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Fear-potentiated startle, but not light-enhanced startle, is enhanced by anxiogenic drugs

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ABSTRACT

Rationale and objectives: The light-enhanced startle paradigm (LES) is suggested to model anxiety, because of the non-specific cue and the long-term effect. In contrast, the fear-potentiated startle (FPS) is suggested to model conditioned fear. However, the pharmacological profiles of these two paradigms are very similar. The present study investigated the effects of putative anxiogenic drugs on LES and FPS and aimed at determining the sensitivity of LES for anxiogenic drugs and to potentially showing a pharmacological differentiation between these two paradigms.
Methods: Male Wistar rats received each dose of the α_2-adrenoceptor antagonist yohimbine (0.25–1.0 mg/kg), the 5-HT_{2C} receptor agonist *m*-chlorophenylpiperazine (mCPP, 0.5–2.0 mg/kg) or the $GABA_A$ inverse receptor agonist pentylenetetrazole (PTZ, 3–30 mg/kg) and were subsequently tested in either LES or FPS.
Results: None of the drugs enhanced LES, whereas mCPP increased percentage FPS and yohimbine increased absolute FPS values. Furthermore, yohimbine increased baseline startle amplitude in the LES, while mCPP suppressed baseline startle in both the LES and FPS and PTZ suppressed baseline startle in the FPS.
Conclusions: In contrast to findings in the FPS paradigm, none of the drugs were able to exacerbate the LES response. Thus, a clear pharmacological differentiation was found between LES and FPS.

1. Introduction

The acoustic startle response can be increased by presenting the startle-eliciting noise in the presence of a cue previously paired with foot shock. This fear-potentiated startle (FPS) paradigm was first described in 1951 (Brown et al., 1951) and has since then greatly increased our understanding of the neural and pharmacological mechanisms that underlie conditioned fear (for review see (Davis et al., 1993) or (Koch, 1999). FPS is pharmacologically well characterized, with selective sensitivity for anxiolytic drugs (Davis et al., 1979; Hijzen et al., 1995; Joordens et al., 1996) and not other classes of psychoactive drugs (Cassella and Davis, 1985; Hijzen et al., 1995). In addition, FPS can be enhanced by anxiogenic drugs (Davis et al., 1979).

More recently, it was shown that the startle response can also be increased by bright light, which in rats proves to be an unconditioned anxiogenic stimulus (Walker and Davis, 1997). In this procedure, which has been termed light-enhanced startle (LES), rats show a potentiated startle response in a brightly illuminated environment, compared to a dark environment. Interestingly, Grillon et al. (1997) showed that in humans, startle is increased when tested in the dark and that this increase appears to be the result of fear or anxiety and not an attentional process. It is suggested that the increase of startle in rats tested in bright light and in humans tested in the dark has an evolutionary basis, that is, rats are nocturnal and are more vulnerable in the light, whereas humans are diurnal and more vulnerable in the dark (Grillon et al., 1997; Walker and Davis, 1997).The LES paradigm has been pharmacologically characterized to some extent. It was shown to be sensitive to various anxiolytic drugs, namely the $GABA_A$ receptor agonist chlordiazepoxide (de Jongh et al., 2002; Walker and Davis, 2002a), the partial 5-HT_{1A} receptor agonist buspirone (Walker and Davis, 1997) and the full 5-HT_{1A} receptor agonist flesinoxan (de Jongh et al., 2002). Sensitivity to anxiogenic drug effects, however, has not been studied in the LES paradigm.

The enhancement of the startle response in the FPS and LES appears to be mediated by different brain regions. Infusions of the AMPA receptor antagonist NBQX into the central nucleus of the amygdala blocked FPS, but not LES, whereas infusion into the bed nucleus of the stria terminalis (BNST) blocked LES and sustained fear responses, but not FPS (Davis et al., 1997; Walker and Davis, 2008; Walker et al., 2009). In addition, a recent study suggested an important role for the anterior cingulate cortex and septo-hippocampal system in the LES response, but not FPS (Veening et al., 2009). In addition to this regional differentiation between LES and FPS, a

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pharmacological differentiation was observed after blockade of CRF receptors. Both the non-specific CRF antagonist α-helical CRF and the specific CRF 1 receptors antagonist GSK876008 blocked LES but not FPS (de Jongh et al., 2003; Walker et al., 2008).

The aim of the present study was to investigate whether anxiogenic drug effects enhance LES and to investigate whether LES and FPS can be pharmacologically differentiated on basis of anxiogenic drug effects. Therefore, we studied the effects of three putative anxiogenic drugs acting on different neurotransmitter systems on both LES and FPS, namely the α_2-adrenoceptor agonist yohimbine, the 5-HT_{2C} receptor agonist *m*-chlorophenylpiperazine (mCPP) and the $GABA_A$ receptor inverse agonist pentylenetetrazole (PTZ). In addition to their effect on FPS, these drugs were already shown to have anxiogenic effects in several rodent (Cole et al., 1995; Davis et al., 1979; Johnston and File, 1989; Kennett et al., 1989; Ramos et al., 2008) and human studies (Charney et al., 1987a,b; Morgan et al., 1993; Rodin and Calhoun, 1970).

