




## Sexually dimorphic and time-dependent influence of active avoidance learning by vilazodone in C57BL/6J mice

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### ABSTRACT

The effects of vilazodone (VZD) on the acquisition of active avoidance behavior were examined in C57BL/6J mice. Both female and male mice were assigned to three groups ( $n = 8$  per group per sex): the vehicle control group (VEH), the 0.5 mg kg<sup>-1</sup> vilazodone lower dose group (VZD0.5) and the 1 mg kg<sup>-1</sup> vilazodone higher dose group (VZD1.0). Spontaneous locomotion and anxiety-like behaviour were assessed after drug administration intraperitoneally in an open field test (OFT). Another cohort of mice was trained in a three-day shuttle box active avoidance test (AAT) after drug administrations with the aim of evaluating the effects of VZD on the acquisition of active avoidance behaviour. In the OFT, VZD decreased freezing time in the corner area in both female and male mice, indicating reduced anxiety-like behaviours. In the AAT, the active avoidance rate was significantly improved on day 1 in female mice and day 2 in male mice, suggesting that VZD facilitated active avoidance learning with sexual dimorphism. Furthermore, the increased active avoidance rates were negatively correlated with freezing time during training. Interestingly, these group differences and correlations diminished on day 3, implying that the facilitation was restricted to early training phases. Collectively, VZD facilitates the acquisition of active avoidance behaviour in mice with distinct sexual dimorphism and temporal dynamics.

**Keywords:** vilazodone, anxiety, active avoidance behaviour, sexual dimorphism

### INTRODUCTION

The 5-hydroxytryptamine (5-HT) system in the brain is one of the key neurotransmitter systems involved in regulating emotions, cognition, motivation and reward processing (1, 2). Psychotherapeutic drugs developed based on modulation of the 5-HT system, such as selective serotonin reuptake inhibitors (SSRIs), are widely used in the clinical treatment of depression and anxiety disorders (3). Vilazodone (VZD), as a newer antidepressant, is unique in that it acts both as a 5-HT reuptake inhibitor and as a partial agonist of the 5-HT<sub>1A</sub> receptor (4). This dual-acting mechanism enables VZD to exhibit antidepressant efficacy comparable to that of traditional SSRIs in clinical settings. It is also used in the

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treatment of anxiety disorders, demonstrating a significant alleviation of generalised anxiety disorder symptoms and a rapid onset of effect relative to placebo (5).

Clinical evidence indicates that some patients with anxiety disorders exhibit excessive avoidance behaviour (6, 7). The acquisition and expression of active avoidance behaviour in experimental animals involves multiple cognitive modules, including fear conditioning, stimulus-response associative learning, decision-making, and the negative reinforcement effect following successful avoidance (8). The neural mechanisms are closely associated with complex circuits involving brain regions such as the prefrontal cortex, amygdala, hippocampus and dorsal striatum (8, 9). Notably, the 5-HT system, particularly the 5-HT<sub>1A</sub> receptor, is densely distributed in these brain regions and has been shown to play an important role in regulating cognitive flexibility and stress responses (10–12). Although the clinical efficacy of VZD has been established, its effects on active avoidance behaviour in laboratory animals have not been reported. Whether its dual action as a 5-HT reuptake inhibitor and a partial agonist of the 5-HT<sub>1A</sub> receptor can modulate this complex behaviour, which relies on multi-regional brain coordination, is worth exploring.

This study aims to investigate the effects of acute VZD on the acquisition of active avoidance behaviour in naïve C57BL/6J mice, while excluding potential interference from depressive or anxiety-like states on learning ability. We hypothesise that VZD, through its dual-acting mechanism, could reduce anxiety levels in mice under acute stress, thereby enhancing learning efficiency in an active avoidance test (AAT). This study will give insight into the potential influence of VZD on learning and motivated behaviours after acute application, which warrants further investigation.

## EXPERIMENTAL

### *Animals*

A total of 96 C57BL/6J mice, comprising 48 females and 48 males aged 8 weeks, were used in this study. Female mice weighed 16–18 g, and male mice weighed 17–22 g. All mice were purchased from Jiangsu GemPharmatech (China). Mice were housed in groups of four per cage with *ad libitum* access to food and water. The animal room was maintained at 20–24 °C with a humidity of 40–60 % under a 12-h light/dark cycle (lights on at 08:00 h). After a one-week acclimation period, behavioural experiments commenced. The experimental procedures were approved by the Laboratory Animal Ethics Committee of Xuzhou Medical University (Xuzhou, Jiangsu, China) and complied with ethical standards and animal welfare requirements.

### *Chemicals and equipment*

The drug doses were selected according to prior studies reporting antidepressant-like effects of VZD at 1 mg kg<sup>-1</sup> in mice (13, 14). VZD (purity ≥ 98 %) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (China) and dissolved in dimethyl sulfoxide (DMSO, Beijing Solarbio Science & Technology, China) to prepare a 1 mg mL<sup>-1</sup> stock solution, which was stored at -20 °C. For administration, the stock solution was diluted with 5 % Tween-80 aq. solution (Beijing Solarbio). A 10-fold dilution yielded a

0.1 mg mL<sup>-1</sup> working solution for the VZD1.0 group (1 mg kg<sup>-1</sup> VZD administration), and a 20-fold dilution yielded a 0.05 mg mL<sup>-1</sup> working solution for the VZD0.5 group (0.5 mg kg<sup>-1</sup> VZD administration). VZD (1.0 or 0.5 mg kg<sup>-1</sup>) or vehicle was administered intraperitoneally. All injections were given at a standardised volume of 0.1 mL per 10 g of body mass. Vehicle (VEH) is composed of 5 % aq. Tween-80 and 10 % DMSO. Animal behaviour was recorded and analysed using the automatic video tracking software VisuTrack (Shanghai Xinruan Information Technology, China).

### *Experimental design*

*Open field test (OFT).* – A total of 24 female and 24 male mice were used in OFT. Males and females were handled and tested in separate sex cohorts. Within each sex, mice were randomly assigned to the following groups ( $n = 8$  per group): control group (VEH) received an equal volume of the vehicle, lower dose group (VZD0.5) received 0.5 mg kg<sup>-1</sup> VZD; higher dose group (VZD1.0) received 1 mg kg<sup>-1</sup> VZD. The OFT was conducted 30 minutes after intraperitoneal injection.

