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Pharmacological interference with metabotropic glutamate receptor subtype 7 but not subtype 5 differentially affects within- and between-session extinction of Pavlovian conditioned fear☆

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ABSTRACT

Fear extinction is defined as the attenuation of a conditioned-fear memory by re-exposing animals to the conditioned stimulus without the aversive stimulus. This process is known to be effectively enhanced via administration of D-cycloserine (DCS), a partial NMDA-receptor agonist. However, other glutamatergic mechanisms, such as interference with metabotropic glutamate receptor (mGluR) subtypes 5 and 7 in the extinction of aversive memories are insufficiently understood. Using the allosteric mGluR5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP), the mGluR7 allosteric agonist *N,N′*-dibenzyhydryl-ethane-1,2-diamine dihydrochloride (AMN082), and DCS for comparison, we aimed to study how pharmacological blockade of mGluR5 and activation of mGluR7 influenced within- and between-session conditioned-fear extinction training and extinction retention in rats. We show that when injected before extinction training, mGluR7 activation with AMN082 enhanced freezing and thereby attenuated within-session fear extinction, whereas both DCS and the mGluR5 receptor antagonist MPEP had no effect on this process. However, these differential drug effects were not long lasting, as no difference in extinction retention were observed 24 h later. Therefore, we assessed whether the compounds affect 24 h consolidation of extinction training following incomplete extinction training (between-session extinction). Similar to DCS, AMN082- but not MPEP-treated rats showed facilitated extinction retention, as exhibited by decreased freezing. Finally, using fluoxetine, we provide evidence that the effect of AMN082 on between-session extinction retention is most likely not via increasing 5-HT transmission. These findings demonstrate that mGluR7 activation differentially modulates conditioned-fear extinction, in dependence on the protocol employed, and suggests drugs with AMN082-like mechanisms as potential add-on drugs following exposure-based psychotherapy for fear-related human disorders.

1. Introduction

Several psychiatric illnesses involve learned components that contribute to the development of the symptom complexes. For instance, in specific phobias, social anxiety disorder and post-traumatic stress disorder (PTSD), conditioned stimuli (learned associations) may elicit fear, anxiety and intrusive memories. Also, in addiction disorders, drug-associated cues can trigger withdrawal responses, craving and relapse effects (Hofmann et al., 2006b; Myers et al., 2010; Rothbaum and Davis, 2003). Such conditioned stimuli can also be formed experimentally by repeatedly pairing initially neutral cues (e.g. odours, tones, visual signals) with an unconditioned stimulus (e.g. physical punishment or onset of drug effect). Consequently, the neutral cue acquires the ability to elicit classical conditioned responses, such as freezing. The most efficient way to reverse conditioned responses is through the process of extinction, which usually involves a protocol of repeated or prolonged exposure to the conditioned stimulus in the absence of the adverse event it once predicted, a procedure termed extinction training (McCallum et al., 2010; Myers et al., 2010; Myers and Davis, 2002).

Like acquisition of Pavlovian conditioning, extinction is also a form of associative learning. It can be sub-divided into mechanistically distinct memory formation processes, most notably encoding,

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consolidation and retention of extinction memory. Encoding occurs during the extinction training session(s), and experimental assessment is often attempted by measuring the amplitude of within-session extinction changes of the behavioural response that was initially triggered by the aversive stimulus, which is a short-term reduction (usually within less than 1 h). Thus, within-session extinction is measured during repeated or prolonged exposure to the conditioned stimulus, but in the absence of the aversive stimulus. Consolidation and retention of extinction, on the other hand, are mostly addressed by measuring between-session extinction, where extinction memory is generally assessed by an extinction retention test usually performed at least 24 h after completion of extinction training (McCallum et al., 2010; Myers et al., 2010).

The brain circuitry underlying fear conditioning and extinction is highly conserved across species (Myers et al., 2010). Therefore, modern research makes extensive use of rodent animal models and aims to uncover the exact neural circuitry as well as molecular and neurophysiological mechanisms underlying the various behavioural characteristics of extinction. Mechanistically, much has been learned about the contribution of various neurotransmitter systems to extinction. In particular, the main excitatory neurotransmitter of the mammalian brain, L-glutamate, and its pre- and postsynaptic receptors have received a lot of attention during the past years (Fendt et al., 2008; Myers et al., 2010; Zushida et al., 2007). The actions of L-glutamate are mediated by ionotropic and metabotropic receptor subtypes (iGluR and mGluR protein families, respectively). *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors constitute the main iGluR subtypes, and their role in extinction has already been studied in depth (Akirav, 2007; Myers et al., 2010; Walker et al., 2002b; Zushida et al., 2007). In fact, one of the best examined pharmacological mechanisms to exert control on fear extinction is partial allosteric activation of ionotropic NMDA receptors at the glycine modulatory site with the clinically approved antibiotic D-cycloserine (DCS). In rodents, systemic administration of DCS before or after extinction training facilitates fear extinction, an effect which could be localized within the basolateral amygdala (Ledgerwood et al., 2003, 2005; Myers et al., 2010; Richardson et al., 2004; Walker et al., 2002b). Interestingly, extinction of conditioned drug craving and withdrawal is facilitated by DCS as well (Botreau et al., 2006; Groblewski et al., 2009; Nic Dhonnchadha et al., 2010; Paolone et al., 2009; Thanos et al., 2009; Torregrossa et al., 2010). Also, at the clinical level, DCS broadly enhances extinction-based psychotherapy, e.g. for fear of heights, social anxiety or panic disorders, and nicotine as well as cocaine addiction (Guastella et al., 2008; Hofmann et al., 2006a; Otto et al., 2010; Price et al., 2009; Ressler et al., 2004; Santa Ana et al., 2009).