In the present experiment, the potentially conditioned fear and anxiety enhancing drug effects were assessed by comparing changes in startle amplitude under several conditions (see also Table 1). Firstly, the enhancement of baseline startle amplitude, as measured during the dark→dark session in the LES paradigm. Secondly, enhancement of startle responding during the light phase in the LES paradigm and during Noise Alone trials following FPS conditioning, which both reflect exacerbation of sustained anxiety (Guscott et al., 2000). Thirdly, enhancement of startle responding to Light Noise trials in the FPS paradigm, which reflects potentiation of conditioned fear. The drug effects on startle responding in these different conditions were all studied to differentiate between drug effects on conditioned fear and anxiety.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Harlan, Horst, The Netherlands), weighing 300–350 g at the beginning of the experiments, were housed in groups of four in a temperature (21 °C±2), humidity (55%±5), and light controlled environment (lights on from 6 AM to 6 PM). Food and water were freely available in the home cages. The experiments were carried out during the light phase of the day–night cycle between 9 AM and 2:30 PM. The study was approved by the ethical committee of the Faculties of Pharmaceutical Sciences, Chemistry and Biology, Utrecht University, The Netherlands.

2.2. Apparatus

Four startle devices were used simultaneously (SR-lab, San Diego instruments, San Diego CA, USA). The startle devices consisted of a Plexiglas cylinder (8.8 cm in diameter and 20.3 cm in length) with a stainless steel grid floor placed on a Plexiglas base. Each startle device was placed in a ventilated sound attenuated cubicle. Cage movements were measured with a piezoelectric film attached to the Plexiglas base of the startle device. A calibration system (San Diego Instruments) was used to ensure comparable startle magnitudes across the four devices throughout the experiment. Startle stimuli, consisting of 50 ms white-noise bursts, were presented through a piezoelectric tweeter situated 15.2 cm from the top of the cylinder. Background noise was 55 dB. Sound intensities were measured using a microphone which was placed on top of the Plexiglas cylinder and fitted to a Bruel and Kjaer sound level meter (Type 2226). Startle amplitudes were sampled each ms during a period of 65 ms beginning at the onset of the startle stimulus. Each startle device was equipped with a white fluorescent bulb (9 W) on the back wall of the sound attenuated cubicle and a stimulus light in the ceiling situated 15.2 cm from the top of the cylinder. The fluorescent bulb produced an illumination level of approximately 900 lx and the stimulus light an illumination level of approximately 180 lx, both measured from inside the Plexiglas cylinder using a Gossen luxmeter (MAVOLUX 5032C). There was no background illumination in any of the experiments.

Table 1
Overview of anxiogenic effects of yohimbine, mCPP and PTZ. Indicated are anxiogenic drug effects on startle magnitude as measured during dark→dark (DD) session (diffuse anxiety), light-enhanced startle and Noise Alone trials following fear-potentiated startle conditioning (exacerbation of general anxiety) and Light Noise trials during fear-potentiated startle (exacerbation of fear).

	Baseline startle	Sustained anxiety		Conditioned fear
	DD session	NA trials	LES	FPS
Yohimbine	↑	X	X	↑
mCPP	↓	↓	X	↑
PTZ	X	↓	X	X

2.3. Procedure

2.3.1. Light-enhanced startle

Light-enhanced startle was performed as previously described (de Jongh et al., 2002; Groenink et al., 2008). In short, animals were placed in the startle chamber and, after a 5 min acclimation period, presented with 30 startle stimuli, 10 each at 90, 95 and 105 dB, with an inter stimulus interval of 30 s. Within every block of three stimuli, the three intensities were presented in a random order, with each intensity being presented only once. These 30 stimuli constituted phase 1. Then, the procedure, including the acclimation period, was repeated. This second set of 30 stimuli constituted phase 2. Depending on the experimental condition, the level of illumination was changed between phase 1 and phase 2. Animals were tested twice a week for four successive weeks, with test days separated by a minimum of 72 h. On one of these test days, the light remained off during both phases (dark→dark). On the other day, a light that produced an illumination level of approximately 900 lx was on during phase 2 (dark→light). Half of the rats started the experiment with the dark→dark session type, the other half began with the dark→light session type. The session type was alternated throughout the experiment. Each drug was tested in a separate group of animals, carried out using the same procedure. In each of the three groups, 4 dosages of the drug (including vehicle) were administered according to a balanced within-subjects design. That is, each rat received each dose of the drug in both session types.

2.3.2. Fear-potentiated startle

Fear-potentiated startle was performed as previously described (Groenink et al., 2008; Hijzen et al., 1995). In short, three separate groups of rats were trained once a day for 2 consecutive days. During each training session, rats were presented with 10 light–shock pairings at an average interval of 4 min (range: 3–5 min). A 0.6 mA foot shock was presented during the last 500 ms of the 3700 ms light period. Shock reactivity, registered by measuring cage movements, was sampled each ms during a period of 200 ms beginning at the onset of foot shock. Each drug was tested in a separate group of animals, carried out using the same procedure. One day after the last training session, the animals in each group received one of 4 dosages of the drug (including vehicle) according to a balanced within-subjects design. After an acclimation period of 5 min, 10 startle stimuli of 105 dB were presented (ISI 30 s), followed by 30 startle stimuli at an ISI of 30 s, 10 each at 90, 95 and 105 dB. Half of the 30 startle stimuli were presented during the last 50 ms of a 3250 ms light period; the other half were delivered in darkness. The six different trial types were presented in a balanced, irregular order across the test session. During the following three weeks, the training and test procedures were repeated 3 times, separated by one week. During these weeks, rats were only trained once a week, followed by a test session the next day.