The open field arena measured 40×40×25 cm. At the beginning of a test session, a mouse was placed in the arena, and its movement was recorded continuously for 10 minutes. The following areas were defined for analysis: central area – the floor of the arena was virtually divided into a 4×4 grid, and the central four squares (rows 2–3, columns 2–3) were designated as the central area; corner area – four fan-shaped areas, each with a radius equal to one-quarter of the side length of the arena, were defined at the four corners. Primary outcome measures included total distance travelled (m), duration (s), number of entries, velocity (m s<sup>-1</sup>), immobility time (s), and freezing time (s) in the central or corner areas (15).

*Active avoidance test (AAT).* – Different 24 female and 24 male mice were used in the shuttle box AAT model. The grouping was identical to that in OFT, with males and females independently divided into VEH, VZD0.5, and VZD1.0 groups ( $n = 8$  per group). During the testing period, mice received the corresponding treatment *via* intraperitoneal injection for 3 consecutive days, and behavioural testing began 30 minutes post-injection.

The two-way shuttle box (Shanghai Xinruan, China) measured 48×21×45 cm and was divided into two equal-sized compartments by a vertical partition with a small gate allowing free passage. The floor consisted of stainless-steel grids (spacing: 1.5 cm) capable of delivering a foot shock (unconditioned stimulus: 0.5 mA). A pure tone generator provided the conditioned stimulus (35 dB, 7000 Hz). All stimuli and behavioural recordings were automatically controlled by a computer and VisuTrack software.

The test consisted of two phases: habituation and training. During the habituation phase (5 days prior to training), mice were handled for 5 minutes daily. A mock *i.p.* injection was performed using a 1-mL syringe needle pressed against the abdomen. Mice were then gently placed into either compartment of the shuttle box and allowed to freely explore for 15 minutes without any stimuli (no tone, no shock). The box was thoroughly cleaned with 75 % ethanol after each session. In the training phase, training began 30 minutes after daily drug injections and continued for 3 consecutive days. Each daily session consisted of 30 trials. A single trial procedure was as follows: trial onset was signalled by the conditioned stimulus (tone), which lasted for 7 s. If the mouse shuttled to the opposite compart-

ment within this 7-second period, it was recorded as an “active avoidance,” the tone was terminated immediately, and no shock was delivered. If no shuttle occurred during the delivery of the tone, an unconditioned stimulus (foot shock, maximum 5 s) was delivered in the compartment occupied by the mouse. Shuttling during the shock period was recorded as an escape, and the shock terminated immediately upon crossing. If the mouse failed to shuttle throughout the entire shock duration, the trial was recorded as a no response (16). A fixed inter-trial interval of 20 s, free of any stimuli, separated consecutive trials.

Primary outcome measures included: active avoidance rate = (number of active avoidances/total trials)  $\times$  100 %; active avoidance latency: the time from conditioned stimulus onset to shuttle completion (s); immobility time per minute = (total immobility time/session duration)  $\times$  60 (s); freezing time per minute = (total freezing time/session duration)  $\times$  60 (s).

### *Statistical analysis*

Data analysis and graph plotting were performed using GraphPad Prism 8.0 software (GraphPad Software, Inc., USA). The values are expressed as the mean  $\pm$  standard error of the mean (SEM). For multiple comparisons between groups, the normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variances was assessed using Bartlett’s test or Levene’s test. If data met assumptions of normality and homogeneity of variances, one-way analysis of variance (ANOVA) was used; otherwise, the non-parametric Kruskal-Wallis test was employed. Pearson’s linear correlation analysis was used for correlation analysis.  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### *Open field test*

Compared with the VEH group, the VZD administration group showed no significant difference in total locomotor distance in either female or male mice (Figs. 1a, 2a), indicating that the tested doses did not affect general motor capacity. This is crucial to determine whether an observed effect in subsequent AAT is due to changes in motivation, anxiety or learning, rather than effects of the VZD on locomotor activity. Consistently, study has shown that VZD, at behaviourally active doses, does not alter general locomotor activity, as measured in the open field test in naïve mice (13). This supports the specificity of VZD’s effects on motivational processes rather than on motor function. Regarding anxiety-like behaviour, VZD exerted sex-dependent modulation. The female VZD1.0 group exhibited a trend toward increased duration in the central area compared with the VEH group, although this did not reach statistical significance (Figs. 1b,c). The velocity in the central area was significantly lower in the VZD1.0 group, compared with the VEH group ( $p < 0.01$ ) (Fig. 1e), while VZD did not alter the movement patterns of male mice in the central area (Figs. 2c-g). This suggests that VZD primarily modifies the behavioural strategy of female mice in an open environment, shifting from rapid escape to more cautious exploration. This interpretation is plausible given that VZD is a 5-HT<sub>1A</sub> receptor partial agonist, and

