extinction. Thus, we tested whether valproic acid (VPA), a dual HDAC inhibitor and enhancer of GABAergic signalling or MS-275, a more specific HDAC inhibitor (Khan et al., 2008; Bantscheff et al., 2011), can rescue deficient extinction in S1 mice.

2. Materials and methods

2.1. Subjects

Subjects were male 3–5 month old 129S1/SvImJ (S1) mice. For experiments performed at the University of Innsbruck, mice (obtained from Charles River, Germany) were housed (4–5 per cage) in a temperature- (22 ± 2 °C) and humidity- (50–60%) controlled vivarium under a 12 h light/dark cycle (lights on at 7:00 A.M.). For experiments conducted at the National Institutes of Health, mice (obtained from The Jackson Laboratory, USA) were housed (2 per cage) in a temperature (22 ± 1 °C)- and humidity (45 ± 15%)-controlled vivarium under a 12 h light/dark cycle (lights on, 6:00 A.M.). All experimental procedures were approved by the Austrian Animal Experimentation Ethics Board and by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use and Austrian Ethical Committees on Animal Care and Use (Bundesministerium für Wissenschaft und Verkehr, Kommission für Tierversuchsangelegenheiten) and followed the National Institutes of Health guidelines outlined in Using Animals in Intramural Research and the local animal care and use committees.

2.2. General procedures for fear conditioning and extinction

For experiments performed at the University of Innsbruck, mice were conditioned in a 25 × 25 × 35 cm chamber with transparent walls and a metal rod floor, cleaned with water and illuminated to 300 lux ('context A'). After a 120 s acclimation period, there were 3× pairings of a 120 s, 80 dB white noise conditioned stimulus (CS) and a 2 s scrambled foot shock unconditioned stimulus (US) (0.6 mA unless stated otherwise), with a 120 s inter-pairing interval. There was a 120 s no-stimulus consolidation period after the final pairing before mice were returned to the home cage. Extinction training was performed the next day in a 25 × 25 × 35 cm cage with a solid grey floor and black walls, cleaned with a 100% ethanol and illuminated to 10 lux ('context B'). After a 120 s acclimation period, there were CS-alone trials (16 unless stated otherwise), with a 5-sec inter-CS interval. Stimulus presentation was controlled by a TSE operant system (TSE, Bad Homburg, Germany). Extinction retrieval was conducted the following day by repeating the extinction training procedure but with 2× CS trials. Freezing was measured as an index of fear (Blanchard and Blanchard, 1969), manually scored based on DVD recordings of the duration of the CS, defined as no visible movement except that required for respiration, and converted to a percentage [(duration of freezing within the CS/total time of the CS) × 100] by a trained observer blind to the animals' group.

For experiments performed at NIAAA, mice were conditioned in a 27 × 27 × 11 cm chamber with a metal-rod floor, cleaned with a 79.5% water/19.5% ethanol/1% vanilla-extract solution ('context A'). After a 180 s acclimation period, there were 3× pairings (60–120 s inter-pairing interval) of the conditioned stimulus (CS; 30 s 80 dB, 3 kHz tone) and the unconditioned stimulus (US; 2 s, 0.6 mA scrambled foot shock), in which the US was presented during the last 2 s of the CS. The session ended 120 s after the final CS–US pairing. Stimulus presentation was controlled by the Med Associates VideoFreeze system (Med Associates, Burlington, VT, USA). 24 h after conditioning, extinction training was conducted in a novel context ('context B') (cylinders with black/white-chequered walls and a solid Plexiglas opaque floor cleaned with a 1% acetic acid/99% water solution) housed in a different room from conditioning. After a 180 s acclimation period, there were 16×