Behavioural Brain Research 353 (2018) 57–61

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

The contribution of contextual fear in the anxiolytic effect of chlordiazepoxide in the fear-potentiated startle test

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ARTICLE INFO

Keywords:
Context
$GABA_A$ receptors
Zolpidem
Chlordiazepoxide

ABSTRACT

This study evaluated the extent to which a reduction in contextual fear contributes to the anxiolytic effect of benzodiazepines in the fear-potentiated startle response. To this end, chlordiazepoxide, an anxiolytic often used as positive control in preclinical drug studies, and zolpidem, known to have sedative properties and to be devoid of anxiolytic effects, were tested in two contexts: the same context as training had taken place and an alternative context. In addition, the level of muscle relaxation was assessed in a grip strength test. Chlordiazepoxide (2.5–10 mg/kg) decreased the fear-potentiated startle response, confirming its anxiolytic activity. In addition, it dose-dependently decreased the overall startle response in the same, but not the alternative context, and did not affect grip strength, indicating that chlordiazepoxide inhibits contextual fear in the absence of non-specific drug effects. Zolpidem (1.0–10 mg/kg) reduced the overall startle response in both contexts equally and decreased grip strength, indicating that its effects on fear-potentiated startle are due to non-specific drug effects, and not anxiolytic effects. The present findings show that chlordiazepoxide reduces contextual conditioned fear in the absence of non-specific drug effects. In addition, they show that training and testing rats in different contexts makes it possible to distinguish between cued, contextual and non-specific drug effects. As exaggerated contextual fear conditioning contributes to the fear generalization processes implicated in pathological anxiety, focus in screening of anxiolytic effects could be directed more towards the suppression of contextual fear and, therefore, this approach would be a valuable addition to standard preclinical screening.

Fear conditioning, a form of associative learning in which an individual learns that a certain stimulus predicts an aversive event [1], has been established an important factor in the aetiology of anxiety disorders [2]. The fear-potentiated startle test, which is based on classical fear conditioning, is considered a reliable tool for detecting anxiolytic properties of compounds in rodents [3]. In this test anxiolytic activity is reflected in an attenuated response to the startle stimuli presented together with the cue-light (cued trials) [4].

In pharmacological fear-potentiated startle studies, chlordiazepoxide, which binds to benzodiazepine allosteric binding sites on $GABA_A$ receptors, is frequently used as a positive control [5,6]. However, a complication within these studies is the strong effect of chlordiazepoxide on the overall startle response per se, which is the average of cued and non-cued trials together. Benzodiazepines have a wide variety of neurological effects of which sedation and muscle relaxation (from here on referred to as 'non-specific drug effects') have been proposed to interfere with the startle response [7]. Therefore a reduction in overall startle response is often considered an artefact. However, as animals also learn to associate the shock with the surrounding context, the level of contextual conditioned fear also affects the magnitude of the overall startle response [8]. Therefore, the overall startle response could also be sensitive to the anxiolytic effects of chlordiazepoxide. So far, however, it is unclear to what extent the reduction in overall startle response results from a reduction in contextual fear or from non-specific drug effects. As contextual conditioned fear is suggested to play a crucial role in pathological anxiety [9], it is of clinical relevance to clarify the anxiolytic activity of know anxiolytics with regard to contextual conditioned fear and to further define the optimal anxiolytic profile of (putative) anxiolytics in the fear-potentiated startle test.

The aim of this study was to disentangle the effects of chlordiazepoxide on contextual and cued fear in the fear-potentiated startle test. To this end, we compared the effects of chlordiazepoxide with the effects of zolpidem. Although zolpidem binds to the same receptor complex as chlordiazepoxide, it is a $GABA_A$ α1-subunit preferential hypnotic known to have sedative properties and weak anxiolytic effects [10,11]. These drugs were tested in two contexts: the same context as training had taken place and an alternative context and the effects on the cued fear response and overall startle response were analyzed.