2.4. Drugs

Yohimbine HCl (0, 0.25, 0.5 and 1.0 mg/kg), *m*-chlorophenylpiperazine HCl (mCPP; 0, 0.5, 1.0, and 2.0 mg/kg) and pentylenetetrazole (PTZ; 0, 3, 10, and 30 mg/kg) were dissolved in 0.9% saline (vehicle) and administered intraperitoneally. mCPP was administered 25 min before testing. Yohimbine and PTZ were administered 10 min before testing. All drugs were given in a volume of 2 ml/kg. Drug and vehicle solutions were freshly prepared each morning.

2.5. Statistics

2.5.1. Light-enhanced startle

Repeated measures ANOVAs were used to analyze overall startle reactivity. Condition (two levels: dark→dark or dark→light session type), phase (two levels: phase 1 and phase 2) and dose (four levels) as within-subject factors. Additionally, to determine drug effects on baseline startle en sustained anxiety, repeated measures ANOVAs were used to analyze the mean startle amplitudes in the dark→dark

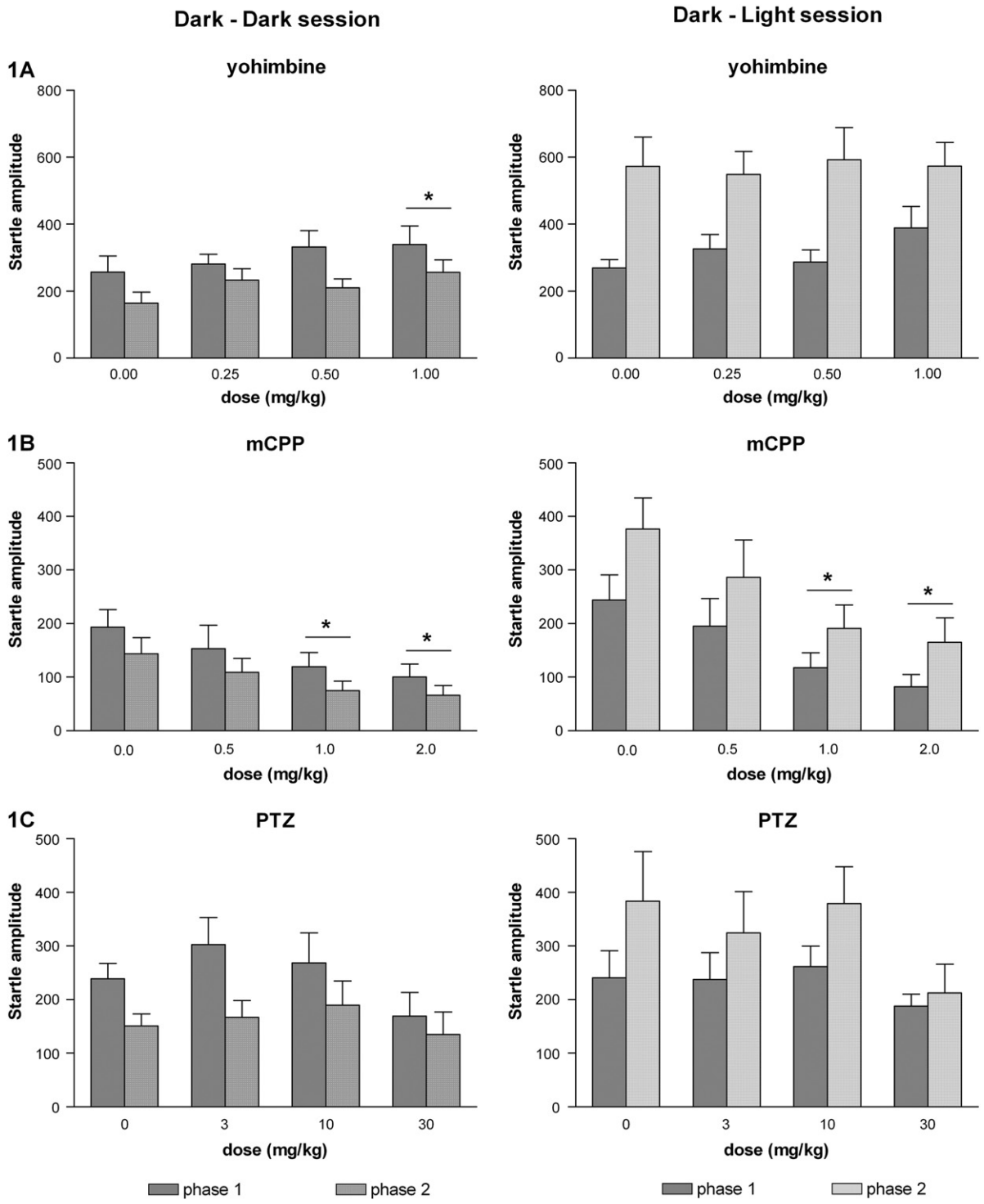

Fig. 1. The effects of yohimbine ($n=11$), mCPP ($n=8$) and PTZ ($n=9$) on light-enhanced startle. The figure shows mean startle amplitudes during phase 1 and phase 2 for the dark→dark session type (left panels) and the dark→light session type (right panels) of animals treated with yohimbine (A), mCPP (B) and PTZ (C). * indicates overall significant difference compared to 0 mg/kg for absolute mean startle amplitudes.

and dark→light session types separately. Phase (two levels: phase 1 and phase 2) and dose (four levels) were used as within-subjects factors. Comparisons between different drug doses were made by simple contrasts. The percentage change [(phase 2 − phase 1)/phase 1] was also calculated for each rat and the mean percentages were subsequently analyzed by repeated measures ANOVA with session type (two levels: dark→dark and dark→light) and dose (four levels) as within-subjects factors. The significance level for all analyses was 5%. Rats that did not show light potentiation in the Dark–Light session type (percentage increase during light phase vs. dark phase <0%) under vehicle conditions and statistical outliers, with startle responses more than two standard deviations away from the mean, were excluded from data analysis (1 for yohimbine, 4 for mCPP and 3 for PTZ). In addition, in the PTZ group, two rats were excluded from the analysis because the highest dose of PTZ resulted in convulsions.

