Post-extinction L-DOPA administration to reduce the return of fear in humans

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Acknowledgements

The authors thank Anita Schick and Benjamin Meyer for blinding the drugs. RK received funding from the German Research Foundation (Deutsche Forschungsgemeinschaft, CRC1193, subproject C01), the State of Rhineland-Palatinate (DRZ program) and the Boehringer Ingelheim Fonds (DRZ program). OT received funding from the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung), the German Research Foundation (Deutsche Forschungsgemeinschaft, CRC1193, subproject C04), and the State of Rhineland-Palatinate (DRZ program). The authors declare to have no conflict of interest.

Abstract

The dopamine precursor L-DOPA is a promising adjunct to exposure therapy in anxiety disorders. In two magnetic resonance imaging (MRI) studies we previously showed that a post-extinction administration of L-DOPA reduces the return of fear. However, when aiming to replicate these results outside the MRI scanner, we found weaker effects – albeit with an adjusted paradigm. In order to discern whether the discrepant results can be explained by the experimental environment (in- vs. outside MRI scanner) or the differences in the paradigms, we here replicated a previous MRI paradigm outside the MRI scanner. In total, N=50 male participants underwent fear conditioning on day 1 and extinction on day 2. We tested the effect of post-extinction L-DOPA compared to placebo administration on differential skin conductance responses (SCR) during test on day 3. In contrast to previous findings in the MRI scanner, there was no reduction of differential SCRs after L-DOPA compared to placebo intake at test. Resembling previous findings outside the MRI scanner, there was a significant relationship between differential SCRs at the end of extinction on day 2 and test on day 3 in the L-DOPA group. This relationship did, however, not differ between groups. Summarizing, our results rather speak against an effect of paradigm and in favour of an effect of MRI environment. Contextual arousal, likely to be elevated in the MRI environment, may influence the effect of L-DOPA. These findings call for future work to investigate the interaction between arousal and catecholamines in the context of extinction memory consolidation.

Keywords

Fear, anxiety, extinction, exposure, memory consolidation, dopamine, post-traumatic stress disorder, cognitive-behavioural therapy

Introduction

To date, fear and anxiety disorders are among the most prevalent psychological disorders in Western societies (Wittchen et al. 2011; Kessler et al. 2012). Cognitive behavioural therapy comprising so-called exposure treatments has been shown to achieve moderate to large effects in treating anxiety disorders (Wolitzky-Taylor et al. 2008). However, even after a successful exposure session, fear may return. The return of fear can be explained considering the learning process suggested to underly the fear-reducing effects of exposure – extinction learning (Bouton 2002; Vervliet et al. 2013). Laboratory research on Pavlovian conditioning and extinction suggests that the repeated presentation of an aversively conditioned stimulus (CS; e.g., a tone) without the unconditioned stimulus it was previously paired with (US, e.g., an electric painful stimulus) does not lead to an erasure of the original CS-US association, or fear memory. Instead, extinction learning creates an inhibitory CS-no US association, or extinction memory (Bouton 2002, 2004). After extinction, the extinction memory competes for behavioural expression with the original fear memory. As a consequence, conditioned fear responses (CRs) can return upon confrontations with the CS in a context different from the extinction context ('contextual renewal'), after the mere passage of time ('spontaneous recovery'), and after unannounced confrontations with the US ('reinstatement' of fear) (Bouton 2002, 2004). One way to prevent the return of fear in both the laboratory and the clinical context is to strengthen the extinction memory and facilitate its long-term retrieval over that of the fear memory (Vervliet et al. 2013).

To this aim, a number of studies tried to pharmacologically target the memory consolidation process occurring after extinction learning (Singewald et al. 2015). We and others showed that a post-extinction administration of L-DOPA, a dopamine precursor, enhances the extinction memory consolidation and reduces the spontaneous recovery, contextual renewal and reinstatement of fear in mice (Haaker et al. 2013; Whittle et al. 2016). Importantly, the post-

extinction administration of L-DOPA had similar effects in humans. In two functional magnetic resonance imaging (fMRI) studies, L-DOPA prevented the contextual renewal (Haaker et al. 2013) and the spontaneous recovery (Gerlicher et al. 2018) of fear. However, post-extinction L-DOPA administration does not unequivocally prevent the return of fear. Another MRI study with a context conditioning paradigm yielded only neural effects (Haaker et al. 2015). In two follow-up studies conducted outside the MRI scanner (Gerlicher et al. 2019), L-DOPA administration was only beneficial in participants with successful extinction. Participants with high CRs at the end of extinction did not profit from the drug treatment.

