Neurobiology of Learning and Memory 142 (2017) 209–217

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Combining D-cycloserine with appetitive extinction learning modulates amygdala activity during recall

Claudia Ebrahimi [a,*], Stefan P. Koch [a], Eva Friedel [a], Ilsoray Crespo [a], Thomas Fydrich [b], Andreas Ströhle [a], Andreas Heinz [a,c], Florian Schlagenhauf [a,d]

[a] Department of Psychiatry and Psychotherapy, Campus Charité Mitte, Charité – Universitätsmedizin Berlin, 10117 Berlin, Germany
[b] Department of Psychology, Humboldt University of Berlin, 10099 Berlin, Germany
[c] Cluster of Excellence NeuroCure, Charité – Universitätsmedizin Berlin, 10117 Berlin, Germany
[d] Max Planck Institute for Human Cognitive and Brain Sciences, 04303 Leipzig, Germany

ARTICLE INFO

Article history:
Received 16 December 2016
Revised 7 May 2017
Accepted 12 May 2017
Available online 13 May 2017

Keywords:
D-cycloserine
Pavlovian conditioning
Extinction recall
Amygdala
vmPFC
Reward

ABSTRACT

Appetitive Pavlovian conditioning plays a crucial role in the pathogenesis of drug addiction and conditioned reward cues can trigger craving and relapse even after long phases of abstinence. Promising preclinical work showed that the NMDA-receptor partial agonist D-cycloserine (DCS) facilitates Pavlovian extinction learning of fear and drug cues. Furthermore, DCS-augmented exposure therapy seems to be beneficial in various anxiety disorders, while the supposed working mechanism of DCS during human appetitive or aversive extinction learning is still not confirmed.

To test the hypothesis that DCS administration before extinction training improves extinction learning, healthy adults (n = 32) underwent conditioning, extinction, and extinction recall on three successive days in a randomized, double-blind, placebo-controlled fMRI design. Monetary wins and losses served as unconditioned stimuli during conditioning to probe appetitive and aversive learning. An oral dose of 50 mg of DCS or placebo was administered 1 h before extinction training and DCS effects during extinction recall were evaluated on a behavioral and neuronal level.

We found attenuated amygdala activation in the DCS compared to the placebo group during recall of the extinguished appetitive cue, along with evidence for enhanced functional amygdala-vmPFC coupling in the DCS group. While the absence of additional physiological measures of conditioned responses during recall in this study prevent the evaluation of a behavioral DCS effect, our neuronal findings are in accordance with recent theories linking successful extinction recall in humans to modulatory top-down influences from the vmPFC that inhibit amygdala activation. Our results should encourage further translational studies concerning the usefulness of DCS to target maladaptive Pavlovian reward associations.

1. Introduction

Drug addiction can be conceptualized as a disorder of persistent maladaptive memory: Environmental cues present during drug intake are associated with the rewarding properties of the drug and can trigger relapse even after long phases of abstinence (Everitt & Robbins, 2005). One way to target these persistent Pavlovian memories is extinction learning, where a previously conditioned cue (CS) is repeatedly presented without its associated reward (unconditioned stimulus, US). Extinction does not erase the maladaptive associations but represents an independent learning process that inhibits the expression of the original CS-US association (e.g., Myers & Davis, 2002). However, several conditions exist that impede extinction recall, causing the conditioned response to recover (Bouton, 2004). Pharmacological agents to enhance extinction learning are therefore of great clinical interest to improve the currently moderate effects of extinction-based addiction treatments (Conklin & Tiffany, 2002; Myers & Carlezon, 2012).

Animal studies using systemic administration of NMDA antagonists revealed an involvement of NMDA-dependent synaptic plasticity in the consolidation of Pavlovian extinction learning (Myers, Carlezon, & Davis, 2011). In line with this, animal models of fear extinction demonstrated that the NMDA receptor partial agonist D-cycloserine (DCS) facilitates extinction learning and

* Corresponding author.
E-mail address: claudia.ebrahimi@charite.de (C. Ebrahimi).

http://dx.doi.org/10.1016/j.nlm.2017.05.008
1074-7427/

deters some relapse effects when administered systemically or directly in relevant structures like the basolateral amygdala or hippocampus either before or immediately after extinction training (Fitzgerald, Seemann, & Maren, 2014). These results were replicated in animal models of drug addiction, where DCS facilitated the extinction of drug-paired cues and contexts (Nic Dhonnchadha & Kantak, 2011). Decreased effectiveness of the drug with increased time delay between extinction and post-training administration, as well as mixed effects on within-session extinction compared to long-term retention, suggest DCS to primarily support memory consolidation by enhancing NMDA receptor signaling (Botreau, Paolone, & Stewart, 2006; Ledgerwood, Richardson, & Cranney, 2003; Nic Dhonnchadha & Kantak, 2011). Anxiety research expanded these findings to clinical trials, demonstrating an overall beneficial effect for DCS-augmented exposure therapy in various anxiety disorders (Bontempo, Panza, & Bloch, 2012; Rodrigues et al., 2014; but see Ori et al., 2015). The few clinical studies combining DCS with cue exposure in addiction are less promising (for review, see Myers & Carlezon, 2012; Otto et al., 2015), although recently DCS-augmented cue exposure with 50 mg of DCS was shown to reduce cue-induced ventral striatal activation (Kiefer et al., 2015) and subjective craving (MacKillop et al., 2015) in alcohol-dependent subjects.