2.5.2. Fear-potentiated startle

A repeated measures ANOVA was used to analyze the mean startle amplitudes on the Noise Alone and Light Noise trials. Trial type (two levels: Noise Alone and Light Noise) and dose (four levels) were used as within-subjects factors. Comparisons between different drug doses were made by simple contrasts. The percentage change [(Light Noise − Noise Alone)/Noise Alone] was also calculated for each rat and the mean percentages were subsequently analyzed by a repeated measures ANOVA with dose (four levels) as within-subjects factor. The significance level for all analyses was 5%.

3. Results

3.1. Light-enhanced startle

The mean startle amplitudes for the dark→dark (DD) session type (left panels) and for the dark→light (DL) session type (right panels) for the three drugs are depicted in Fig. 1.

In all three groups tested significant light-enhanced startle was induced during the DL session (session × phase interactions: Yohimbine [$F(1, 10) = 29.5$; $p<0.001$], mCPP [$F(1, 7) = 26.2$; $p<0.001$] and PTZ [$F(1, 8) = 17.9$; $p<0.01$]). Specific analyses of behavioural responses under vehicle conditions revealed significant light-enhanced startle also under control conditions specifically (phase 2 vs. phase 1 during DL session: Yohimbine [$T(1, 10) = -3.795$, $p<0.01$], mCPP [$T(1, 7) = -4.619$, $p<0.01$] and PTZ [$T(1, 7) = -2.632$, $p<0.05$]).

In the yohimbine group, analyses of DD and DL session types separately showed that yohimbine did not influence the light-enhanced startle response. However, yohimbine increased overall startle amplitude in the DD session (DD session: main effect dose [$F(3, 30) = 3.1$; $p<0.05$]), but not in the DL session. Simple contrasts revealed that this effect of yohimbine in the DD session type was mediated by the 1.0 mg/kg dose [$F(1, 10) = 10.7$; $p<0.01$].

In the mCPP group, analyses of the DD and DL session types separately showed that mCPP had no effect on the light-enhanced startle response. However, mCPP decreased overall startle amplitude in both session types (DD session type: [dose $F(3, 5) = 3.4$; $p<0.05$]; DL session type: [dose $F(3, 5) = 8.4$; $p<0.001$]). Simple contrasts revealed that, in both session types, overall startle amplitude was decreased after 1.0 mg/kg and 2.0 mg/kg mCPP (1.0 mg/kg: DD session type [$F(1, 7) = 29.2$; $p<0.001$]; DL session type [$F(1, 7) = 16.7$; $p<0.01$] and 2.0 mg/kg: DD session type [$F(1, 7) = 7.4$; $p<0.05$]; DL session type: [$F(1, 7) = 14.5$; $p<0.01$]).

In the PTZ group, analyses of the two session types separately showed that PTZ had no effect on absolute light-enhanced startle responding. In addition, PTZ did not affect overall startle amplitude in the DL session types, although there was a trend towards an effect on overall startle amplitude in the DD session type [DD session type: [$F(3, 24) = 2.507$, $p<0.1$]. This trend in the DD session type could not be specifically ascribed to a specific dose.

Fig. 2 depicts the mean percentage change in startle potentiation in the DD session type and in the DL session type for the three drugs. In all three groups significant light-enhanced startle was induced (main effect session type: yohimbine [$F(1, 10) = 28.4$; $p<0.001$, mCPP [$F(1, 7) =$