A parsimonious explanation for the discrepancy between the results in- and outside the MRI scanner may be that paradigm differences increased the power to detect effects of L-DOPA in the fMRI studies. Specifically, we employed different reinforcement rates and more trials during fear conditioning in the MRI studies (24 and 10 conditioning trials in Haaker et al. 2013; Gerlicher et al. 2018) than the non-MRI studies (5 conditioning trials in both studies in Gerlicher et al. 2019). The longer fear acquisition was associated with – not necessarily causal of – greater CRs during the memory test on the day after extinction (effect sizes $CS+ > CS-$ in the MRI: $\eta_p^2 = .26$, Haaker et al. 2013; $\eta_p^2 = .58$, Gerlicher et al. 2018; outside the MRI: $\eta_p^2 = .20$ and $\eta_p^2 = .14$, Gerlicher et al. 2019). Critically, greater CRs at test (i.e., a greater main effect of stimulus) come with greater power to detect ordinal interaction effects, such as the potential reduction of $CS+$ responses after L-DOPA intake (Maxwell 2004). Alternatively, the different effects may have also been a direct consequence of the environment in which the experiments were conducted. Several studies report that placing participants in an MRI scanner is associated with increases in cortisol and salivary alpha amylase, a marker of noradrenergic activity (van Stegeren et al. 2006; Eatough et al. 2009; Muehlhan et al. 2011; Peters et al. 2011; Lueken et al. 2012; Visser et al. 2015). Noradrenaline has been shown to enhance extinction learning and consolidation in animals

(Abraham et al. 2012) and administrations of cortisol affect human extinction learning (Merz et al. 2017) and enhance extinction memory consolidation in humans (Brueckner et al. 2019). Noradrenaline and cortisol increases associated with testing in the MRI could thus have had effects on extinction learning or memory consolidation in their own right or may have interacted with the effect of L-DOPA (Ranjbar-Slamloo and Fazlali 2020). Thus, it remains unclear whether paradigm differences or changes in stress hormones associated with testing participants in an MRI environment may have led to the discrepant results on the effect of L-DOPA on extinction memory retrieval.

The present study constitutes a first step to identify the cause of the stronger effects of L-DOPA in the MRI scanner (for another ongoing approach in which we directly assess levels of cortisol and salivary alpha amylase, see Hu et al. 2018). Specifically, we conducted an exact replication of one of the MRI studies outside the MRI scanner. We reasoned that employing the same paradigm (i.e., same trial number and reinforcement rate) can exclude paradigm differences as potential explanation for the different results. As in one of the original MRI studies (Gerlicher et al. 2018), participants underwent differential cued fear conditioning on day 1 (see Figure 1). During conditioning, a geometric symbol (CS+) was paired with an aversive electrotactile stimulus (US) in 50% of all trials (i.e., 5 out of 10 trials). A second CS was never paired with the US and served as a control stimulus (CS-). Both CSs were super-imposed on a background image (context A) and presented 10 times each during conditioning. We assessed skin conductance responses (SCR) to CS+ and CS- as CRs. Approximately 24 h later (day 2), we presented the two CSs again in the absence of the US (i.e., in extinction) for 15 trials each on a different background image (context B). To ensure that L-DOPA does not affect extinction learning but merely acts on memory consolidation, participants received either L-DOPA or a placebo pill after extinction. We tested the effect of this manipulation on CRs in the extinction context B on day 3

during 10 CS+ and CS- trials. Because extinction (day 2) and test (day 3) occurred in the same context, this corresponded to a test of spontaneous recovery of fear.