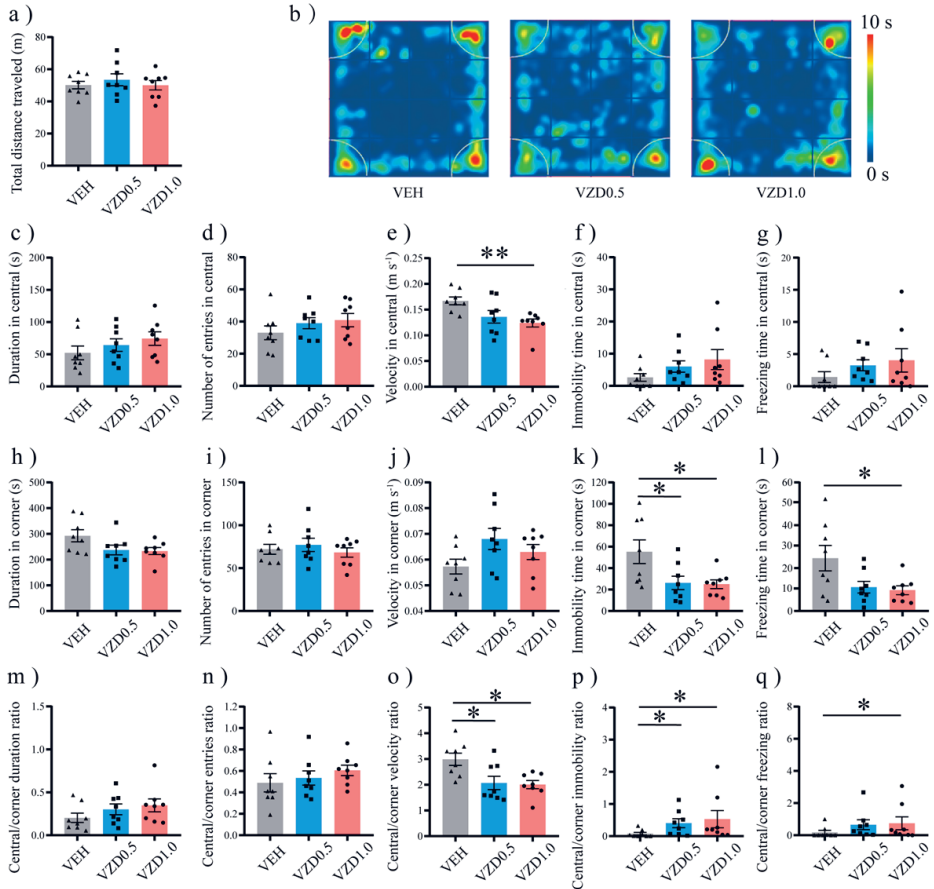


Fig. 1. Results of the OFT in female mice: a) total distance travelled in the OFT; b) heat map illustrating visit duration in different areas of the open field. Behavioural indices in the central area: c) visit duration; d) number of entries; e) velocity; f) immobility time; g) freezing time. Behavioural indices in the corner area: h) visit duration; i) number of entries; j) velocity; k) immobility time; l) freezing time; m–q) ratios of corresponding indices between the central area and the corner area. The values are expressed as the mean  $\pm$  SEM. Statistical significance was determined using the Kruskal-Wallis test for data in panels c, m–q, and by one-way ANOVA for all other indices. Significant differences *vs.* VEH group: \* $p < 0.05$ , \*\* $p < 0.01$ . VEH – vehicle, VZD0.5 – vilazodone 0.5 mg kg<sup>-1</sup>, VZD1.0 – vilazodone 1 mg kg<sup>-1</sup>.

activation of 5-HT<sub>1A</sub> receptors is known to promote exploratory activity (17) and to facilitate adaptive responses to potentially threatening environments (18).

Furthermore, VZD significantly reduced immobility and freezing time of female mice in the corner area (Figs. 1k,l), an effect also observed in male mice (Figs. 2k,l). The corner area is typically regarded as a “safe area” (19, 20); reducing freezing in this area implies that VZD may decrease excessive vigilance in a “safe environment”, thereby allowing mice

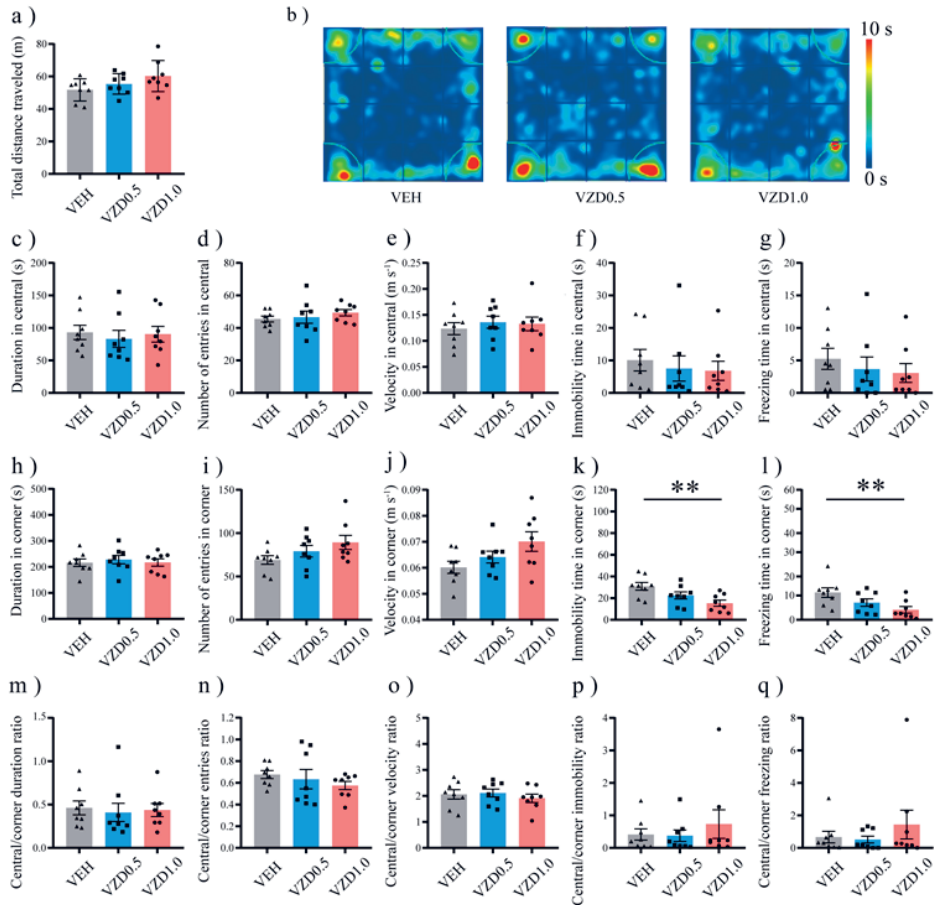


Fig. 2. Results of the OFT in male mice: a) total distance travelled in the OFT; b) heat map illustrating visit duration in different areas of the open field. Behavioural indices in the central area: c) visit duration; d) number of entries; e) velocity; f) immobility time; g) freezing time. Behavioural indices in the corner area: h) visit duration; i) number of entries; j) velocity; k) immobility time; l) freezing time; m–q) ratios of corresponding indices between the central area and the corner area. The values are expressed as the mean  $\pm$  SEM. Statistical significance was determined using the Kruskal-Wallis test for data in panel o, and by one-way ANOVA for all other indices. Significant difference *vs.* VEH group: \*\* $p < 0.01$ . VEH – vehicle, VZD0.5 – vilazodone 0.5 mg kg<sup>-1</sup>, VZD1.0 – vilazodone 1 mg kg<sup>-1</sup>.

to accumulate motivational resources for subsequently venturing into the “dangerous” open area (21). This modulation of threat sensitivity in a safe context aligns with recent evidence that VZD can alleviate anxiety-like behaviours by promoting neuroplasticity, potentially *via* mechanisms such as the Wnt/ $\beta$ -catenin signalling pathway (22). This inhibition of specific types of freezing behaviour also provides a crucial clue for interpreting the performance of mice in the shuttle box. Additionally, the ratio of velocity in the central area to that in the corner area was significantly higher in the VEH group compared with

