Pharmacological enhancement of mGluR5 facilitates contextual fear memory extinction

Ferzin Sethna1 and Hongbing Wang2,3

1Genetics Program, 2Department of Physiology, 3Neuroscience Program, Michigan State University, East Lansing, Michigan 48824, USA

Behavioral exposure therapy, which involves extinction of the previously acquired fear, has been used to treat anxiety-related symptoms such as post-traumatic stress disorder. It has been hypothesized that proextinction pharmacotherapeutics may enhance the efficacy of exposure therapy. Systemic administration of the metabotropic glutamate receptor 5 (mGluR5)-positive allosteric modulator 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) facilitated the extinction of contextual fear memory. Notably, CDPPB also enhanced the initial fear memory formation, and had no effect on memory retrieval. Our data suggest that positive regulation of mGluR5 may offer a new method to enhance exposure therapy through facilitating extinction without adversely affecting other aspects of memory process.

[Supplemental material is available for this article.]

Experiencing an extremely traumatic event can sometimes lead to dysregulated fear, which manifests itself as post-traumatic stress disorder (PTSD) or other fear-related symptoms such as anxiety disorder (Kearns et al. 2012). It is estimated that PTSD and anxiety disorders affect 5%–10% and 18% of the population, respectively, in the USA. Majority of people who experience trauma show hyperarousal, which declines over time. However, the hyperarousal in PTSD patients remains persistent, possibly due to impaired fear memory extinction (Kearns et al. 2012; Parsons and Ressler 2013). In order to reduce fear response linked to certain situations, individuals must incorporate new learning and form a corrective memory such that the situation is no longer associated with the traumatic event (Myers and Davis 2007; Maren 2011). This is the same process that occurs in memory extinction, during which the frequency or magnitude of the conditioned response decreases upon repeated or prolonged exposure to a cue (i.e., the conditioned stimulus) in the absence of the unconditioned stimulus. Indeed, extinction has been successfully used in exposure therapy, which involves gradual exposure to the fear-causing stimulus in the absence of an aversive event (Quirk et al. 2010; Kearns et al. 2012). Thus, application of proextinction agents along with exposure therapy may improve the efficacy and outcome of PTSD treatment (Choi et al. 2010).

Glutamate transmission, which is mediated by ionotropic and metabotropic glutamate receptors (iGluR and mGluR), regulates both long-term potentiation (LTP) and long-term depression (LTD) as well as learning and memory formation. Potentiation of iGluRs including NMDAR and AMPAR normally leads to the facilitation of learning and extinction (Tang et al. 1999; Yamada et al. 2009; Myers et al. 2011). Inhibition of these receptors impairs learning and extinction. Notably, the partial NMDAR agonist d-cycloserine (DCS) has been effectively used in combination with exposure therapy for several fear and anxiety abnormalities such as panic disorder, social anxiety disorder, obsessive-compulsive disorder, and PTSD (Kushner et al. 2007; Otto et al. 2010; Myers et al. 2011; Difede et al. 2014). Recently, targeting the modulatory mGluRs has been increasingly popular for therapeutic approaches due to fewer serious side effects such as memory loss, disorientation, psychosis, and hallucinations (Cleva and Olive 2011). Among the mGluRs, mGluR5 is found to physically interact with NMDAR, and its activation potentiates NMDAR function (Awad et al. 2000; Cleva and Olive 2011). Genetic deletion and pharmacological inhibition of mGluR5 leads to impairments in the acquisition of fear memory and extinction (Lu et al. 1997; Rodrigues et al. 2002; Xu et al. 2009; Fontanez-Nuin et al. 2011). Thus, positive modulation of mGluR5 may represent an attractive approach to facilitate both the initial learning and the inhibitory learning during extinction. In support of this idea, the mGluR5-positive allosteric modulator (PAM) CDPPB (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide) enhances the extinction learning following methamphetamine (Kufahl et al. 2012), ethanol (Gass et al. 2014), and cocaine self-administration (Cleva et al. 2011). CDPPB also facilitates the extinction of conditioned place preference memory associated with cocaine (Gass and Olive 2009). Consistent with the notion that the prefrontal cortex (PFC) is involved in inhibitory learning during extinction, neuroplasticity, and neuronal firing in the PFC are altered following the extinction of drug seeking and the administration of CDPPB (Lecourtier et al. 2007; Knackstedt et al. 2010; Ghasemzadeh et al. 2011; Gass et al. 2014). However, the effect of mGluR5 PAM on regulating fear memory extinction remains unknown.