If paradigm differences (e.g., different reinforcement rate, different number of conditioning trials) are at the core of the discrepancies between results in- vs. outside the MRI scanner, we expect to observe the same results as in Gerlicher et al. (2018) and significantly smaller CRs at test after L-DOPA compared to the placebo administration. Alternatively, if the environment in which L-DOPA was administered (in- vs. outside MRI-scanner) affected extinction learning or memory consolidation, or interacted with the effect of L-DOPA, we expect to observe the same result as in two previous studies conducted outside the MRI scanner (Gerlicher et al. 2019). Specifically, L-DOPA should only be effective in participants with low differential CRs at the end of extinction.

Figure 1 Experimental design. The experiment took place on three consecutive days and comprised differential (CS+, CS-) fear conditioning in context A on day 1 and fear extinction in context B and subsequent placebo or L-DOPA administration on day 2. On day 3, we tested whether post-extinction L-DOPA relative to placebo administration affected extinction memory retrieval, i.e., differential conditioned responses (CRs) in the extinction context B.

Methods

Participants

In total 56 male participants were recruited for the experiment. Two participants had to be excluded due to a positive drug test result. Three participants were excluded due to technical

problems (N=2 problems with the experiment script, N=1 no SCR recording). As a precaution, one additional person was excluded due to his belief of having experienced an electrotactile stimulus during extinction on day 2, leaving a sample of N=50 male participants (mean age $\pm$ SD: 27.5 ± 2.3 years; placebo N=26, L-DOPA N=24) for statistical analysis. Recruitment was restricted to male participants, as extinction memory consolidation can be affected by oestrous cycle phase and, specifically, dopaminergic manipulations can have opposing effects depending on the oestrous cycle (Lebron-Milad and Milad 2012; Cover et al. 2014; Rey et al. 2014). A board-certified physician screened participants for contraindications of L-DOPA intake, current physiological, neurological or psychiatric disorders, excessive consumption of nicotine (>10 cigarettes/day), alcohol (>15 glasses of beer/wine per week), or cannabis (>1 joint/month) and participation in other pharmacological studies. Potential drug consumption was assessed via urine test (M-10/3DT; Diagnostik Nord, Schwerin, Germany). The experiment was approved by the local ethics committee (Ethikkommission der Landesärztekammer, Rhineland-Palatinate, Germany) and conducted in accordance with the Declaration of Helsinki.

Drug treatment

A person not involved in data collection, assigned participants randomly to the L-DOPA or placebo group with the restriction that groups were matched on Spielberger's State-Trait-Anxiety Inventory (STAI-T; Spielberger et al. 1970) and Anxiety Sensitivity Index (ASI-3; Taylor et al. 2007) questionnaire scores. For statistical comparisons of placebo and L-DOPA groups on questionnaire scores, see Table 1. Participants were administered either 150/37,5 mg levodopa-benserazide (Levodopa-Benserazid ratiopharm®, Germany; for dosage see Haaker et al. 2013, 2015) or an identically looking capsule filled with mannitol and aerosol (i.e., placebo). Drugs were prepared and provided by the pharmacy of the University Medical Center Mainz. Participants were asked to refrain from eating, consuming caffeinated drinks and smoking two hours prior to drug

intake. Drug administration was double-blind. The short half-life of L-DOPA in plasma (~90 minutes) (Contin and Martinelli 2010) allowed us to specifically test the effect of L-DOPA on memory consolidation, without risking potential confounding effects of L-DOPA at test on day 3, that is, 24 h after drug intake.

Table 1 Demographic information and questionnaire scores. Participants in the L-DOPA group did not differ significantly from participants in the placebo group with respect to age, trait anxiety (STAI-T), anxiety sensitivity (ASI), body mass index (BMI), or subjective rating of the US intensity. Despite the fact that US intensity was rated as comparably uncomfortable in both groups, placebo-treated participants chose significantly higher US intensities (mA). This difference in US intensity did not affect the results (for analyses including US intensity as covariate, see Supplementary Information).