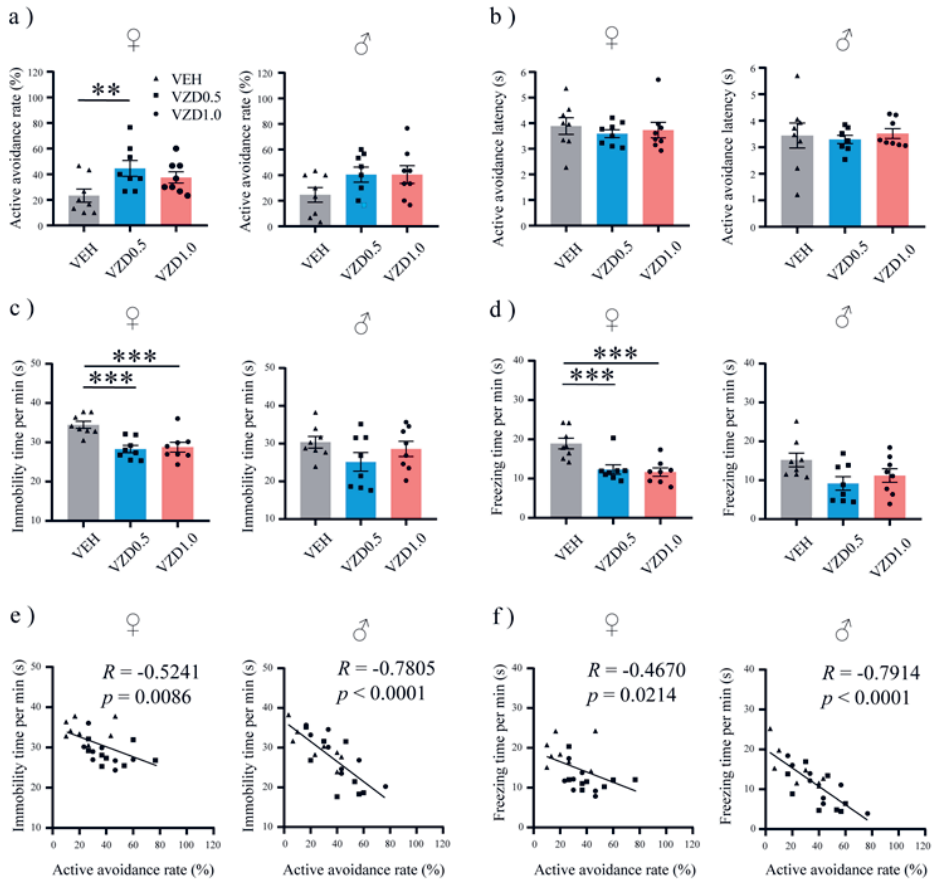


Fig. 3. The performance during AAT in female and male mice on day 1: a) the active avoidance rate; b) active avoidance latency; c) immobility time per minute; d) freezing time per minute. Correlation analysis between the: e) active avoidance rate and immobility time per minute and f) freezing time per minute, for all mice (female and male data presented in the left and right panels). The values are expressed as the mean  $\pm$  SEM. Statistical significance was determined using the one-way ANOVA. Significant difference vs. VEH group: \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .  $R$  – Pearson correlation coefficient, VEH – vehicle, VZD0.5 – vilazodone 0.5 mg kg<sup>-1</sup>, VZD1.0 – vilazodone 1 mg kg<sup>-1</sup>.

both VZD administration groups ( $p < 0.05$ ) (Fig. 1o). This supports the notion that VZD modifies the qualitative pattern of exploration and risk-assessment rather than simply increasing or decreasing general activity or anxiety in a uniform way.

#### Shuttle-box active avoidance test

*Effects of VZD on AAT in female and male mice on day 1.* – On the training day 1 of the AAT, the active avoidance rate was significantly higher in female mice in the VZD0.5 group compared with the VEH group ( $p < 0.01$ ), with an increasing trend in the VZD1.0

group (Fig. 3a, left panel). In contrast, male mice in both the VZD groups showed a non-significant increasing trend in the active avoidance rate (Fig. 3a, right panel). Direct comparison between vehicle-treated female and male mice on day 1 revealed no significant difference in the active avoidance rate. This does not align with some previous reports of superior avoidance acquisition in females under lower stress conditions (16, 23). The comparable, and relatively low, baseline performance in both sexes in our study may be attributable to the relatively high intensity of the foot shock (0.5 mA) used as the unconditioned stimulus, which has been shown to impede the acquisition of active avoidance behaviour in an intensity-dependent manner (16). This sexual dimorphism may be related to fundamental differences in the interaction between the 5-HT and neurohormonal systems in males and females (24, 25). Estrogen is known to modulate the expression and function of 5-HT<sub>1A</sub> receptors (26, 27), which could render females more sensitive to drugs with combined 5-HT<sub>1A</sub> receptor agonist activity (like VZD), leading to responses on training day 1. No significant differences were observed among the groups in terms of active avoidance latency in both genders (Fig. 3b). This result indicates that VZD's facilitatory effect on active avoidance learning in female mice is not attributable to a general acceleration of motor response time. VZD improved the probability of making an avoidance response but did not affect the speed of that response once initiated, implying that the facilitation is more cognitive or motivational in nature.

Compared with the VEH group, both the VZD groups from female mice demonstrated a significant reduction in immobility time per minute and freezing time per minute during the test session ( $p < 0.001$ ) (Figs. 3c, left panel, 3d, left panel). Correlation analysis further revealed significant negative correlation on the training day 1 between the active avoidance rate and both immobility time per minute ( $R = -0.5241$ ,  $p < 0.05$ ) and freezing time per minute ( $R = -0.4670$ ,  $p < 0.05$ ) (Figs. 3e, left panel, 3f, left panel). In male mice, neither the VZD0.5 group nor the VZD1.0 group significantly altered immobility time per minute or freezing time per minute compared with the VEH group (Figs. 3c, right panel, 3d, right panel). However, correlation analysis revealed a significant negative correlation between the active avoidance rate and both immobility time per minute ( $R = -0.7805$ ,  $p < 0.001$ ) and freezing time per minute ( $R = -0.7914$ ,  $p < 0.001$ ) (Figs. 3e, right panel, 3f, right panel). Our data support the hypothesis that "reducing freezing promotes active avoidance learning" (28). Correlation analyses revealed a strong negative correlation between the active avoidance rate and either immobility or freezing time per minute during the test. This indicates that less freezing in response to the conditioned stimulus is associated with a higher probability of executing an active avoidance response (28, 29). From a behavioural perspective, the freezing response is an instinctive reaction to an inescapable threat, but it can severely interfere with the learning of adaptive active avoidance behaviour (8, 28, 30). VZD, *via* its dual-acting mechanism of 5-HT reuptake inhibition and 5-HT<sub>1A</sub> receptor partial agonism, may rapidly modulate neural activity in the prefrontal-amygdala pathway in response to stressful stimuli (10, 31), shifting the behavioural mode from passive "freezing" to active "coping", thereby facilitating active avoidance learning. Collectively, we observed sex differences in VZD's effect on active avoidance behaviour.