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https://doi.org/10.1016/j.bbr.2018.06.035
Received 14 May 2018; Received in revised form 28 June 2018; Accepted 29 June 2018
Available online 30 June 2018
0166-4328/

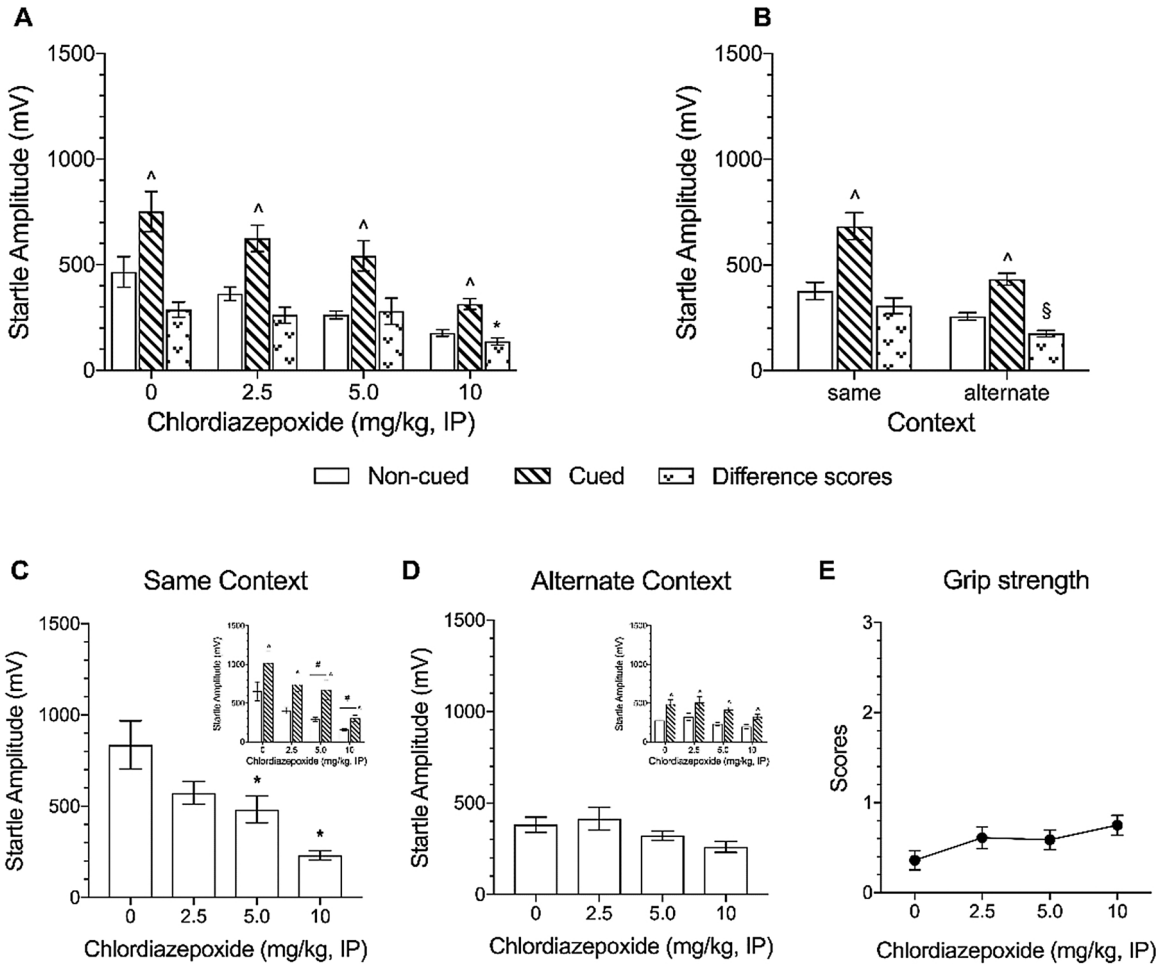

Fig. 1. Effects of chlordiazepoxide in the fear-potentiated startle and grip strength test. The figure shows the effects of chlordiazepoxide (A, n = 24 per dose) and context (B, n = 48 per context) on the response to non-cued and cued trials and difference scores. Lower panel shows the effects of chlordiazepoxide on the overall startle response, non-cued trials and cued trials in the same (C, n = 12 per dose) and alternate context (D, n = 12 per dose) and grip strength (E, n = 22 per dose). Data are shown as mean ± SEM. ^ $p < 0.05$ compared to non-cued trials. Both * and # indicate $P < 0.05$ compared to vehicle condition. § $p < 0.05$ compared to the same context condition.