	Placebo (N = 26)	L-DOPA (N = 24)	t-value	p-value
	mean (SD)	mean (SD)		
Age (years)	27.0 (1.9)	28.1 (2.6)	1.62	.11
BMI (kg/m ²)	18.3 (1.8)	19.1 (2.5)	1.37	.18
STAI-T	36.1 (7.3)	36.1 (6.6)	.00	.99
ASI-3	17.1 (9.0)	17.8 (8.2)	.29	.77
US intensity (mA)	17.9 (13.2)	10.0 (6.9)	-2.63	.01*
US rating (0-10)	7.8 (.75)	7.5 (.91)	-1.31	.20

Questionnaires

For sample characterization and matching of groups, participants were asked to fill out Spielberger's State-Trait-Anxiety Inventory (STAI; Spielberger et al. 1970) and the Anxiety Sensitivity Index (ASI-3; Taylor et al. 2007) before the start of the experiment on day 1. State anxiety (STAI-S; Spielberger et al. 1970) was also assessed before and after the experiment on

day 2 and 3 (see Supplementary Information Table 1). Due to a technical issue with the recording software, STAI-S questionnaire scores on day 2 and 3 for one participant are missing.

Stimuli

Two black geometric symbols, a square and a rhombus (square turned by 90 degrees), served as CSs. The CSs were presented in the centre of a computer screen and super-imposed on background pictures of either a kitchen or a living room, which served as contexts A or B, respectively. Assignment of symbols to CS+/CS- and rooms to contexts A/B was counter-balanced between participants and groups. In order to minimize differences between the fMRI experiment (Gerlicher et al. 2018) and the present study, we employed the same stimuli that had been adjusted for contrast and luminance differences using the SHINE toolbox (Willenbockel et al. 2010). A painful electrotactile stimulation consisting of three square-wave pulses (50 ms inter-stimulus interval) of 2 ms was employed as US. Pain stimuli were generated by a DS7A electrical stimulator (Digitimer Ltd, Welwyn Garden City, UK) and delivered on the right dorsal hand through a surface electrode with platinum pin (Specialty Developments, Bexley, UK).

Experimental Procedure.

Day 1 - Conditioning: Upon arrival participants completed questionnaires on trait and state anxiety (Spielberger et al. 1970), anxiety sensitivity (Taylor et al. 2007), and demographic data. Electrodes were attached and US intensity was calibrated to a level rated as “maximally painful, but still tolerable”. Familiarization consisted of two CS presentations in both contexts and a practical training of US expectancy ratings. We instructed participants that the experiment was distributed across three days, that one symbol would never be followed by an electrical stimulation, and that their task was to find out what applied to the other symbol. Each day, the paradigm started with US expectancy ratings for each CS (Supplementary Figure 1).

Subsequently, the context picture was on screen for 5 seconds before the first CS was presented. The context remained on screen continuously throughout the experiment. CSs were presented for 4.5 seconds. The delivery of the US co-terminated with CS presentation. Inter-trial intervals (ITIs) lasted 17, 18, or 19 s with a mean of 18.5 s. Trial order was randomized in such a way that not more than two trials of the same type (i.e., CS+, CS-) succeeded each other. During conditioning in context A participants were presented with 10 CS+ and 10 CS- trials, and 5 out of 10 CS+ presentations (i.e., 50%) were reinforced with the US. After conditioning, participants again rated their US expectancy. Subsequently, electrodes were detached and participants filled out a contingency questionnaire. The experiment lasted approximately 1 h on day 1.

Day 2 – Extinction: The extinction session took place approximately 24 h (± 2 h) after conditioning. Before the start of the extinction session participants filled out the STAI-S, and SCR and US electrodes were attached. We did not re-calibrate the US, but informed participants that the individual US intensity calibrated on day 1 would be applied again and that the experiment would simply continue. During the extinction session 15 CS+/CS- trials were presented in context B. Subsequently, electrodes were detached and participants were administered either a placebo or a L-DOPA pill. Subsequently, participants remained under observation for 60 min and were provided with magazines for reading. After 60 min participants filled out a questionnaire assessing potential side-effects of L-DOPA and the STAI-S. The experiment lasted 2.5 h on day 2.

Day 3 - Test: Approximately 24 h (± 2 h) later, participants filled out the side-effect questionnaire and the STAI-S again. After electrode attachment participants were again only instructed that the US intensity of day 1 would be applied and that the experiment would simply continue. During test

10 CS+/10 CS- were presented in context B in extinction. That is, no further US was administered. The experiment lasted maximally 1 h on day 3.

