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Pharmacological modulation of metabotropic glutamate receptor subtype 5 and 7 impairs extinction of social fear in a time-point-dependent manner

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ABSTRACT

Pharmacological modulation of metabotropic glutamate receptor subtype 5 (mGluR5) and 7 (mGluR7) was shown to attenuate the acquisition and to facilitate the extinction of cued and contextual, non-social, fear. Using the allosteric mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and the allosteric mGluR7 agonist *N*,*N*'-dibenzyhydryl-ethane-1,2-diamine dihydrochloride (AMN082), we aimed to study how pharmacological blockade of mGluR5 and activation of mGluR7 influence acquisition and extinction of social fear in mice. We could show that when administered before social fear conditioning, neither MPEP nor AMN082 affected acquisition and extinction of social fear, suggesting that mGluR5 inactivation and mGluR7 activation do not alter social fear. However, when administered before social fear extinction, both MPEP and AMN082 impaired social fear extinction and extinction recall. These findings suggest that mGluR5 inactivation and mGluR7 activation are unlikely to prevent the formation of traumatic social memories. Furthermore, medication strategies aimed at augmenting exposure-based therapies for psychiatric disorders associated with social deficits *via* modulation of mGluR5 and mGluR7 must be pursued cautiously because of their potential to delay social fear extinction processes.

1. Introduction

Several psychiatric disorders, such as posttraumatic stress disorder (PTSD), panic disorder, specific phobias and social anxiety disorder (SAD) involve learned components. Memories triggered by trauma-associated cues induce fear and anxiety and contribute to the development and maintenance of symptoms. Such traumatic memories can also be induced experimentally by repeatedly pairing initially neutral cues (*e.g.* odors, tones, visual stimuli, social stimuli) with an unconditioned stimulus (*e.g.* physical punishment or onset of drug effect). Consequently, the neutral cue acquires the ability to elicit conditioned responses, such as fear and anxiety. Therapeutic approaches aimed at reducing the impact of trauma-associated cues in eliciting maladaptive fear and anxiety responses in psychiatric disorders are likely to be beneficial. The most efficient way to reduce conditioned responses is through the process of extinction, which usually involves repeated exposures to the conditioned stimulus in the absence of the adverse event it once predicted [1,2].

Research on the role of glutamate and its ionotropic and metabotropic (mGluR) receptors in extinction have led to the development of pharmacotherapies which enhance the efficacy of extinction-based protocols in clinical populations [2]. For example, augmentation of exposure therapy with D-cycloserine, a ionotropic *N*-methyl-D-aspartate (NMDA) receptor agonist, was shown to improve some anxiety symptoms in SAD [3,4], PTSD [5,6], panic disorder [7], and acrophobia [8]. Studies on the contribution of mGluRs to fear extinction have only appeared recently, and knowledge at this time is still limited. Preclinical research suggested that mGluRs may represent promising candidates for pharmacologically improving the outcome of exposure-based therapy. As such, negative allosteric modulation of mGluR5 with 2-methyl-6-(phenylethynyl)-pyridine (MPEP) demonstrated effects against acquisition and retention of conditioned fear [9–12]. Similarly, allosteric activation of the mGluR7 subtype with *N*,*N*'-dibenzyhydryl-ethane-1,2-diamine dihydrochloride (AMN082) blocked acquisition of conditioned fear, but also facilitated extinction of conditioned aversion and fear in two amygdala-dependent paradigms (*i.e.* conditioned taste aversion and fear-potentiated startle) [13,14]. However, it is unclear, whether pharmacological modulation of mGluR5 and mGluR7 might also alter coping with, and recovery from, a traumatic social experience. Only a few studies investigated the effects of MPEP on social

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behaviors and most of these reported that MPEP has the potential to normalize deficits in social interaction. As such, MPEP decreased inter-male aggression and increased social investigation in highly aggressive OF1 mice [15]. It also increased social investigation in Balb/c mice [16], and mice lacking the excitatory synaptic signaling scaffold IRSp53 [17], which were shown to have deficits in social interaction and communication. However, MPEP did not improve social interaction deficits in autistic-like BTBR mice [18] and even decreased social investigation in Swiss Webster mice, which show normal social interaction [16]. So far, only one study investigated the effects of AMN082 on social behavior. In more detail, Navarro et al. [19] have shown that AMN082 decreased inter-male aggression without affecting social investigation in highly aggressive OF1 mice.

In the present study, we aimed to investigate whether pharmacological modulation of mGluR5 and mGluR7 affects acquisition and extinction of conditioned social fear in CD1 mice, which otherwise show normal social interaction [20–22]. In order to induce social fear, we used the social fear conditioning (SFC) paradigm, which was established to mimic the major behavioral symptoms of SAD, *i.e.* reduced social investigation and avoidance of con-specifics, as indicative of social fear [23,24]. In this model, social fear is induced by administration of mild electric foot shocks during the investigation of a con-specific. Importantly, treatment of socially fear-conditioned (SFC^+) mice with medication used for SAD patients, such as diazepam and paroxetine, reversed social fear, providing predictive validity to the SFC model [23]. Repeated exposure of the SFC^+ mice to unknown con-specifics leads to a gradual decline in the fear response, a process termed social fear extinction. MPEP or AMN082 were administered either prior to SFC (also referred to as acquisition) or social fear extinction in order to determine whether the drugs affected acquisition or extinction of social fear.