This raises the question of the precise working mechanism of DCS in human extinction learning. Experimental designs suitable to address this issue typically involve three phases: conditioning of CS-US associations, extinction learning, and extinction recall; all spaced at least 24 h apart (Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Klumpers et al., 2012). This allows learning to consolidate, manipulate extinction independent of conditioning, and test DCS effects during extinction recall in a drug-free state.

The proposed mechanism that DCS enhances extinction consolidation is not clearly confirmed in humans (Brom et al., 2015; Guastella et al., 2007; Klumpers et al., 2012; Kuriyama, Honma, Soshi, Fujii, & Kim, 2011); moreover, the neuronal changes that may underlie DCS-augmented extinction are currently unknown. While two human laboratory studies (Guastella et al., 2007; Klumpers et al., 2012) reported that DCS administration before extinction learning failed to attenuate conditioned fear responses during simple recall, that is, CS-presentations in the extinction context (spontaneous recovery), Kuriyama et al. (2011) found 100 mg of DCS to attenuate SCRs after a reactivation procedure (i.e., recall after a CS-US reactivation trial), while no group differences were observed during simple recall. Recently, Brom et al. (2015) administered 125 mg of DCS or placebo after extinction learning of conditioned sexual responses in females. While no group differences emerged during simple extinction recall, the DCS group showed attenuated conditioned responses when tested outside the extinction context, indicating that DCS reduced the context specificity of extinction learning. Especially in the appetitive domain, more research is needed to evaluate the usefulness of DCS as supporting pharmacological strategy to improve extinction-based treatments.

We therefore investigated the effect of 50 mg of DCS during extinction learning in a double-blind, placebo-controlled 3-day design, using a Pavlovian conditioning procedure with monetary wins and losses to probe appetitive and aversive extinction learning. To our knowledge, this is the first human study examining the neuronal correlates of DCS-augmented appetitive extinction learning. We assumed DCS to facilitate extinction of both the appetitive and aversive CS. We hypothesized attenuated SCRs and CS-evoked BOLD response after a reactivation procedure during extinction recall in areas implicated in Pavlovian conditioning, like the amygdala and hippocampus (Quirk & Mueller, 2008), in the DCS compared to the placebo group.

2. Materials and methods

2.1. Subjects

Forty-seven healthy, right-handed volunteers participated in this study. Subjects were examined by medical professionals and excluded in case of current or past psychiatric (DIAX-CIDI; Wittchen & Pfister, 1997), neurological or internal medical disorders (e.g., diabetes mellitus, increased blood pressure, or liver and renal dysfunctions). Further exclusion criteria were pregnancy, positive urinary drug screening, color blindness or weakness (Ishihara color-test; Ishihara, 1917), and abnormalities in hematology and resting electrocardiogram (ECG). Participants were instructed to refrain from alcohol on all days. The required learning criterion of explicit contingency awareness—shown to be necessary for trace conditioning, where CS and US are spaced by a time delay (Clark & Squire, 1998; Knight, Nguyen, & Bandettini, 2006; Weike, Schupp, & Hamm, 2007)—was met by 38 subjects immediately after conditioning on day 1. Of these, six participants were excluded from fMRI analysis due to slice misplacement or excessive signal loss, leaving 32 subjects with adequate data quality on all days (16 women, mean age = 27 ± 1 year SEM, range: 19–39 years; see also Supplementary Fig. S1 for a participant flow chart). Groups did not differ in terms of age, sex, education, or neuropsychological characteristics (see Supplementary Table S1). Participants provided written informed consent for study participation. The study was approved by the local ethics committee (LAGeSo, Berlin, Germany) and registered as a clinical trial at EudraCT (EudraCT-Nr.: 2006-004860-29).

2.2. Stimuli and procedure

Subjects underwent conditioning, extinction, and extinction recall on three consecutive days. They were randomized to receive either 50 mg of DCS or placebo 1 h before extinction under double-blind conditions (Fig. 1A). A Pavlovian trace conditioning and extinction paradigm with monetary outcomes was used (Fig. 1B + C).

Conditioning (day 1). In each trial, a CS was presented for 1.5 s followed by a fixed 3-s trace interval and a subsequent outcome stimulus for 1.5 s (100% reinforcement). The inter-trial interval (ITI) ranged from 3 to 10 s (exponentially distributed with mean 4.5 s; Fig. 1C). The paradigm included three conditions with 16 trials each:

(1) *appetitive condition:* CS ($CS+_{app}$) followed by appetitive US (US_{app}),
(2) *aversive condition:* CS ($CS+_{avers}$) followed by aversive US (US_{avers}), and
(3) *neutral condition:* neutral cue (CS−) followed by neutral outcome (noUS).

Geometric shapes (cycle, square, pentagon) combined with a tone (500, 550, 600 Hz) served as cues and were randomly assigned to conditions over participants. The US consisted of a 2€ coin image with plus or minus signs (US_{app}, US_{avers}), while the neutral outcome was a blurred coin image (noUS). Trial order was pseudo-randomized over subjects and sessions within the constraint of a maximum of three consecutive presentations of the same condition.

Participants were instructed to attend to the relations between cues and outcomes and were informed they would receive the cumulated money after the session. To maintain attention and obtain an additional measure of learning, participants engaged in a cued outcome discrimination task: In each trial, subjects discrim-