Skin conductance response (SCR)

During a separate screening session before the start of the experiment, we tested whether participants were skin conductance non-responders. To this aim, we attached two electrodes of the eSense skin response device (Mindfield® Biosystems Ltd, Berlin, Germany) to the medial phalanges of the index and middle finger of the non-dominant hand. Participants were then asked to take a deep breath, or the experimenter clapped his hands without announcement. Both deep breathing and acoustic startle result in a deflection of the skin conductance, not seen in skin conductance non-responding individuals. This test of skin conductance responding does not bias the selection of participants, as it is independent of the experimental manipulation (Lonsdorf et al. 2019). None of the participants screened for the present experiment had to be excluded according to this criterion. During the experiment, electrodermal activity was recorded from the thenar and hypothenar of the non-dominant hand using self-adhesive Ag/AgCl electrodes (EL-509, BIOPAC® Systems Inc., Goleta, California, USA) filled with an isotonic electrolyte medium and the Biopac MP150 with EDA100C device. The raw signal was amplified and low-pass filtered with a cut-off frequency of 1 Hz. Using a custom-made analysis script, we manually scored the first local minimum in the skin conductance time course in a window from 900 to 4000 ms after CS onset as response onset. The following local maximum was scored as response peak and SCRs as peak-to-onset difference. Importantly, the experimenter scoring the data was blinded to stimulus type (CS+/CS-) of each SCR and group belongingness (placebo/L-DOPA) of each participant. Responses smaller than 0.02 μ S were scored as zero and remained in the analysis. If more than 75% of trials of one day had to be scored as zero, data of that participant/day was

considered invalid. This applied to $n = 0/1/1$ (day 1/day 2/day 3) participants, leaving data of $N=50/49/49$ participants for statistical analysis. Raw SCRs were log-transformed (+1) and range-corrected within each participant and day before statistical analysis (Lykken and Venables 1971).

US expectancy ratings

Before the start and after the end of each experimental phase we asked participants to indicate their expectancy to receive an electro tactile stimulus for each CS presented on a grey background on the computer screen. Using a computer mouse, participants could move a cursor and select a position on a visual analogue scale ranging from 0 (=no expectation) to 100 (=very high expectation). Results of US expectancy ratings are provided in Supplementary Figure 1.

Statistical analysis

For statistical analysis of the SCR data, we operationalized CRs at the end of conditioning, beginning and end of extinction, and at test according as in previous work and averaged SCRs across the last 20% of conditioning trials on day 1 (i.e., two trials), the first and last 20% of extinction trials on day 2, (i.e., three trials, respectively) and across the entire test phase on day 3 (i.e., ten trials) (Gerlicher et al., 2018, 2019; Haaker et al., 2013, 2015). These a-priori determined procedures are an attempt to prevent post-hoc biases in the operationalization of physiological measures (Ney et al. 2018). To exclude group differences at the end of conditioning on day 1, the beginning or end of extinction on day 2 and to test the effect of L-DOPA on conditioned fear at test on day 3 we used repeated measures ANOVA with within-subject factor stimulus (CS+/CS-) and between-subject factor group (L-DOPA/placebo). In order to assess whether the effect of L-DOPA on conditioned fear at test on day 3 was dependent on conditioned fear at the end of extinction on day 2, we used a multiple linear regression analysis with differential CRs at the end of extinction, group and their interaction as independent and differential CRs at

test as dependent variable. Since extinction success could have been confounded by initial fear acquisition or fear memory recall, we also included differential CRs at the end of conditioning and at the beginning of extinction into the regression analysis (as in Gerlicher et al. 2019). In order to assess the relationship between differential CRs at the end of extinction and differential CRs at test within each group, regression analysis was followed up with an analysis of simple slopes (Aiken and West 1991). Effects were considered significant when $p < .05$ (two-sided tests).