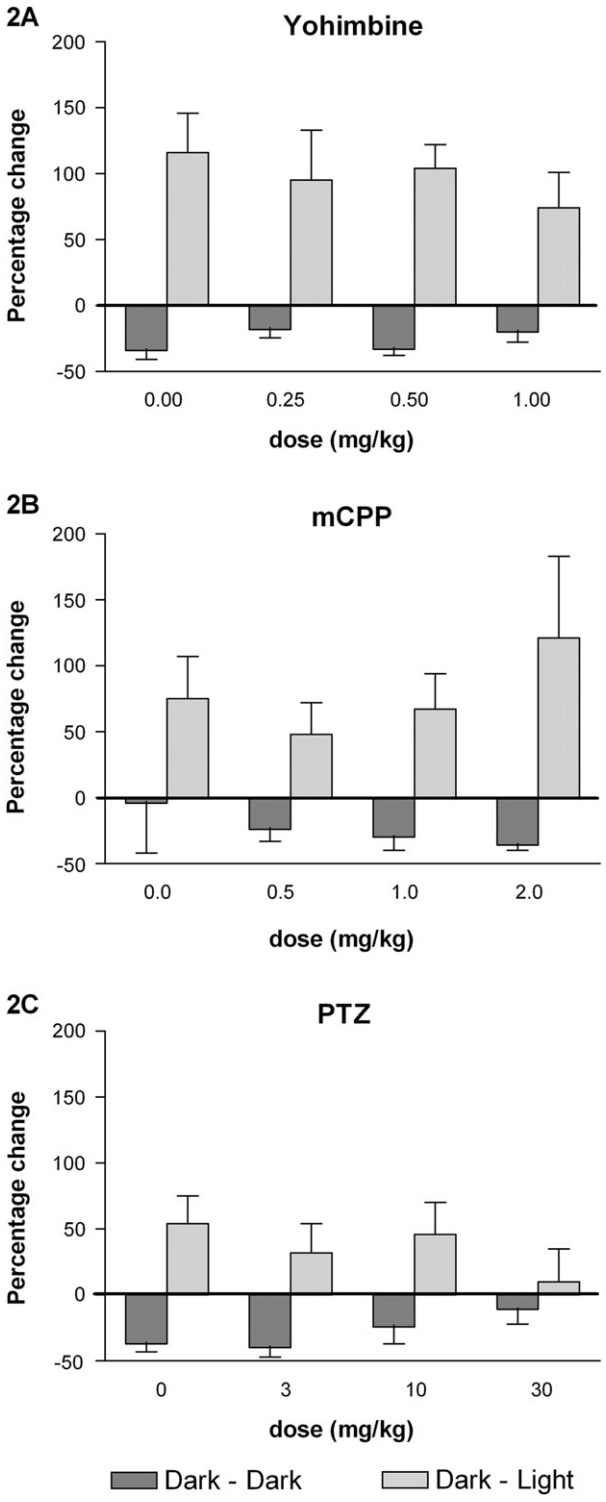

Fig. 2. The effects of yohimbine ($n = 11$), mCPP ($n = 8$) and PTZ ($n = 9$) on light-enhanced startle. The figure shows the mean percentage decrease in the dark→dark session type and the mean percentage increase in the dark→light session type for yohimbine (A) mCPP (B) and PTZ (C).

21.8; $p<0.01$] and PTZ [$F(1,8)=22.9$; $p<0.001$]). However, none of the drugs affected percentage light-enhanced startle.

3.2. Fear-potentiated startle

In Fig. 3, the left panels show mean startle amplitudes on Noise Alone and Light Noise trials animals treated with yohimbine (A), mCPP (B) and PTZ (C). Right panels show the mean percentage potentiation.

In all three groups fear-potentiated startle was induced successfully (trial type: Yohimbine [$F(1,10)=23.9$; $p<0.001$], mCPP [$F(1,9)=83.5$; $p<0.001$] and PTZ $F(1,11)=18.4$; $p<0.001$]). In all three groups, specific analyses of behavioural responses in the vehicle condition separately also revealed significant fear potentiation under control