Testing subjects in an alternative context prevents the expression of contextual conditioned fear [12] and, therefore, can provide a more effective means of separating anxiolytic effects from non-specific drug effects. Grip strength was determined as a proxy of muscle relaxation.

192 male rats (Wistar HsdCpd: WU, Harlan Laboratories BV, Horst, Netherlands), 6 weeks old on arrival, were housed in groups of four in a temperature (21 °C ± 2), humidity (55% ± 5), and light controlled room (lights on from 6 A M to 6 PM). Rats were randomly allocated to the cages upon arrival. Food and water were available *ab libitum* in the home cages. For each compound 96 rats were tested. Chlordiazepoxide was tested in four cohorts of 24 rats, zolpidem was tested in two cohorts of 48 rats. Rats were randomly allocated to the experimental conditions (that is, drug dose and context). Experiments were carried out during the light phase of the day-night cycle between 9 A M and 4 PM. All studies were approved by the Ethical Committee for Animal Research (DEC, Utrecht University, Netherlands) and conducted according to the European Directive 2010/63/EU.

After one-week acclimation period to the housing conditions, rats were exposed to a startle set-up habituation session (SR-lab, San Diego instruments, San Diego, CA, USA) during a habituation session (30 startle stimuli, 10 × 95, 100, and 110 dB, ISI: 30 s, no grid floors). Four days later rats received the fear-potentiated startle training which started with 5 min acclimation, after which 10 light-shock pairings were presented with an average interval of 4 min (range: 3–5 min; 0.6 mA during the last 500 ms of the 3700 ms light period). 24 h after this training, rats were blindly injected with one of four doses of chlordiazepoxide HCl (vehicle, 2.5, 5, and 10 mg/kg, 20 min before the test) or zolpidem (vehicle, 1.0, 3.0, and 10 mg/kg, 15 min before test). Chlordiazepoxide (Pharbita B.V., Zaandam the Netherlands) was dissolved in 0.9% sodium chloride and zolpidem (Sunovion Pharmaceuticals Inc. Marlborough Massachusetts, USA) was suspended in Gelatin Mannitol (0.5%/5%) and both compounds were administered intraperitoneally (i.p.) in a volume of 2 ml/kg. Half of the rats were tested in the same context as during training (a grid floor and standard white walls), while the other half were tested in the alternate context (a PVC board floor and striped walls). The fear-potentiated test started with an acclimation period of 5 min, after which 10 startle stimuli were presented for habituation purposes (110 dB, ISI 30 s), Subsequently, 60 startle stimuli (20 × 95, 100, and 110 dB, ISI 30 s) were presented, half of which were delivered during the last 50 ms of a 3250 ms light cue (cued trials), the other half were delivered in the absence of the cue (non-cued trials). The six different trial types were presented in a balanced, irregular order throughout the test. During all sessions, a background noise of 70 dB was presented, which is 2 dB above environmental background noise. For further details of equipment, training and test, see [13].

Grip strength was measured immediately after the fear-potentiated startle test. The rat was placed on the cage lid and gently pulled backwards. The response was scored by an observer blinded to drug treatment (two observers in the chlordiazepoxide study to validate the

score). The four-point scale ranged from '0' normal grip resistance to '3' clearly less resistance [14]. This manual observation was validated against an automated grip test meter (BIOSEB *in vivo* Research Instruments, Vitrolles, France).

Startle peak values of each trial type were averaged for statistical analyses (SPSS, IBM SPSS Statistics version 24, Inc., Chicago, IL). For each compound, data were analysed unblinded using repeated measures ANOVAs with trial type (cued vs non-cued) and intensity (95 dB, 100 dB, and 110 dB) as within-subject factors, and context (same vs alternate) and dose (vehicle and three different dosages) as between-subjects factors. If adequate, overall analyses were followed by subsequent ANOVAs and T-tests. In addition, startle difference scores (difference between cued and non-cued trials, measure of absolute cued fear-potentiated startle response) and grip strength data were analyzed with One-way ANOVA. Eight animals in the chlordiazepoxide experiment were not measured in the grip strength test due to time-schedule limitations. Dunnett's post hoc analyses were performed to compare drug treatments with the vehicle group. Significance was set at P-values < 0.05

In both experiments, fear-potentiated startle was successfully induced (cue effect, chlordiazepoxide $F_{1,88} = 151.3$, $p < 0.001$; zolpidem $F_{1,88} = 127.5$, $p < 0.001$).