*Effects of VZD on AAT in female and male mice on day 2.* – On the training day 2, no significant intergroup differences were found in female mice in the active avoidance rate, latency, immobility time per minute, or freezing time per minute. Correlation analyses also yielded no statistically significant results (Figs. 4a–f, left panel). By contrast, male mice

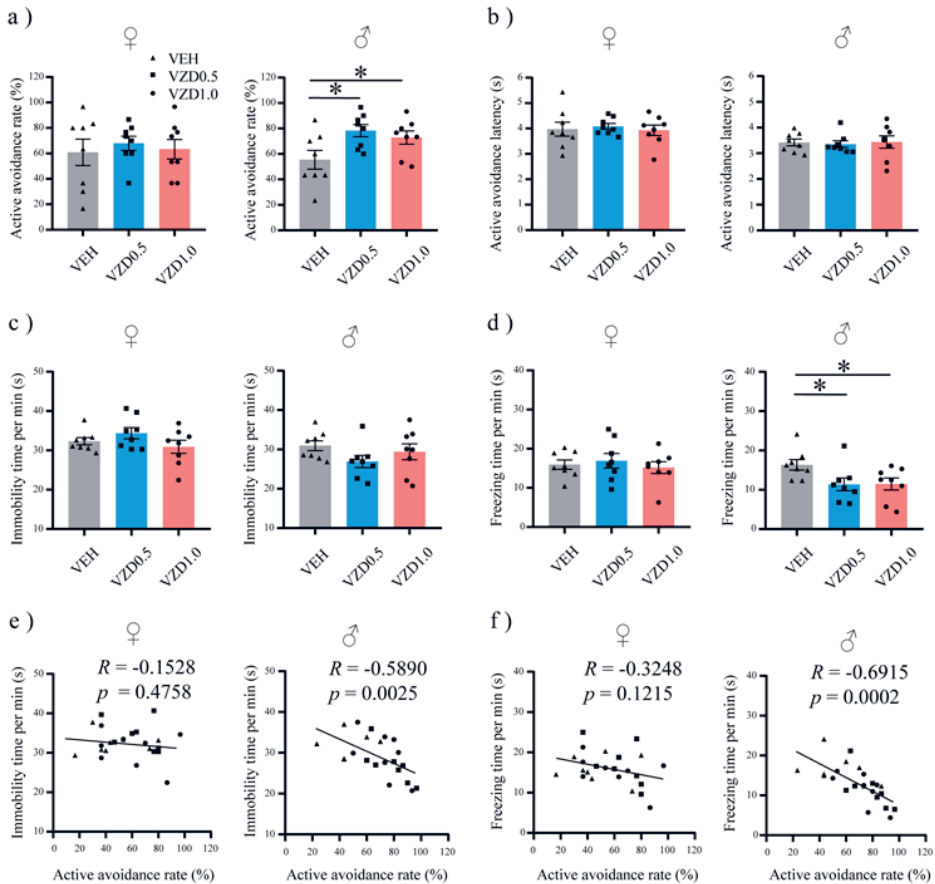


Fig. 4. The performance during AAT in female and male mice on day 2: a) the active avoidance rate; b) active avoidance latency; c) immobility time per minute; d) freezing time per minute. Correlation analysis between the: e) active avoidance rate and immobility time per minute and f) freezing time per minute, for all mice (female and male data presented in the left and right panels). The values are expressed as the mean  $\pm$  SEM. Statistical significance was determined using the one-way ANOVA. Significant difference *vs.* VEH group: \* $p < 0.05$ .  $R$  – Pearson correlation coefficient, VEH – vehicle, VZD0.5 – vilazodone 0.5 mg  $\text{kg}^{-1}$ , VZD1.0 – vilazodone 1 mg  $\text{kg}^{-1}$ .

from the VZD0.5 group and VZD1.0 group exhibited a significantly higher active avoidance rate ( $p < 0.05$ ) (Fig. 4a, right panel) and a significantly reduced freezing time per minute ( $p < 0.05$ ) (Fig. 4d, right panel) compared with the VEH group. This pattern of results demonstrates that VZD's facilitation of active avoidance is not a global effect but is gated by sex. The active avoidance rate showed significant negative correlations with both immobility time per minute ( $R = -0.5890, p < 0.01$ ) (Fig. 4e, right panel) and freezing time per minute ( $R = -0.6915, p < 0.001$ ) (Fig. 4f, right panel). It suggests that VZD's efficacy is tied to its capacity to disinhibit behaviour by reducing freezing and this effect is prominently observed in male mice during their peak learning phase.