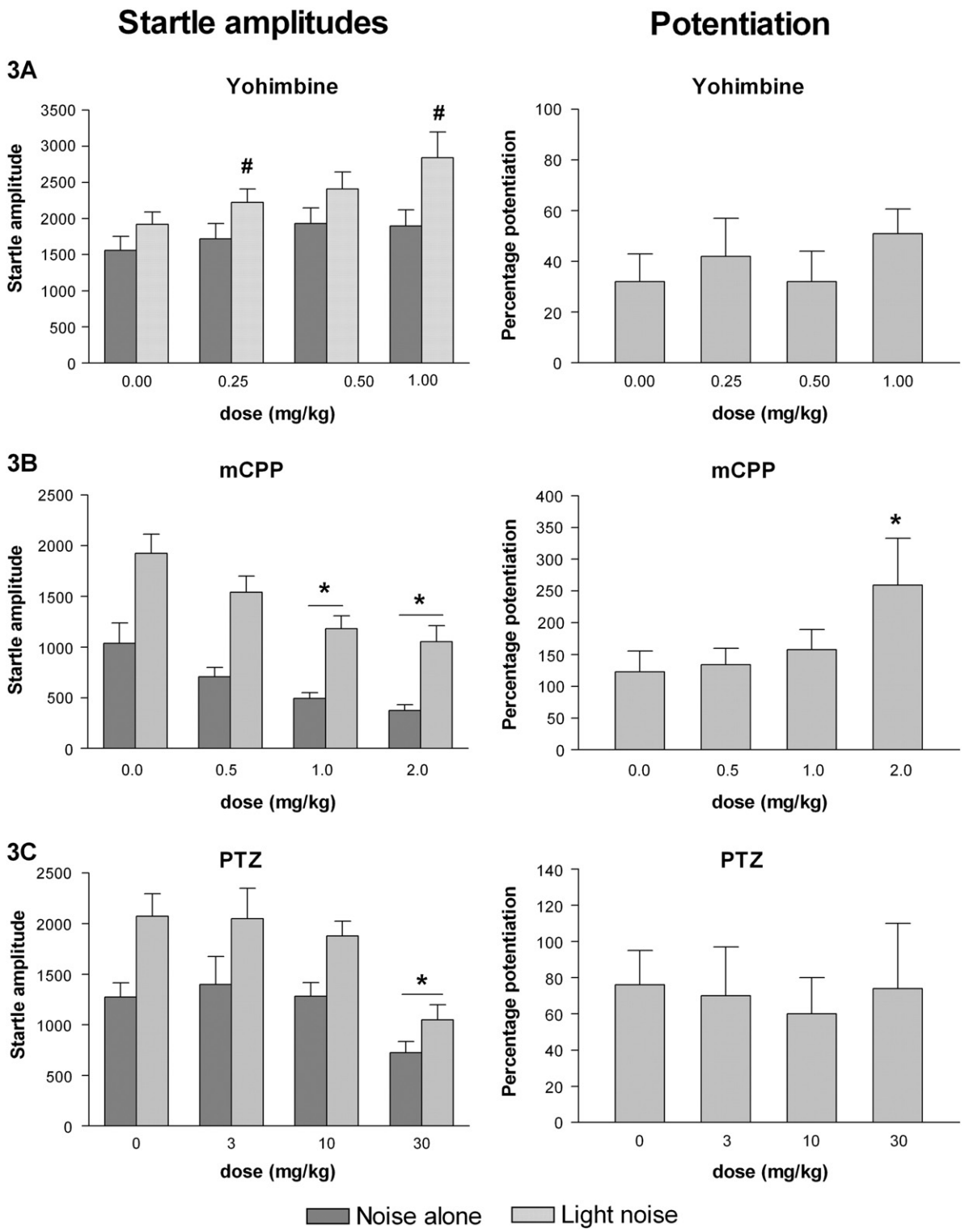

Fig. 3. The effects of yohimbine ($n=11$), mCPP ($n=10$) and PTZ ($n=12$) on fear-potentiated startle. Left panels show mean startle amplitudes on Noise Alone and Light Noise trials and right panels show the mean percentage potentiation of animals treated with yohimbine (A), mCPP (B) and PTZ (C). # indicates overall significant effect compared to 0 mg/kg during fear potentiation trials (left panel). * indicates significant difference compared to 0 mg/kg for mean percentage potentiation (right panel).

conditions (Noise Alone vs. Light Noise: Yohimbine [$T(1, 10) = -2.818$, $p<0.05$], mCPP [$T(1, 9) = -4.515$, $p<0.001$] and PTZ [$T(1, 11) = -3.412$, $p<0.01$]).

In the yohimbine study, overall analysis showed that yohimbine increased startle amplitude dependent on trial type (dose × trial type [$F(3, 8) = 2.8$; $p<0.1$]). Further analyses of Noise Alone and Light Noise trials separately, showed that this effect was specifically mediated by a significant increase in startle magnitude during the fear potentiation trials following 0.25 mg/kg and 1.0 mg/kg yohimbine (Light Noise trials: dose [$F(3, 30) = 4.301$; $p<0.05$]; simple contrasts: 0.25 mg/kg [$F(1, 10) = 5.138$; $p<0.05$]; 0.5 mg/kg [$F(1, 10) = 4.055$; $p<0.1$; 1.0 mg/kg [$F(1, 10) = 9.222$; $p<0.05$]), whereas Noise Alone trials were unaffected. Yohimbine did not significantly increase percentage fear potentiation.

In the mCPP study, overall analyses revealed that mCPP significantly decreased mean startle amplitude independent of trial type at the 1.0 mg/kg and 2.0 mg/kg doses (main effect dose [$F(3, 27) = 13.8$; $p<0.001$]; simple contrasts: 1.0 mg/kg [$F(1, 9) = 13.7$; $p<0.01$] and 2.0 mg/kg [$F(1, 9) = 45.2$; $p<0.001$]). In addition, percentage fear potentiation was increased by 2.0 mg/kg mCPP (main effect dose [$F(3, 27) = 3.4$; $p<0.05$]; simple contrasts: 2.0 mg/kg [$F(1, 9) = 6.5$; $p<0.05$]).

In the PTZ group, overall analysis showed that PTZ reduced startle amplitude independent of trial type (main effect dose [$F(3, 33) = 8.8$; $p<0.001$]). Simple contrasts revealed that this reduction was significant at 30 mg/kg [$F(1, 11) = 31.8$; $p<0.001$]. PTZ had no effect on percentage fear potentiation.

4. Discussion

This study evaluated the effects of three putative anxiogenic drugs on baseline startle amplitude and the potentiated startle response in the light-enhanced and fear-potentiated startle paradigms. In line with previous research, yohimbine and mCPP increased the potentiated startle response in the FPS paradigm. However, these drugs did not potentiate light-enhanced startle in the LES paradigm. In addition, yohimbine increased baseline startle amplitude in the LES study, whereas mCPP decreased overall startle amplitude in both the LES and FPS paradigm and PTZ decreased overall startle amplitude in the FPS paradigm. PTZ had no effect on potentiated startle response in either the FPS or the LES paradigm. An overview of the drug effects on startle amplitude in the LES and FPS paradigms found in the current study is given in Table 1.

4.1. Effect of yohimbine on baseline startle amplitude and startle potentiation

Yohimbine increased fear potentiation in the FPS paradigm when displayed as absolute startle potentiation. Startle potentiation in the LES paradigm, however, was not affected by yohimbine in the dose range tested, showing a pharmacological differentiation between FPS and LES. The lack of effect of yohimbine on LES was somewhat surprising. Systemic administration of yohimbine results in activation of the BNST, lateral septum and cingulate cortex, all brain areas implicated in LES (Davis and Shi, 1999; Singewald et al., 2003; Veening et al., 2009). In addition, decreasing noradrenergic signaling in the BNST via local administration of clonidine inhibits LES (Schweimer et al., 2005).

A possible explanation for the lack of effect of yohimbine on LES could be the specific anxiety-like state that is induced or exacerbated by yohimbine. It has been proposed that yohimbine potentiates neural mechanisms mediating flight. Flight, freezing and defensive threat/attack are the responses to immediate threat and constitute the fear/defense pattern (Blanchard and Blanchard, 1989). A potential threat elicits the anxiety/defense pattern, with risk assessment as the dominant response (Blanchard and Blanchard, 1989). As FPS is proposed to measure short duration conditioned fear and LES is proposed to measure sustained anxiety responses to a potentially threatening context, this differential influence of yohimbine on specific anxiety-like states may explain the differential sensitivity of the FPS and LES paradigm to detect yohimbine-induced effects. Interestingly, studies in patient groups underline these specific effects of yohimbine: While panic disorder patients show increased reports of arousal and anxiety compared to healthy subjects following yohimbine administration (Charney et al., 1987a), generalized anxiety disorder patients are not more responsive than healthy subjects to yohimbine-induced arousal and anxiety (Charney et al., 1989).