Chlordiazepoxide treatment had a differential effect on cued and non-cued trials (cue x dose $F_{3,88} = 3.2$, $p = 0.026$), which was independent of the context (cue x dose x context $F_{3,88} < 1$). As shown in Fig. 1A, chlordiazepoxide reduced the fear-potentiated startle response with increasing doses. None of the dosages completely blocked the fear-potentiated startle response, as responses to cued and non-cued trials remained significantly different at all doses (Fig. 1A). However, subsequent analyses of absolute difference scores showed that 10 mg/kg chlordiazepoxide reduced the difference scores compared to vehicle controls (dose effect $F_{3,92} = 2.9$, $p = 0.038$; post-hoc analyses: 10 mg/kg vs vehicle $p = 0.032$; Fig. 1A).

The effect of chlordiazepoxide on the overall startle response ($F_{3,88} = 10.9$, $p < 0.001$) was dependent on the context in which rats were tested (dose x context $F_{3,88} = 4.6$, $p = 0.005$). In the same context, chlordiazepoxide attenuated the overall startle response at 5.0 and 10 mg/kg relative to vehicle treatment (dose effect $F_{3,44} = 9.1$, $p < 0.001$; Fig. 1C), whereas chlordiazepoxide had no effect on the overall startle response in the alternate context (dose effect $F_{3,44} = 2.6$, $p = 0.07$; Fig. 1D). In addition, chlordiazepoxide had no significant effect in the grip strength test ($F_{3,84} = 2.0$, $p = 0.13$; Fig. 1E).

The fear-potentiated startle response was dependent on the context in which the test was performed (cue x context $F_{1,88} = 11.2$, $p = 0.001$). In both contexts, the response to cued trials was significantly higher than to non-cued trials, but the absolute difference score was lower in the alternate than in the same context ($T_{94} = 3.3$, $p = 0.002$, Fig. 1B).

The effect of zolpidem on the startle response was dependent on trial type (cue x dose $F_{3,88} = 5.9$, $p = 0.001$), but independent of context (cue x dose x context $F_{3,88} < 1$). Zolpidem significantly reduced the response to non-cued trials at 3.0 and 10 mg/kg (dose effect, $F_{3,88} = 9.3$, $p < 0.001$; Fig. 2A), whereas the response to cued trials was reduced at 10 mg/kg (dose effect, $F_{3,88} = 8.6$, $p < 0.001$). Subsequent analyses of absolute difference scores showed that administration of 3.0 mg/kg zolpidem significantly increased the absolute difference scores compared to vehicle treatment ($F_{3,92} = 5.6$, $p = 0.001$).

In addition, zolpidem significantly decreased the overall startle response at 10 mg/kg (dose effect $F_{3,88} = 9.2$, $p < 0.001$; Fig. 2C and D), independent of context (context x dose $F_{3,88} < 1$). Furthermore, zolpidem significantly reduced grip strength at 10 mg/kg ($F_{3,92} = 4.5$, $p = 0.006$; Fig. 2E).

The level of fear-potentiated startle was dependent on the context in which the test was performed (cue x context $F_{1,88} = 6.9$, $p = 0.010$). In both contexts the response to cued trials was significantly higher than to non-cued trials, however, the absolute difference score was lower in the alternate context ($T_{94} = 2.5$, $p = 0.016$, Fig. 2B).

This study aimed to distinguish between non-specific, contextual fear and cued fear effects of chlordiazepoxide in the fear-potentiated startle paradigm. The results show that, in line with previous research, chlordiazepoxide shows an anxiolytic profile, displayed as a decrease in fear-potentiated startle response. In addition, chlordiazepoxide only decreased the overall startle response when tested in same context, suggesting that this reduction in overall startle results from a decrease in contextual conditioned fear and not from non-specific drug effects. This idea is supported by the finding that zolpidem reduced the overall startle response equally in both contexts. In addition, in both experiments the fear-potentiated startle response was lower in the alternate context, as compared to the same context, indicating that the level of cue conditioned fear expression is context-dependent.