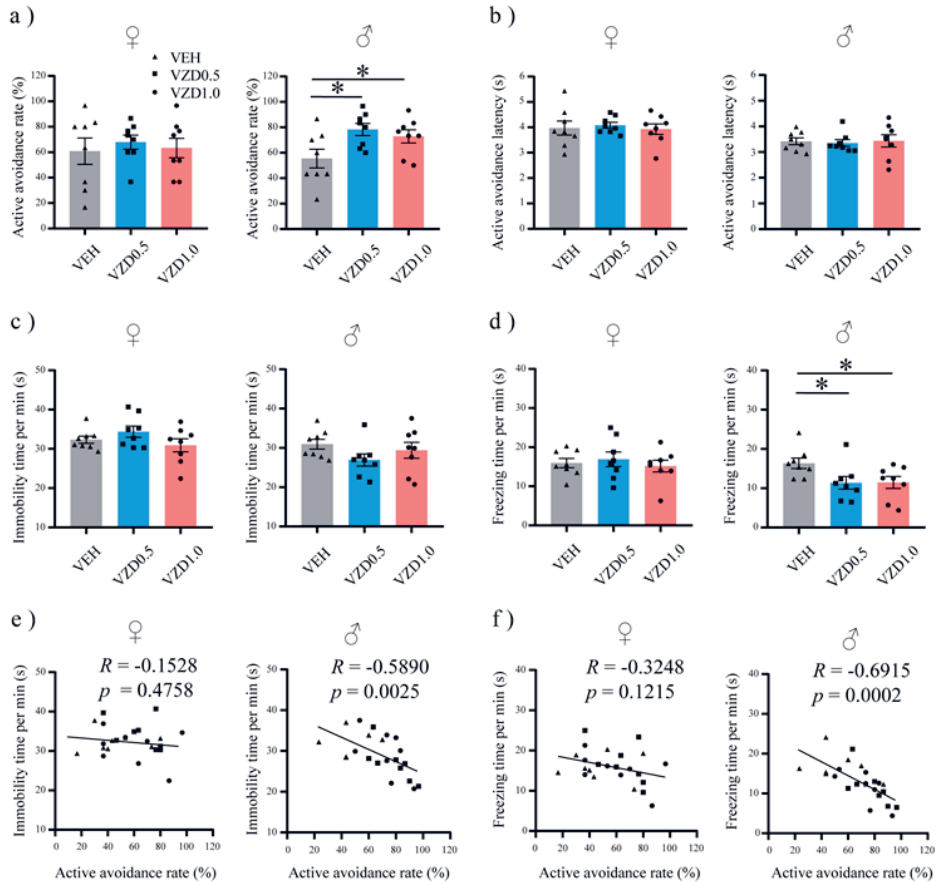


Fig. 5. The performance during AAT in female and male mice on day 3: a) the active avoidance rate; b) active avoidance latency; c) immobility time per minute; d) freezing time per minute. Correlation analysis between the: e) active avoidance rate and immobility time per minute and f) freezing time per minute, for all mice (female and male data presented in the left and right panels). The values are expressed as the mean  $\pm$  SEM. Statistical significance was determined using the Kruskal-Wallis test for data in a, left panel, and by one-way ANOVA for all other indices.  $R$  – Pearson correlation coefficient, VEH – vehicle, VZD0.5 – vilazodone 0.5 mg kg<sup>-1</sup>, VZD1.0 – vilazodone 1 mg kg<sup>-1</sup>.

*Effects of VZD on AAT in female and male mice on day 3.* – During the training day 3, no significant differences were found among all groups in the active avoidance rate, latency, immobility time per minute, or freezing time per minute. Correlation analyses for these days also yielded no statistically significant results (Figs. 5a–f). The pro-learning effect of VZD was most pronounced during the early stages of training and diminished by the training day 3 as the learning curves of the control groups increased. This phenomenon suggests that VZD administered acutely in healthy animals may primarily accelerate early-phase learning rather than enhancing the ultimate performance level. Our finding that acute VZD administration enhances the early-phase acquisition of active avoidance behaviour is consistent

with clinical observations of accelerated behavioural adaptation following the initiation of antidepressant treatment (32, 33). The facilitation of active avoidance acquisition demonstrated by VZD here provides preclinical behavioural evidence for its efficacy in improving motivational deficits and behavioural retardation in depressed patients.

Furthermore, our findings regarding VZD's influence on early-phase avoidance learning align with and extend existing preclinical evidence on its behavioural and potential anxiolytic effects. Adamec *et al.* (34) demonstrated that prophylactic administration of VZD could block stress-potentiated startle response in a predator stress model at higher doses (20–40 mg kg<sup>-1</sup>), suggesting a potential role in mitigating hypervigilance following severe stress, albeit without affecting anxiety-like behaviour in the elevated plus maze. This indicates that VZD's effects may be behaviour- and paradigm-specific, consistent with our observation of enhanced acquisition in an active avoidance task.

### *Limitations of the study and future prospects*

Our findings suggest that VZD promotes avoidance learning not merely by reducing anxiety, but by enabling a shift in behavioural strategy, from passive freezing to active coping. There are certain limitations in our study. First, we only observed the acute effects of VZD administration; the consequences of long-term administration remain unclear. Second, the study focused on behavioural changes and lacked an in-depth investigation into the specific mechanisms underlying VZD's pharmacological actions. Finally, this study tested VZD's effects only in naïve C57BL/6J mice; future research could validate these findings in classic depression/anxiety models, such as the chronic unpredictable mild stress or social defeat stress paradigms. Concurrently, employing techniques like fibre photometry or microdialysis to monitor real-time dynamic changes in neurotransmitters such as serotonin and dopamine in brain regions like the amygdala and prefrontal cortex during behavioural testing would allow direct verification of our hypotheses at the circuit and molecular levels.

## CONCLUSIONS

In summary, this study reveals that VZD facilitates the acquisition of active avoidance behaviour in naïve C57BL/6J mice, exhibiting distinct sexual dimorphism and dynamic temporal patterns. Crucially, we found that its pro-learning effect is highly correlated with the reduction of stress-induced freezing behaviour. This supports our core hypothesis that VZD effectively reduces anxiety and behavioural suppression triggered by aversive stimuli through its dual-acting mechanism, thereby biasing behavioural choice towards active coping strategies, ultimately manifesting as enhanced learning efficiency. This research opens new avenues for exploring VZD's role in avoidance-based learning tasks.

*Ethical approval.* – Laboratory Animal Ethics Committee of Xuzhou Medical University (Xuzhou, Jiangsu, China), approval number: 202312T007.

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*Conflicts of interest.* – The authors declare no conflict of interest.

*Author's contribution.* – Conceptualisation, K.H., A.Z., and J.M.; investigation, K.H., A.Z., and H.Y.; analysis, K.H. and H.Y.; writing, original draft preparation and supervision, K.H., A.Z., Y.S., X.K., and J.M. All the authors revised the final manuscript and approved the final version.