Results

Questionnaires and US intensity

Groups did not differ with respect to questionnaire scores on STAI-T or ASI or with respect to body mass index (BMI) (see Table 1). Placebo-treated participants selected a significantly higher US intensity than L-DOPA-treated participants ($t_{48} = -2.63$, $p = .01$, Cohen's $d = .78$). However, the subjective intensity of the US as rated on a visual analogue scale from 0-10 did not differ significantly between groups ($t_{48} = -1.31$, $p = .20$). That means, even though participants in the placebo group chose stronger electrotactile stimuli, they did not experience the US as more aversive than participants in the L-DOPA group. We computed all subsequent analyses with and without US intensity as covariate (see Supplementary Information). The main results were not affected by this group difference.

Skin conductance responses

During fear conditioning on day 1, CRs were successfully acquired as indicated by greater SCR to the CS+ than the CS- in both the L-DOPA and the placebo group (Figure 2; stimulus: $F_{1,48} = 93.66$, $p < .001$, partial $\eta^2 = .66$; group: $F_{1,48} = .70$, $p = .41$, stimulus*group: $F_{1,48} = .65$, $p = .42$; $n = 50$). On day 2, participants retrieved the acquired CS-US association and CRs did not differ

significantly between groups at the beginning (stimulus: $F_{1,47}=35.98$, $p<.001$, partial $\eta^2=.43$; group: $F_{1,47}=.12$, $p=.73$; stimulus*group: $F_{1,47}=3.20$, $p=.08$; $n=49$). At the end of extinction SCRs were still significantly greater for the CS+ than the CS- but did not differ significantly between groups (stimulus: $F_{1,47}=11.43$, $p=.001$, partial $\eta^2=.20$; group: $F_{1,47}=.09$, $p=.77$; stimulus*group: $F_{1,47}=.19$, $p=.67$; $n=49$). Participants were administered a pill of placebo or L-DOPA directly after extinction learning on day 2. At test on day 3, both groups showed significantly greater SCRs to the CS+ than the CS- (stimulus: $F_{1,47}=59.55$, $p<.001$, partial $\eta^2=.56$; $n=49$). However, in contrast to our findings with the same paradigm in the MRI scanner, there was no significant difference between groups during test (group: $F_{1,47}=.05$, $p=.82$; stimulus*group: $F_{1,47}=.25$, $p=.62$). Hence, despite the usage of the same paradigm, L-DOPA administration outside the MRI scanner did not improve extinction memory retrieval and paradigm differences can likely be excluded as potential explanation for the lack of effect.

Figure 2 Trial-by-trial SCRs (Z-scored within participant and day) during fear conditioning on day 1, extinction on day 2 and test on day 3 in **(a)** placebo-treated participants ($n=25$) and **(b)** L-DOPA-treated participants ($n=25$). There were no significant differences between the placebo or L-DOPA groups in CRs at the end of fear conditioning, beginning or end of extinction, or at test. Data are presented as mean $\pm$ standard error of the mean (s.e.m).

Next, we tested whether the effect of L-DOPA on extinction memory retrieval was dependent on individual extinction success, as observed in two previous studies outside the MRI scanner (Gerlicher et al. 2019). We indeed replicated our observation of a significant relationship between differential CRs at the end of extinction on day 2 and differential CRs during test on day 3 in L-DOPA-treated participants (simple-slope analysis: $\beta_{\text{endfear}}=.39$, $SE=.13$, $t_{42}=3.04$, $p=.004$; see Fig. 3). As before, L-DOPA-treated participants with low differential CRs at the end of extinction, that is, successful extinction, showed low differential CRs at test. This result suggests that L-DOPA

conserves individual extinction success. In the present study, this relationship did, however, not differ significantly between groups ($\beta_{\text{endfear} \times \text{group}} = .18$, $SE = .17$, $t_{42} = 1.06$, $p = .30$). Instead, there was also a positive, albeit non-significant, relationship between differential CRs at the end of extinction and differential CRs at test in the placebo group (simple-slope analysis: $\beta_{\text{endfear}} = .20$, $SE = .12$, $t_{42} = 1.62$, $p = .11$).

Figure 3. Relationship between differential CRs at the end of extinction on day 2 and at test on day 3. (a) There was no significant relationship between differential CRs at the end of extinction on day 2 and differential CRs at test on day 3 in the placebo group. **(b)** After post-extinction L-DOPA administration there was a significant positive relationship between differential CRs at the end of extinction and test (as in Gerlicher et al. 2019). Participants with low differential CRs (SCR CS+>CS-), i.e., successful extinction, showed low differential CRs (SCR CS+>CS-) at test, and vice versa. Relationships did not differ significantly between groups.