To the best of our knowledge, this is the first study on the effect of zolpidem in the fear-potentiated startle test. We found that zolpidem decreased the overall startle response equally in the same and alternate context. In addition, zolpidem reduced muscle strength in the grip strength test, confirming the muscle relaxing effect of zolpidem. Zolpidem is known for its sedative and hypnotic effects in a range of human [15,16] and animal tests [17]. In addition, it has been associated with reduced baseline startle responses in prepulse inhibition in rats [18]. These effects can be attributed to its preferential affinity for α1 subunit-containing $GABA_A$ receptors [19]. With the use of two different contexts, we showed that zolpidem does not affect contextual conditioned fear in rats and that the effects of zolpidem in the fear-potentiated startle test can be selectively attributed to its non-specific drug effects.

In the present study, chlordiazepoxide attenuated the overall startle response in the same context without altering this response in the alternate context. This lack of effect in the alternate context was not due to the floor effect. Because the overall startle response at 10 mg/kg in the same context was much lower than that of the different dose groups in the alternate context. In addition, chlordiazepoxide did not affect muscle relaxation in the grip strength test. The combined results from the chlordiazepoxide and zolpidem experiments indicate that, even though fear-potentiated startle is subject to the sedative effects of compounds acting on the benzodiazepine binding site, the effects of the non-selective benzodiazepine chlordiazepoxide on overall startle response in the standard fear-potentiated startle paradigm, within the dose range tested, are selectively mediated by a reduction in contextual conditioned fear and not due to a sedative or muscle relaxing effect. This finding is in contrast to Guscott et al. [12] who did report an effect of chlordiazepoxide on non-cued trials in a different context. A possible explanation for this discrepancy could be the differences in training protocol used for the different context experiment in the Guscott study and the removal of the grid floor in the alternate context in our study. In the Guscott study, the used set up resulted in a relatively high response to non-cued trials and diminished sensitivity of cued-trials to the anxiolytic effects of chlordiazepoxide. This may suggest that the differences in training protocol and changes in contextual cues may have affected the discreteness of cued fear conditioning and thereby affected the general state of anxiety of the animals. This may have increased the sensitivity of non-cued trials for the anxiolytic effects of CDP in the Guscott study. Nevertheless, both studies highlight the importance of including context as an experimental factor when analyzing anxiolytic activity; Guscott et al. did so by studying different anxiolytics with limited sedative and/or motor relaxant effects. We confirm those findings and add to their study by showing the lack of context-dependent effects of zolpidem, in the presence of sedative and motor relaxant effects. To the best of our knowledge, this is the first study in rodents that disentangled contextual fear and non-specific drug effects of chlordiazepoxide by comparing its effects to that of a known sedative and muscle relaxing α1-selective hypnotic.

In line with previous fear-potentiated startle studies, chlordiazepoxide attenuated the expression of cued conditioned fear in the present