## REFERENCES

1. J. Moncrieff, R. E. Cooper, T. Stockmann, S. Amendola, M. P. Hengartner and M. A. Horowitz, The serotonin theory of depression: A systematic umbrella review of the evidence, *Mol. Psychiatry* **28**(8) (2023) 3243–3256; <https://doi.org/10.1038/s41380-022-01661-0>
2. G. Ślifirski, M. Król and J. Turlo, 5-HT receptors and the development of new antidepressants, *Int. J. Mol. Sci.* **22**(16) (2021) Article ID 9015 (31 pages); <https://doi.org/10.3390/ijms22169015>
3. C. Wang, N. Zhang, Y. Shao, T. Li, M. Zhang, M. Gao, Y. Liang, Y. Wang, T. Xue, Y. Shi, H. Chen and C. Cao, Pathway-selective 5-HT<sub>1A</sub>R agonist as a rapid antidepressant strategy, *Cell* **188**(25) (2025) 7222–7237; <https://doi.org/10.1016/j.cell.2025.10.022>
4. Y. Jiang, Y. Qu, Z. Du, M. Ou, Y. Shen, Q. Zhou, L. Tian and H. Zhu, Exploring adverse events of vilazodone: Evidence from the FAERS database, *BMC Psychiatry* **24**(1) (2024) Article ID 371 (9 pages); <https://doi.org/10.1186/s12888-024-05813-0>
5. N. Zareifopoulos and I. Dylja, Efficacy and tolerability of vilazodone for the acute treatment of generalized anxiety disorder: A meta-analysis, *Asian J. Psychiatr.* **26** (2017) 115–122; <https://doi.org/10.1016/j.ajp.2017.01.016>
6. K. L. Szuhany and N. M. Simon, Anxiety disorders: A review, *JAMA* **328**(24) (2022) 2431–2445; <https://doi.org/10.1001/jama.2022.22744>
7. B. W. Penninx, D. S. Pine, E. A. Holmes and A. Reif, Anxiety disorders, *Lancet* **397**(10277) (2021) 914–927; [https://doi.org/10.1016/s0140-6736\(21\)00359-7](https://doi.org/10.1016/s0140-6736(21)00359-7)
8. A. Fernández-Teruel and A. Tobeña, Revisiting the role of anxiety in the initial acquisition of two-way active avoidance: Pharmacological, behavioural and neuroanatomical convergence, *Neurosci. Biobehav. Rev.* **118** (2020) 739–758; <https://doi.org/10.1016/j.neubiorev.2020.08.014>
9. C. B. Klune, C. M. Goodpaster, M. W. Gongwer, C. J. Gabriel, J. An, R. Chen, N. S. Jones, O. H. Williams, M. Shari, M. Ramirez, A. Hacking, T. Andrade, L. A. Schwarz and L. A. DeNardo, Developmentally distinct architectures in top-down pathways controlling threat avoidance, *Nat. Neurosci.* **28**(4) (2025) 823–835; <https://doi.org/10.1038/s41593-025-01890-w>
10. H. Esaki, Y. Sasaki, N. Nishitani, H. Kamada, S. Mukai, Y. Ohshima, S. Nakada, X. Ni, S. Deyama and K. Kaneda, Role of 5-HT<sub>1A</sub> receptors in the basolateral amygdala on 3,4-methylenedioxyamphetamine-induced prosocial effects in mice, *Eur. J. Pharmacol.* **946** (2023) Article ID 175653 (7 pages); <https://doi.org/10.1016/j.ejphar.2023.175653>
11. Y.-F. Jiang, J. Liu, J. Yang, Y. Guo, W. Hu, J. Zhang, X.-M. La, W. Xie, H.-S. Wang and L. Zhang, Involvement of the dorsal hippocampus 5-HT<sub>1A</sub> receptors in the regulation of depressive-like behaviors in hemiparkinsonian rats, *Neuropsychobiology* **79**(3) (2020) 198–207; <https://doi.org/10.1159/000505212>
12. L. Wang, Z. Zhu, W. Hou, X. Zhang, Z. He, W. Yuan, Y. Yang, S. Zhang, R. Jia and F. Tai, Serotonin signaling through prelimbic 5-HT<sub>1A</sub> receptors modulates csds-induced behavioral changes in adult female voles, *Int. J. Neuropsychopharmacol.* **22**(3) (2019) 208–220; <https://doi.org/10.1093/ijnp/pyy093>
13. C. M. de Arruda, D. L. Doneda, V. V. de Oliveira, R. A. L. da Silva, Y. A. V. de Matos, I. L. Fernandes, C. A. H. Rohden, G. G. Viola, F. Rios-Santos, E. de Lima, Z. da Silva Buss and S. Vandresen-Filho, Involvement of kynurenine pathway and N-methyl-D-aspartate receptors in the antidepressant-

- like effect of vilazodone in the tail suspension test in mice, *Pharmacol. Biochem. Behav.* **218** (2022) Article ID 173433 (7 pages); <https://doi.org/10.1016/j.pbb.2022.173433>
14. E. B. Fraga-Junior, I. L. Fernandes, C. A. H. Rohden, D. L. Doneda, H. N. Ynoue, F. Rios-Santos, E. Lima, Z. S. Buss and S. Vandresen-Filho, Attenuation of the levels of pro-inflammatory cytokines prevents depressive-like behavior during ethanol withdrawal in mice, *Brain. Res. Bull.* **191** (2022) 9–19; <https://doi.org/10.1016/j.brainresbull.2022.10.014>
  15. M. L. Seibenhener and M. C. Wooten, Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice, *J. Vis. Exp.* (96) (2015) e52434 (9 pages); <https://doi.org/10.3791/52434>
  16. T. Macheda, H. C. Snider, J. B. Watson, K. N. Roberts and A. D. Bachstetter, An active avoidance behavioral paradigm for use in a mild closed head model of traumatic brain injury in mice, *J. Neurosci. Methods* **343** (2020) Article ID 108831 (9 pages); <https://doi.org/10.1016/j.jneumeth.2020.108831>
  17. M. Sakaue, Y. Ago, C. Sowa, Y. Koyama, A. Baba and T. Matsuda, The 5-HT<sub>1A</sub> receptor agonist MKC-242 increases the exploratory activity of mice in the elevated plus-maze, *Eur. J. Pharmacol.* **458**(1–2) (2003) 141–144; [https://doi.org/10.1016/s0014-2999\(02\)02786-3](https://doi.org/10.1016/s0014-2999(02)02786-3)
  18. K. C. Klemenhagen, J. A. Gordon, D. J. David, R. Hen and C. T. Gross, Increased fear response to contextual cues in mice lacking the 5-HT<sub>1A</sub> receptor, *Neuropsychopharmacology* **31**(1) (2006) 101–111; <https://doi.org/10.1038/sj.npp.1300774>
  19. P. Simon, R. Dupuis and J. Costentin, Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions, *Behav. Brain. Res.* **61**(1) (1994) 59–64; [https://doi.org/10.1016/0166-4328\(94\)90008-6](https://doi.org/10.1016/0166-4328(94)90008-6)
  20. L. Prut and C. Belzung, The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review, *Eur. J. Pharmacol.* **463**(1–3) (2003) 3–33; [https://doi.org/10.1016/s0014-2999\(03\)01272-x](https://doi.org/10.1016/s0014-2999(03)01272-x)
  21. M. L. Kisipan, R. O. Ojoo, T. I. Kanui and K. S. P. Abelson, Bodyweight, locomotion, and behavioral responses of the naked mole rat (*Heterocephalus glaber*) to lipopolysaccharide administration, *J. Comp. Physiol. A* **208**(4) (2022) 493–504; <https://doi.org/10.1007/s00359-022-01557-y>
  22. R. A. El-Kadi, N. F. Abdelkader, H. F. Zaki and A. S. Kamel, Vilazodone alleviates neurogenesis-induced anxiety in the chronic unpredictable mild stress female rat model: Role of Wnt/ $\beta$ -catenin signaling, *Mol. Neurobiol.* **61**(11) (2024) 9060–9077; <https://doi.org/10.1007/s12035-024-04142-3>
  23. A. Spröwitz, J. Bock and K. Braun, Sex-specific positive and negative consequences of avoidance training during childhood on adult active avoidance learning in mice, *Front. Behav. Neurosci.* **7** (2013) Article ID 143 (11 pages); <https://doi.org/10.3389/fnbeh.2013.00143>
  24. R. Ravenelle, J. Lee, C. Fernandes-Henriques, J. Liu, A. K. Friedman, E. Likhtik and N. S. Burghardt, Serotonergic modulation of the BNST-CeA pathway reveals sex differences in fear learning, *Nat. Neurosci.* **28**(9) (2025) 1897–1909; <https://doi.org/10.1038/s41593-025-02025-x>
  25. T. J. Philippe, L. Bao, M. E. Koblanski and V. Viau, Sex differences in serotonin 5-HT<sub>1A</sub> receptor responses to repeated restraint stress in adult male and female rats, *Int. J. Neuropsychopharmacol.* **25**(10) (2022) 863–876; <https://doi.org/10.1093/ijnp/pyac046>
  26. E. G. Jacobs, L. M. Holsen, K. Lancaster, N. Makris, S. Whitfield-Gabrieli, A. Remington, B. Weiss, S. Buka, A. Klibanski and J. M. Goldstein, 17 $\beta$ -estradiol differentially regulates stress circuitry activity in healthy and depressed women, *Neuropsychopharmacology* **40**(3) (2015) 566–576; <https://doi.org/10.1038/npp.2014.203>
  27. S. Gupta, M. Wang, Y. Azuma and N. A. Muma, Regulation of serotonin 1A receptor SUMOylation by SENP2 and PIASx $\alpha$ , *Int. J. Mol. Sci.* **22**(24) (2021) Article ID 13176 (14 pages); <https://doi.org/10.3390/ijms222413176>