Our findings confirm the findings of yohimbine increased fear potentiation described by Davis et al. (1979), although in the present study effects were found only at the highest dose (1.0 mg/kg). An additional difference is that the effect of yohimbine was found only when looking at the absolute, but not the proportional, difference scores. It has been suggested that assigning drug effects on basis of absolute difference scores is less trustworthy than on basis of proportional difference scores because absolute difference scores can be confounded by baseline startle effects (Walker and Davis, 2002b). Responding to Noise Alone trials in the FPS study, however, was unaffected by yohimbine, which strengthens the idea that the effect on fear potentiation was not a due to confounding baseline effects, but a specific effect of yohimbine on fear-related responding. In addition, in the current study drug effects on exacerbation fear responses were analyzed by looking at interaction effects. A significant drug × trial type interaction means that a drug affected the Light Noise trials significantly different as compared to Noise Alone trials. These interaction effects seem to resemble a really specific effect on potentiated startle responding, irrespective of drugs effects on overall startle responding.

The finding of yohimbine-induced increases in baseline startle response in the LES fits a previous study in rats (Kehne and Davis, 1985). A similar pattern has been detected in humans, although this was primarily found in psychiatric patients suffering from anxiety disorders (Morgan et al., 1993). The question remains, however, what this increase in baseline startle responding reflects. It might reflect general arousal induced by autonomic activation. In post-traumatic stress disorder patients, yohimbine increased baseline startle amplitude, without altering the level of self reported anxiety (Morgan et al., 1995). The yohimbine-induced increase in baseline startle may even simply reflect excitation of spiny motor neurons, as a study by Kehne and Davis (1985) indicated that the yohimbine-induced effect on baseline startle was specifically mediated by norepinephrine release within the spinal cord (Kehne and Davis, 1985). Both possibilities would indicate that yohimbine-induced effects on baseline startle are independent of anxiety state. On the other hand, it has also been shown that local infusion of yohimbine into the central amygdala increases the startle response similar to shock-induced sensitization of the startle response. This shock-induced sensitization of the startle response could be blocked by local infusion of the α_2-adrenergic agonist ST-91 (Fendt et al., 1994). These findings suggest that yohimbine is able to induce a negative affective state (anxiety state) similar to that induced during shock-induced sensitization. It may be that this kind of anxiety-inducing effect is involved in the increased startle response found in the current study. Further research should look into the specific yohimbine-induced state that is reflected by increased baseline startle.

In summary, based on the current findings yohimbine seems to specifically alter startle responding related to fear/defense patterns, resulting in increased FPS. In addition, baseline startle is increased following yohimbine administration. Yohimbine, however, does not exacerbate sustained anxiety, as measured during LES and Noise Alone trials in the FPS paradigm.

4.2. Effect of mCPP on baseline startle amplitude and startle potentiation

mCPP increased percentage potentiation in the FPS paradigm, but did not affect startle potentiation in the LES paradigm. This would suggest that mCPP specifically influences conditioned fear responses and not the type of anxiety measured in the LES paradigm. Interestingly, a study by Mora et al. (1997) showed that mCPP specifically enhances fear-like responding, but not anxiety-like responding, in the elevated T-maze. A similar differentiation was found in a study with healthy subjects, wherein mCPP markedly enhanced fear in a conditioned fear paradigm, but did not influence a more generalized form of anxiety during a public speaking task (Graeff et al., 1996). However, this line of evidence contrasts the effects of mCPP in unconditioned anxiety-like paradigms, like the elevated plus maze and social interaction test, and it brain activation pattern (Fone et al., 1996; Kennett et al., 1989; Singewald et al., 2003; Veening et al., 2009). Thus, alternatively, it might be that the anxiogenic response to bright light just cannot be exacerbated by pharmacological treatment.

In contrast to the current study, Mansbach and Geyer (1988) were unable to detect an effect on fear potentiation. This contrast is most likely explained by the dose range tested. The increment in FPS in the current study was found only at the highest dose (2.0 mg/kg), while the highest dose tested by Mansbach and Geyer was 1.0 mg/kg (Mansbach and Geyer, 1988).

The suppressing effect of mCPP on overall startle responding in both FPS and LES is consistent with a previously reported reduction in baseline startle in the FPS paradigm (Mansbach and Geyer, 1988). It has been suggested that baseline startle responding in the FPS paradigm (Noise Alone trials) might be sensitive to effects of anxiolytic drugs as a result of the induction of contextual fear during FPS training (Guscott et al., 2000). The reduced baseline startle amplitude in the FPS following mCPP administration, however, is unlikely due to blockade of contextual fear, as baseline startle responding in the LES paradigm, which does not involve conditioned contextual fear, is also decreased following mCPP administration. The mCPP-induced effects on baseline startle are probably best explained by locomotor suppression (Fone et al., 1996; Kennedy et al., 1993; Kennett et al., 1989) and overall behavioural suppression (Jones et al., 2002). Unfortunately, considering the finding that mCPP did have an anxiogenic effect on Noise Alone trials in mice that were insensitive to the locomotor suppressing effects of mCPP (Risbrough and Geyer, 2005), it cannot be excluded that the overall mCPP-mediated behavioural suppression may have prevented the detection of possible anxiogenic effects of mCPP within the current study. In addition, the profound effect of mCPP on overall startle responding results in a discrepancy between absolute and proportional difference scores, that could possibly lead to misinterpretation of the FPS data.