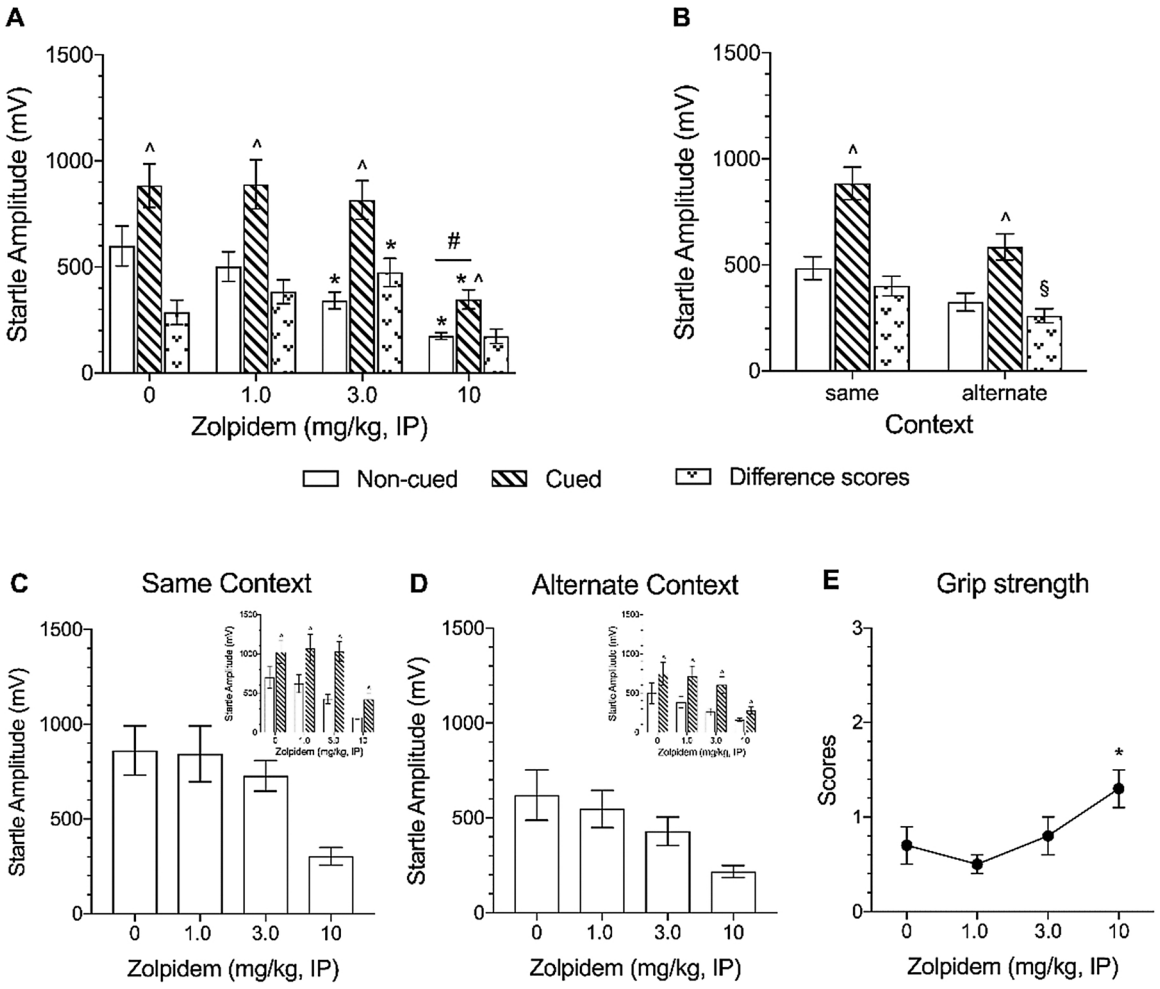

Fig. 2. Effects of zolpidem in the fear-potentiated startle and grip strength test. The figure shows the effects of zolpidem (A, n = 24 per dose) and context (B, n = 48 per context) on the response to non-cued and cued trials and difference scores. The lower panel shows the effects of zolpidem on the overall startle response, non-cued trials and cued trials in the same and alternate context (C and D, n = 12) and grip strength (E, n = 24). Data are shown as mean ± SEM. ^ $p < 0.05$ compared to non-cued trials. * $p < 0.05$ compared to the vehicle condition. § $p < 0.05$ compared to the same context condition. # $p < 0.05$ difference in overall startle response relative to the vehicle condition.

study. Interestingly, the anxiolytic effect of chlordiazepoxide on contextual conditioned fear is apparent at lower doses than its anxiolytic effect on cued fear, which is consistent with [12]. This may suggest that chlordiazepoxide, and possibly anxiolytic benzodiazepines in general, may act more effectively on contextual conditioned fear than on cued conditioned fear. Several human studies have reported that other benzodiazepines, such as diazepam, oxazepam, and alprazolam, are not effective in reducing the fear-potentiated startle response in humans but significantly reduce contextual fear [20,21]. This suggests that the drug effects on contextual conditioned fear may actually show high translational value and studying this type of fear more consistently may improve our understanding of the mechanisms underlying pathological anxiety.

It is noteworthy that a lower cued fear response was observed in rats tested in the alternate context. This finding shows the facilitating role of contextual information on the expression of cued fear, already reported in both rodents and humans [12,22,23]. This further strengthens the translational value of the fear-potentiated startle paradigm.