2. Materials and methods

2.1. Animals

Male CD1 mice (Charles River, Sulzfeld, Germany, 30–35 g) were individually housed for 1 week and transferred to observation cages (30 × 23 × 36 cm) 3 days before experiments started. Age- and weight-matched male CD1 mice were used as social stimuli. Mice were kept under standard laboratory conditions (12:12 light/dark cycle, lights on at 06:00 h, 22 °C, 60% humidity, food and water *ad libitum*). Experiments were performed during the light phase, between 08:00 and 12:00, in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Oberpfalz and the guidelines of the NIH. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Social fear conditioning (SFC) paradigm

SFC was performed with a computerized fear conditioning system (TSE System GmbH, Bad Homburg, Germany) as previously described [23,24]. Briefly, the conditioning chamber consisted of a transparent Perspex box (45 × 22 × 40 cm) enclosed in a wooden chamber to reduce external noise and visual stimulation. The floor consisted of a removable stainless steel grid connected to a shock delivery unit used for manual application of foot shocks. A video camera at the top of the chamber enabled video recording.

2.3. SFC (day 1)

For the acquisition of social fear, mice were placed in the conditioning chamber and, after a 30-s habituation period, an empty wire mesh cage (7 × 7 × 6 cm) was placed as a non-social stimulus near one of the short walls. After 3 min, the non-social stimulus was replaced by an identical cage containing an unfamiliar male mouse. Unconditioned mice (SFC^-) were allowed to investigate this social stimulus for 3 min, whereas conditioned mice (SFC^+) were given a 1-s electric foot shock (0.7 mA) each time they investigated, *i.e.* made direct contact with the social stimulus. Mice received between 1 and 5 foot shocks, with a variable inter-shock interval, depending on when direct social contact was made. The number of foot shocks was assessed as a measure of distress. Mice were returned to their home cage when no further social contact was made for 2 min. The time mice spent investigating the non-social stimulus, as a pre-conditioning measure of non-social anxiety, was analyzed from videos by an observer blind to the treatment using the JWatcher program (V 1.0, Macquarie University and UCLA).

2.4. Social fear extinction (day 2)

To investigate whether SFC^+ mice displayed social fear and whether this fear could be extinguished, social investigation was assessed in the home cage 1 day after SFC. Mice were exposed to 3 non-social stimuli, *i.e.* empty cages identical to the cage used on day 1, to assess non-social investigation as a parameter of non-social fear. Mice were then exposed to 6 unfamiliar social stimuli, *i.e.* male mice enclosed in wire mesh cages, to assess social investigation as a parameter of social fear. Each stimulus was placed near a short wall of the home cage and presented for 3 min, with a 3-min inter-exposure interval. Non-social investigation was defined as direct sniffing of the empty cage, whereas social investigation was defined as direct sniffing of the cage and/or of the social stimulus inside the cage.

2.5. Extinction recall (day 3)

To investigate whether repeated exposure to social stimuli during social fear extinction leads to a complete reversal of social fear, social investigation was assessed in the home cage 1 day later. Mice were exposed to 6 unfamiliar social stimuli for 3 min, with a 3-min inter-exposure interval, to assess social investigation as a parameter of social fear.

2.6. Drugs

MPEP and AMN082 (discovered by Novartis Pharma AG, Basel, Switzerland) were freshly dissolved in 0.5% methylcellulose (AMIMED, Allschwil, Switzerland) and administered intraperitoneally (i.p.) at a volume of 10 ml/kg. The doses of MPEP (10 mg/kg) and AMN082 (4 mg/kg) were chosen based on previous studies [19,25–27].

2.7. Statistical analysis

For statistical analysis PASW/SPSS (Version 21) was used. Data were analyzed by one-way (factor group) or three-way (factors conditioning × treatment × stimulus) ANOVA for repeated measures, followed by a Bonferroni's post-hoc analysis whenever appropriate. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effects of MPEP and AMN082 administration prior to SFC on social fear

To investigate whether MPEP and AMN082 influence acquisition of social fear, mice (n = 6/group) were injected i.p. with either vehicle (Veh; 10 ml/kg 0.5% methylcellulose), MPEP, or AMN082 30 min before SFC (Fig. 1A).

Mice showed similar investigation of the non-social stimuli during SFC ($F(5,30) = 0.60$; $p = 0.70$), reflecting similar pre-conditioning non-social anxiety. All SFC^+ mice received a similar number of foot shocks during SFC ($F(2,15) = 0.65$; $p = 0.54$), reflecting similar dis-