In summary, although it cannot be excluded that certain drug effects were concealed by behavioural suppression, the current results suggest that mCPP specifically increases conditioned fear, as measured with FPS, and does not influence sustained anxiety, as measured with LES and during Noise Alone trials following fear conditioning.

4.3. Effect of PTZ on baseline startle amplitude and startle potentiation

PTZ had no effect on startle potentiation in neither the LES nor the FPS paradigm. The lack of effect on startle potentiation was unexpected, as PTZ was already found to be anxiogenic in various measures of anxiety-like behaviour (Johnston and File, 1989; Ramos et al., 2008) and $GABA_A$ receptor activation with for example the anxiolytic chlordiazepoxide inhibits both LES and FPS (Johnston and File, 1989; Ramos et al., 2008). Several studies, however, failed to detect an anxiogenic effect of PTZ (De Vry et al., 1993; Rodgers et al., 1995; Treit, 1987). In addition, previous studies in the FPS paradigm with other ligands of the $GABA_A$ receptor complex have also shown variable effects of $GABA_A$ receptor blockade. For example, the full $GABA_A$ inverse receptor agonist DMCM (methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate) and lindane, a neurotoxin that interferes with the chloride channel of the $GABA_A$ receptor, increased FPS (Hijzen and Slangen, 1989), whereas the partial $GABA_A$ inverse receptor agonist FG-7142 decreased the FPS response (Hart et al., 1998). The present findings suggest that, although stimulation of $GABA_A$ receptors with for example the anxiolytic chlordiazepoxide (CDP) has an anxiolytic profile in both the FPS and LES paradigm (de Jongh et al., 2002; Guscott et al., 2000), $GABA_A$ receptor inactivation with PTZ has no readily detectable effect on fear- and anxiety-related startle responding.

PTZ suppressed the overall startle response in both the FPS and LES at the 30 mg/kg dose. PTZ has been reported to suppress locomotion (Jones et al., 2002) and in mice, administration of 30 mg/kg PTZ severely disrupted behaviour, indicative of a preconvulsant state (Rodgers et al., 1995). In the present experiment, two rats had to be excluded from the study, because the highest dose of PTZ caused convulsions in these rats. As with mCPP, it is likely that PTZ-induced motor suppression interfered with the execution of the startle response at the highest dose tested.

A possible limitation of this study is that no positive control was used in the LES paradigm. However, this was also not possible due to the limited pharmacological validation of the LES paradigm. To the best of our knowledge, no reports have been made on drugs that are able to enhance the LES response. Another possible limitation of this study is the use of a within-subject design. It could be argued that this has prevented the detection of drug effects on LES and FPS, as exposure to an anxiogenic drug might sensitize behavioural responses in subsequent tests and these sensitized responses might interfere with possible drug effects. Analysis of time effects on both baseline startle responding and potentiated startle responding, however, did not reveal any sensitization in response to the drugs tested. Therefore, it seems unlikely that the design of the current study has prevented the detection of possible additional drug effects. The more as a highly significant effect of experimental manipulation was found under vehicle conditions.

In addition, it might be possible that the exclusion of non-responders from the LES studies has interfered with the detection of anxiogenic drug effects, because of a ceiling effect. Excluding non-responders from analysis could have resulted in the selection of animals that already show relatively high anxiety levels (startle magnitudes) under control conditions, and therefore the range left to further enhance the startle response in response to an anxiogenic drug may be relatively small as compared to animals that show low anxiety levels under control conditions. A median split analysis of the LES data on basis of %LES under vehicle conditions (resulting in a low LES-reactive and a high LES-reactive group) did not reveal any differential drug effects in the low and high reactive group nor did it reveal drug × group interactions for any of the drugs, excluding a possible ceiling effect. It must be mentioned, though, that group size in this analysis was relatively small (4–6 animals per group). Another argument against absence of effects in LES due to ceiling effects is that the drugs did not only fail to enhance LES, they also did not increase responding to Noise Alone trials, a measure of sustained anxiety related to the LES response.

To the best of our knowledge, this is the first study on the effect of anxiogenic drugs on LES. Although the FPS paradigm has been extensively validated with different classes of psychoactive drugs, the sensitivity of the LES paradigm for detecting drug effects has hardly been investigated. So far, it has been shown that the LES response can be attenuated by several anxiolytic drugs (de Jongh et al., 2002; Walker and Davis, 2002a) and that CRF is specifically involved in the LES response, as it is blocked by CRF antagonists (de Jongh et al., 2003; Walker et al., 2008). In the current study, none of the drugs influenced LES. Therefore, it could be concluded that the LES paradigm is not able

to detect the specific anxiety states induced by yohimbine, mCPP and PTZ. On the other hand, one could argue that the anxiety state induced in the LES paradigm cannot be exacerbated by an additional anxiogenic manipulation. This hypothesis may be true for pharmacological manipulations. However, it has already been shown that LES can be enhanced by behavioural stress manipulations (Jonkman et al., 2007; Tazumi et al., 2005), although opposite effects of stress manipulations on LES have also been reported (Bijlsma et al., 2010; de Jongh et al., 2005).

Altogether, current findings show that the putative anxiogenic drugs yohimbine and mCPP potentiate FPS, but not LES. PTZ, however, had no effect on the potentiated startle response in either FPS or LES. The differential influence of yohimbine and mCPP on LES and FPS suggests that these drugs specifically enhance conditioned fear responses, as measured during FPS, and not sustained anxiety, as measured during LES.

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