Discussion

The present study investigated whether a post-extinction L-DOPA administration can improve extinction memory retrieval. Specifically, we sought to clarify whether paradigm differences could account for previously observed discrepancies in effect of L-DOPA between studies investigating

its administration in- compared to outside the MRI scanner. To this aim, we replicated a previous MRI paradigm (Gerlicher et al. 2018) as closely as possible outside MRI. In contrast to previous findings inside the scanner (Haaker et al. 2013; Gerlicher et al. 2018), we here found no evidence for improved extinction memory retrieval on the group level after L-DOPA compared to placebo administration. As the effect size of the effect of stimulus (CS+>CS-) at test was large and comparable to the one observed in the previous MRI study employing the same paradigm (Gerlicher et al. 2018), we can exclude that paradigm differences or an associated lack of power can explain the lack of fear reduction after L-DOPA administration in the present study. However, as in previous studies outside MRI (Gerlicher et al. 2019), we again observed a significant positive relationship in the L-DOPA group between extinction success, as expressed in our end-fear metric, and the later retrieval of the extinction memory formed during learning. That is, L-DOPA-treated participants with low differential CRs at the end of extinction continued to show low differential CRs during test 24 h later. Conversely, L-DOPA-treated participants with high differential CRs at the end of extinction showed high differential CRs at test. This relationship was not significant in the placebo group, but the relationships between extinction success and retrieval at test did not differ significantly between groups in the present study.

A dependency of the effect of a pharmacological extinction enhancer on extinction success has previously not only been reported for L-DOPA, but also for the partial NMDA receptor agonist d-Cycloserine (DCS) and methylene blue, an agent that increases mitochondrial cytochrome oxidase activity in the brain (Smits et al. 2013b, a, 2014; Telch et al. 2014). These pharmacological interventions have in common that they can act on memory consolidation processes occurring after learning (Ledgerwood et al. 2003; Gonzalez-Lima and Bruchey 2004; Hikind and Maroun 2008). Assuming that the current behavioural expression of fear indicates the currently active memory trace on which a pharmacological intervention will act (Eisenberg et al.

2003), L-DOPA may enhance specifically the consolidation of the active extinction memory trace in participants showing a complete reduction of fear at the end of an extinction session. In contrast, in participants with high fear at the end of extinction, the extinction session may not have established an extinction memory trace but instead merely reactivated the fear memory acquired earlier during conditioning (Merlo et al. 2014). Upon reactivation, fear memories become labile and malleable again, and subsequent memory re-consolidation processes are necessary to stabilize them again (Nader et al. 2000). Thus, when administered after incomplete extinction, L-DOPA, or other consolidation enhancers, may inadvertently enhance reconsolidation and potentially strengthen the undesirable fear memory trace. Therefore, in potential future clinical applications, it may be safest to only administer L-DOPA after an exposure session in which the practitioner can ensure that fear expression is low.

With two human MRI studies showing a general reduction of cued fear expression after L-DOPA intake on the group level, independent of extinction success (Haaker et al. 2013; Gerlicher et al. 2018), and three studies conducted outside MRI showing that the beneficial effect of L-DOPA hinges on whether or not participants extinguished successfully (albeit groups did not differ significantly in the present study), our findings suggest that factors associated with testing in an MRI environment influence the effect of L-DOPA on extinction memory consolidation. It is conceivable that testing participants in an MRI scanner results in the release of endogenous stress hormones such as noradrenaline and cortisol, which may have their own effects on extinction memory consolidation or interact with the effect of L-DOPA on this process. We are currently undertaking an exact replication of the MRI study by Gerlicher et al. (2018), which also provided the paradigm for the current study, during which we assess individual differences in salivary alpha-amylase, a marker of noradrenergic activity, and cortisol (Hu et al. 2018). Eventually, however, only experimental manipulations of noradrenaline and/or cortisol together

with L-DOPA administration will be able to determine whether either noradrenaline or cortisol or their combination amplify the effect of L-DOPA on extinction memory consolidation. If the effect of L-DOPA is indeed stronger and independent of extinction success when levels of noradrenaline and/or cortisol are high, this would not preclude, but rather advocate for, its application as adjunct to exposure treatments in clinical practice, as exposure sessions naturally induce stress and thereby also lead to a release of stress hormones.