Unexpectedly, an increase in difference score was observed after treatment with the middle dose of zolpidem (3.0 mg/kg). Such an increase could be interpreted as an anxiogenic effect. Since zolpidem has not been associated with anxiogenic actions, it seems more likely that in the presence of the aversive cue-light, the non-specific drug effects of zolpidem were less strongly expressed than during non-cued trials, because of, for example the arousal-inducing effect the cue-light may have [24]. But importantly, since the actions of zolpidem were independent of the test context, it is unlikely that this reduced response to non-cued trials reflects a decrease in contextual fear.

In conclusion, the design used in the present study enabled us to unmask the effects of chlordiazepoxide on contextual fear, by separating these from its non-specific effects. This dissociation was further confirmed by comparing the drug profile of the anxiolytic chlordiazepoxide to that of the hypnotic and sedative zolpidem. According to the present findings, chlordiazepoxide reduces contextual conditioned fear in the absence of non-specific drug effects and at lower doses than cued fear.

Given the important role of exaggerated contextual fear conditioning in the fear generalization processes implicated in anxiety disorders, focus in preclinical screening of anxiolytic effects could be directed more towards the suppression of contextual fear. Hence, this approach would be a relevant addition to standard screening tools.

Acknowledgement

This work was supported by the funding for PhD students from China Scholarship Council (201306300062, 2013-2017).

References

[1] S. Maren, Neurobiology of pavlovian fear conditioning, Annu. Rev. Neurosci. 24 (2001) 897–931, http://dx.doi.org/10.1146/annurev.neuro.24.1.897.

[2] D. Tinoco-Gonzalez, M.A. Fullana, D. Torrents-Rodas, A. Bonillo, B. Vervliet, M.J. Blasco, M. Farre, R. Torrubia, Conditioned fear acquisition and generalization in generalized anxiety disorder, Behav. Ther. 46 (2015) 627–639, http://dx.doi.org/10.1016/j.beth.2014.12.004.

[3] M. Davis, Fear-potentiated startle in rats, Curr. Protoc. Neurosci. 11A (2001), http://dx.doi.org/10.1002/0471142301.ns0811as14 Chapter 8, Unit 8.

[4] M. Fendt, M.S. Fanselow, The neuroanatomical and neurochemical basis of conditioned fear, Neurosci. Biobehav. Rev. 23 (1999) 743–760, http://dx.doi.org/10.1016/S0149-7634(99)00016-0.

[5] D.J. Bill, A. Fletcher, B.D. Glenn, M. Knight, Behavioral studies on WAY100289; A novel 5-HT3 receptor antagonist; in two animal models of anxiety, Eur. J. Pharmacol. 218 (1992) 327–334.

[6] A.L. Vale, S. Green, Effects of chlordiazepoxide, nicotine and d-amphetamine in the rat potentiated startle model of anxiety, Behav. Pharmacol. 7 (1996) 138–143.

[7] R.J.E. Joordens, T.H. Hijzen, B.W.M.M. Peeters, B. Olivier, Fear-potentiated startle response is remarkably similar in two laboratories, Psychopharmacology (Berl.) 126 (1996) 104–109, http://dx.doi.org/10.1007/BF02246344.

[8] S. Campeau, M.D. Hayward, B.T. Hope, J.B. Rosen, E.J. Nestler, M. Davis, Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear, Brain Res. 565 (1991) 349–352, http://dx.doi.org/10.1016/0006-8993(91)91669-R.

[9] S. Lissek, C. Grillon, Learning models of PTSD, Oxford handb. Trauma Stress Disord. (2012), http://dx.doi.org/10.1093/oxfordhb/9780195399066.013.0013.

[10] P. Salvà, J. Costa, Clinical pharmacokinetics and pharmacodynamics of zolpidem, Clin. Pharmacokinet. 29 (1995) 142–153, http://dx.doi.org/10.2165/00003088-199529030-00002.

[11] F. Sancar, S.S. Ericksen, A.M. Kucken, J.A. Teissére, C. Czajkowski, Structural determinants for high-affinity zolpidem binding to GABA-a receptors, Mol. Pharmacol. 71 (2007) 38–46, http://dx.doi.org/10.1124/mol.106.029595.