In contrast to iGluRs, studies on the contribution of mGluRs to fear extinction have only appeared very recently, and the knowledge at this time is still limited. In the mammalian central nervous system, mGluRs exist as eight receptor subtypes with multiple pharmacological sites and modes of action (Conn and Niswender, 2006; Flor et al., 2002; Niswender and Conn, 2010). In the present study, we focus on two mGluR-directed mechanisms that represent promising potential for neurological and possibly psychiatric disorders (Bird and Lawrence, 2009; Krystal et al., 2010; O'Connor et al., 2010; Yang, 2005), which also emerge in the field of conditioned fear and extinction research: first, negative allosteric modulation of mGluR5 (e.g. with the prototypical drug MPEP), a mechanism that is under clinical investigation in several nervous system disorders and has also demonstrated effects against acquisition and retention of conditioned fear responses (Fontanez-Nuin et al., 2010; Gasparini et al., 2008; Kim et al., 2007; Riedel et al., 2000; Schulz et al., 2001; Xu et al., 2009); second, allosteric activation of the mGluR7 subtype, e.g. with the agent AMN082 that shows preclinical antiparkinsonian-, anxiolytic-, and antidepressant-like activity (Greco et al., 2010; O'Connor et al., 2010; Palucha et al., 2007; Stachowicz et al., 2008). Interestingly, AMN082 blocks acquisition of conditioned fear but also facilitates extinction of conditioned aversion and fear in two amygdala-dependent paradigms [i.e. conditioned taste aversion and fear-potentiated startle (Fendt et al., 2008; Siegl et al., 2008)]. Furthermore, a recent study, also targeting a closely related metabotropic glutamate receptor, revealed that mGluR8-deficient mice had a significant decrease in the freezing response to the conditioning context (Fendt et al., 2010). Thus, the available evidence suggests that mGluRs may represent promising candidates for pharmacologically improving the outcome of exposure-based therapy.

Therefore, the primary aim of our present study was to assess whether pharmacological interference with mGluR5 and mGluR7 differentially affects the stages of extinction memory, as assessed by conditioned freezing. Further, we compare the effects of the mGluR-directed mechanisms to those of DCS. Overall, our studies aim to inform future clinical anxiety and drug addiction trials about MPEP- and AMN082-like agents as possible pharmacological add-on aids to exposure-based psychotherapy in man.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Charles River, Sulzfeld, Germany) weighing 220–250 g were housed in groups of four and kept under standard laboratory conditions (12:12 light/dark cycle, lights on at 6 am, 22 °C, 60% humidity and given free access to water and standard rat chow). All behavioral procedures took place during the light phase (8 am–2 pm). Experimental procedures were approved by the local government of the Oberpfalz (Bavaria, Germany) and followed the European Communities Council directive (86/609/EEC).

2.2. Drugs

All drugs were administered intraperitoneally (i.p.) at a volume of 1 ml/kg. DCS (from Sigma-Aldrich, Germany) was freshly dissolved in saline. AMN082 and MPEP (synthesized by Novartis Pharma AG; Basel, Switzerland) were freshly dissolved in 0.5% methylcellulose (AMIMED, Allschwil, Switzerland). The dose of DCS (15 mg/kg) was selected because it has previously been reported to facilitate fear extinction in rats (Bertotto et al., 2006; Langton and Richardson, 2008; Ledgerwood et al., 2003; Walker et al., 2002b). The dose of AMN082 (10 mg/kg) was chosen based on previously published studies, which all showed a very narrow useful dose-range for AMN082 (Bahi et al., in press; Fendt et al., 2008; Mitsukawa et al., 2005; Stachowicz et al., 2008). Higher doses of AMN082 in rats (20–60 mg/kg) induced motor side-effects such as mild ataxia or body tremor and/or showed reduced efficacy in several behavioural tests (compared to 10 mg/kg; see e.g. Bahi et al., in press). Lower doses than 10 mg/kg of AMN082 in rats are usually not efficacious in behavioural models (Bahi et al., in press; Fendt et al., 2008). In addition, previous studies have shown that 10 mg/kg of MPEP was also a safe and effective dose in fear conditioning experiments and did not affect spontaneous locomotor activity (Backstrom et al., 2004; Fontanez-Nuin et al., 2010; Herzig and Schmidt, 2004; Schulz et al., 2001; Varty et al., 2005). Fluoxetine (Sigma- Aldrich, Germany) was freshly dissolved in saline. The selected dose of fluoxetine (10 mg/kg) has previously been reported to alter fear conditioning (Burghardt et al., 2007; Santos et al., 2006).

2.3. Apparatus

The cued-fear experiments were performed in two different contexts, A and B, which differed in visual, tactile and olfactory cues. Fear conditioning occurred in context A, which consisted of a transparent perspex box (45 × 45 × 40 cm) with a transparent lid. The floor was made up of 25 × 0.6 cm stainless steel bars set 1.2 cm apart. Context A was cleaned with a neutral smelling detergent before each trial. Extinction training and retention occurred in context B, which consisted of a black perspex box (45 × 45 × 40 cm) with a smooth floor. Context B was cleaned with a lemon-scented detergent before each trial. The boxes were enclosed in a wooden chamber to reduce external noise and visual stimulation. A low level of background noise was produced by ventilation fans within the chamber. Illumination (300 lx for context A and 20 lx for context B) was provided by four white LEDs. Auditory stimuli were delivered through a speaker attached approximately 30 cm above the floor of the box. A video camera was placed at the top of the chamber and allowed the recording of the animal's behavior. As a measure of fear, freezing/inactivity, defined