Next to a potential interaction of stress hormones with the effect of L-DOPA in the MRI, an alternative explanation for the different effects in- and outside the scanner could be that individuals recruited for MRI experiments differ from participants recruited for non-MRI experiments. It has recently been reported that MRI participants exhibit on average lower trait anxiety scores (Charpentier et al. 2020; Sjouwerman et al. 2020) showing a narrower distribution of scores, with less scores above 45 (Charpentier et al. 2020). Lower trait anxiety scores in MRI participants may be due to self-selection or the exclusion of claustrophobic participants. Such a sampling bias can potentially bias the results. As an example, previous research has shown that a pharmacological disruption of fear memory reconsolidation exerted stronger effects in individuals with low compared to high trait anxiety (Soeter and Kindt 2013). The present sample did, however, not differ significantly from the sample recruited for the previous MRI study with respect to the mean or the distribution of trait anxiety scores and we did not observe a significant effect of trait anxiety on the present results (see Supplementary Figure 2). However, also in the present non-MRI sample trait anxiety scores appear to have a slightly broader distribution than in the MRI sample. Future research may consider using stratified sampling to ensure that the samples in studies in- and outside the MRI scanner are comparable.

In order to account for inter-individual differences in overall level of skin conductance we commonly standardize the resulting SCRs (log-transformation and range correction). Several

authors pointed out that interaction effects, such as the stronger relation between CRs at the end of extinction and test in the L-DOPA compared to the placebo group, can be dependent on the scaling of the variables (Wagenmakers et al. 2012; Rohrer and Arslan 2020). That is, some interactions are removable by linear transformations of the measurement scale, such as standardization. If a data transformation can remove an interaction, the interaction is not model independent and not interpretable (for detailed explanation, see Wagenmakers et al. 2012). One could therefore ask whether transforming SCRs linearly affected the past results (Gerlicher et al. 2019). However, the previously reported interactions (Gerlicher et al. 2019) refer to cross-over interactions robust under different scaling assumptions (see Supplementary Figure 3), suggesting that - independent of SCR scaling - post-extinction L-DOPA administration was beneficial after successful but not non-successful extinction learning in these studies.

A clear limitation of the present study is the exclusion of female participants from the study sample. As in our previous work, we opted for excluding female participants due to known interactions of the oestrous cycle with extinction memory consolidation and dopaminergic effects thereof (Lebron-Milad and Milad 2012; Cover et al. 2014; Rey et al. 2014). Given the higher prevalence of anxiety disorders in women than men, the investigation of the effect of L-DOPA on extinction memory consolidation in both sexes is, however, an important target. In a first investigation of the effect of L-DOPA in women, Cisler et al. (2020) recruited participants explicitly diagnosed with PTSD. The authors found no effect of L-DOPA on spontaneous recovery of fear but reduced (stimulus-independent) reinstatement after L-DOPA compared to placebo administration. This is first evidence that L-DOPA may have beneficial effects in women diagnosed with PTSD. Whether these effects may be dependent on oestrous cycle, as indicated by animal research (Rey et al. 2014), is a question for future research.

To summarize, the present study aimed to rule out paradigm differences as potential explanation for differences in the effect of L-DOPA on extinction memory consolidation. Despite having employed the same paradigm as a previous MRI study, we did not replicate the results found inside the MRI scanner and did not observe a reduction of fear after post-extinction L-DOPA administration on the group level. As in previous studies outside the MRI scanner, there was, however, a significant positive relationship between extinction success and fear expression at test, suggesting that L-DOPA conserves what has been learned during the extinction session. This relationship did not differ significantly between groups. The present results suggest that factors associated with testing in an MRI environment, such as the release of stress hormones, may affect the results of a pharmacological extinction enhancer and emphasize the need to investigate these interactions.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Data availability statement.

The data described in this article are openly available in the Open Science Framework at <https://osf.io/jv835/>

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