Here, we examined the effect CDPPB on contextual fear memory extinction. All experiments were performed with 2- to 3-mo-old male C57BL6 mice, and all manipulations were in compliance with the guidelines of Institutional Animal Care and Use Committee at Michigan State University. As shown in Figure 1A1, mice were first trained by contextual fear conditioning, during which animals received a single mild footshock (0.7 mA, 2 sec).
Twenty-four hours later, mice receiving intraperitoneal injection of vehicle (10% DMSO) or CDPPB (20 mg/kg in 10% DMSO) were subjected to a 15-min extinction session. Both groups showed significant freezing (Fig. 1A2) and reduced ambulatory movement (Supplemental Fig. S1) during the first 3 min of the extinction session, indicating that potentiation of mGluR5 by CDPPB did not affect memory retrieval (or expression). Compared with the vehicle-injected group, mice receiving CDPPB showed more decrease in freezing (Fig. 1A2; $F_{(1,13)} = 5.01, P < 0.05$) and more increase in movement (Supplemental Fig. S1; $F_{(1,13)} = 6.39, P < 0.05$) within the 15-min extinction session.

To test the retention of contextual memory extinction, we examined the conditioned fear response 24 h following the extinction session (Fig. 1A1). The facilitated fear extinction in the CDPPB group was preserved, as indicated by less freezing (Fig. 1A1; $F_{(1,13)} = 5.01, P < 0.05$) and more movement (Supplemental Fig. S1; $F_{(1,13)} = 6.39, P < 0.05$) within the 15-min extinction session.

It is possible that CDPPB administered before extinction could simply degrade the contextual memory and thus result in reduced fear during the subsequent retention test. To test this possibility, we injected another cohort of mice with vehicle or CDPPB 24 h after training and then tested the contextual fear memory 24 h after the injection (Fig. 2A). The vehicle- and CDPPB-injected mice showed similar freezing (Fig. 2B1) as well as movement (Fig. 2B2) during testing. This indicates that CDPPB in the absence of the extinction protocol does not alter contextual fear memory.

Previous studies have suggested that memory extinction and the initial memory formation may be regulated by opposing mechanisms. For instance, enhancement of cAMP signaling impairs memory formation but facilitates extinction (Abel et al. 1997; Iségas et al. 2006); enhancement of cAMP facilitates fear memory formation but impairs extinction (Wang et al. 2004; Monti et al. 2006).

We further examined whether mGluR5 potentiation also has procognitive effect on the initial fear memory formation (Fig. 3A). After receiving CDPPB or vehicle, mice were trained by contextual fear conditioning, during which a mild footshock was delivered (Fig. 3B). The CDPPB- and vehicle-injected mice showed comparable response to the footshock, which stimulated running behavior leading to a momentary increase in locomotion (Fig. 3B; $F_{(1,12)} = 0.53, P > 0.05$). This indicates that CDPPB does not alter sensitivity to the shock-induced pain sensation. When tested 24 h after the contextual training, CDPPB-injected group displayed more freezing (Fig. 3C) and less movement (Supplemental Fig. S2), together, these data indicate that enhancement of mGluR5 activity may facilitate the extinction of the old fear memory (Fig. 1A) without hampering new fear memory formation (Fig. 3).
Our data demonstrate that positive modulation of mGluR5 enhances behavioral flexibility, as indicated by that CDPBP facilitates both the initial learning and inhibitory learning during fear memory extinction. This is consistent with the observation that mGluR5 PAMs also enhance synaptic flexibility by facilitating both LTP and LTD (Ayala et al. 2009). Recent studies have shown that mGluR5 PAMs (e.g., CDPBP and ADX47273) enhance both hidden platform and reversal platform learning in the Morris water maze test (Ayala et al. 2009; Xu et al. 2013). By using another mGluR5 PAM, Xu et al. (2013) has found that pretreatment administration of ADX47273 fails to enhance the single-session extinction of both contextual and cued fear memory. Further, pre- or post-extinction injection of ADX47273 also does not affect the multisession extinction of contextual fear memory. It is unknown whether ADX47273 and CDPBP have different pharmacological modality, and whether higher dose of ADX47273 would show preextinction effect. It is important to note that the study by Xu et al. (2013) trained mice with three footshocks but we used one shock. It is possible that the stronger conditioned fear may be less sensitive to positive modulation of mGluR5. Interestingly, when ADX47273 is given between the brief memory reactivation/retrieval session and the subsequent extinction session, it causes a weaker recall of the cued fear memory without affecting the freezing behavior within the extinction session. Thus, ADX47273 may facilitate the consolidation of extinction when administered during the reactivation–reconsolidation window (Xu et al. 2013).

In summary, this study, for the first time, demonstrates the effects of mGluR5-positive modulation in both the initial contextual fear memory formation and extinction. As CDPBP enhances fear memory extinction without adversely affecting other aspects of the learning process, our results suggest the application of mGluR5 PAMs as a new potential strategy to enhance the efficacy of exposure therapy and treat anxiety disorders.

Competing interest statement
The authors declare no conflict of interest.

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