[12] M.R. Guscott, G.P. Cook, L.J. Bristow, Contextual fear conditioning and baseline startle responses in the rat fear-potentiated startle test : a comparison of benzodiazepine / γ-aminobutyric acid-a receptor agonists, Behav. Pharmacol. 11 (2000) 495–504.

[13] L. Groenink, E.Y. Bijlsma, B. Olivier, Fear-potentiated startle and light-enhanced startle models in drug discovery, Curr. Protoc. Pharmacol. 5 (48) (2008), http://dx.doi.org/10.1002/0471141755.ph0548s41 1-5.48.15.

[14] G. Alma, Behavioural Observation Test, (2010).

[15] T. Nakajima, T. Sasaki, K. Nakagome, S. Takazawa, E. Ikebuchi, Y. Ito, Y. Miyazawa, M. Tanaka, O. Kanno, Comparison of the effects of zolpidem and zopiclone on nocturnal sleep and sleep latency in the morning: a cross-over study in healthy young volunteers, Life Sci. 67 (2000) 81–90, http://dx.doi.org/10.1016/S0024-3205(00)00603-2.

[16] S.E. Tom, E.M. Wickwire, Y. Park, J.S. Albrecht, Nonbenzodiazepine sedative hypnotics and risk of fall-related injury, Sleep 39 (2016) 1009–1014, http://dx.doi.org/10.5665/sleep.5742.

[17] L.S. Mathiasen, N.R. Mirza, R.J. Rodgers, Strain- and model-dependent effects of chlordiazepoxide, L-838,417 and zolpidem on anxiety-like behaviours in laboratory mice, Pharmacol. Biochem. Behav. 90 (2008) 19–36, http://dx.doi.org/10.1016/j.pbb.2008.01.014.

[18] P. Mierzejewski, M. Kolaczkowski, M. Marcinkowska, A. Wesolowska, J. Samochowiec, M. Pawlowski, P. Bienkowski, Antipsychotic-like effects of zolpidem in Wistar rats, Eur. J. Pharmacol. 773 (2016) 51–58, http://dx.doi.org/10.1016/j.ejphar.2016.01.009.

[19] Y.A. Blednov, S. Jung, H. Alva, D. Wallace, T. Rosahl, P. Whiting, R.A. Harris, Deletion of the alpha 1 or beta 2 subunit of GABA a receptors reduces actions of alcohol and other drugs, Pharmacology 304 (2003) 30–36, http://dx.doi.org/10.1124/jpet.102.042960.tion.

[20] J. Baas, C. Grillon, K. Böcker, A. Brack, C. Morgan, L. Kenemans, M. Verbaten, Benzodiazepines have no effect on fear-potentiated startle in humans, Psychopharmacology (Berl.) 161 (2002) 233–247, http://dx.doi.org/10.1007/s00213-002-1011-8.

[21] C. Grillon, J.M.P. Baas, D.S. Pine, S. Lissek, M. Lawley, V. Ellis, J. Levine, The benzodiazepine alprazolam dissociates contextual fear from cued fear in humans as assessed by fear-potentiated startle, Biol. Psychiatry 60 (2006) 760–766, http://dx.doi.org/10.1016/j.biopsych.2005.11.027.

[22] G. Winocur, M. Moscovitch, M. Sekeres, Memory consolidation or transformation: context manipulation and hippocampal representations of memory, Nat. Neurosci. 10 (2007) 555–557, http://dx.doi.org/10.1038/nn1880.

[23] N.C. Huff, J.A. Hernandez, M.E. Fecteau, D.J. Zielinski, R. Brady, K.S. LaBar, Revealing context-specific conditioned fear memories with full immersion virtual reality, Front. Behav. Neurosci. 5 (2011) 1–8, http://dx.doi.org/10.3389/fnbeh.2011.00075.

[24] E.R. Samuels, R.H. Hou, R.W. Langley, E. Szabadi, C.M. Bradshaw, Modulation of the acoustic startle response by the level of arousal: comparison of clonidine and modafinil in healthy volunteers, Neuropsychopharmacology 32 (2007) 2405–2421, http://dx.doi.org/10.1038/sj.npp.1301363.