28. J. Wendt, A. Löw, M. Weymar, M. Lotze and A. O. Hamm, Active avoidance and attentive freezing in the face of approaching threat, *Neuroimage* **158** (2017) 196–204; <https://doi.org/10.1016/j.neuroimage.2017.06.054>
29. F. H. Klaassen, L. Held, B. Figner, J. X. O'Reilly, F. Klumpers, L. D. de Voogd and K. Roelofs, Defensive freezing and its relation to approach-avoidance decision-making under threat, *Sci. Rep.* **11**(1) (2021) Article ID 12030 (12 pages); <https://doi.org/10.1038/s41598-021-90968-z>
30. E. T. Rolls, Emotion, motivation, decision-making, the orbitofrontal cortex, anterior cingulate cortex, and the amygdala, *Brain Struct. Funct.* **228**(5) (2023) 1201–1257; <https://doi.org/10.1007/s00429-023-02644-9>
31. Y.-H. Chen, J.-L. Wu, N.-Y. Hu, J.-P. Zhuang, W.-P. Li, S.-R. Zhang, X.-W. Li, J.-M. Yang and T.-M. Gao, Distinct projections from the infralimbic cortex exert opposing effects in modulating anxiety and fear, *J. Clin. Invest.* **131**(14) (2021) e145692 (17 pages); <https://doi.org/10.1172/jci145692>
32. V. Orgeta, P. Leung, R. Del-Pino-Casado, A. Qazi, M. Orrell, A. E. Spector and A. M. Methley, Psychological treatments for depression and anxiety in dementia and mild cognitive impairment, *Cochrane Database Syst. Rev.* **4**(4) (2022) Cd009125 (156 pages); <https://doi.org/10.1002/14651858.CD009125.pub3>
33. J. R. Strawn, J. A. Mills, V. Suresh, T. S. Peris, J. T. Walkup and P. E. Croarkin, Combining selective serotonin reuptake inhibitors and cognitive behavioral therapy in youth with depression and anxiety, *J. Affect. Disord.* **298**(Part A) (2022) 292–300; <https://doi.org/10.1016/j.jad.2021.10.047>
34. R. Adamec, G. D. Bartoszyk and P. Burton, Effects of systemic injections of vilazodone, a selective serotonin reuptake inhibitor and serotonin 1<sub>A</sub> receptor agonist, on anxiety induced by predator stress in rats, *Eur. J. Pharmacol.* **504**(1–2) (2004) 65–77; <https://doi.org/10.1016/j.ejphar.2004.09.009>

## Supplementary material

Fig. 5. The performance during AAT in female and male mice on Day 3

a) Active avoidance rate (%)									
	♀				♂				
	VEH	VZD0.5	VZD1.0		VEH	VZD0.5	VZD1.0		
1	56.67	70.00	70.00	1	80.00	93.33	80.00		
2	80.00	66.67	90.00	2	60.00	76.67	70.00		
3	70.00	23.33	73.33	3	83.33	56.67	83.33		
4	100.00	40.00	83.33	4	86.67	66.67	83.33		
5	33.33	23.33	90.00	5	70.00	96.67	100.00		
6	66.67	100.00	86.67	6	33.33	30.00	83.33		
7	36.67	50.00	86.67	7	63.33	36.67	56.67		
8	86.67	96.67	83.33	8	43.33	90.00	80.00		
Kruskal-Wallis test: P = 0.1362				$F_{2,23} = 1.195, P = 0.3225$					

b) Active avoidance latency (s)									
	♀				♂				
	VEH	VZD0.5	VZD1.0		VEH	VZD0.5	VZD1.0		
1	4.34	4.71	3.59	1	3.45	2.63	4.07		
2	3.31	3.77	2.88	2	4.06	3.52	4.17		
3	4.43	3.75	4.35	3	2.92	3.06	4.25		
4	2.57	4.13	3.71	4	3.19	3.40	3.66		
5	4.96	4.35	3.17	5	3.51	2.65	2.46		
6	3.45	2.05	3.34	6	3.68	4.08	3.65		
7	4.15	3.50	3.72	7	4.36	3.16	3.90		
8	3.28	3.36	4.24	8	2.85	3.05	2.26		
$F_{2,23} = 0.1390, P = 0.8710$				$F_{2,23} = 0.8267, P = 0.4512$					

c) Immobility time /min (s)									
	♀				♂				
	VEH	VZD0.5	VZD1.0		VEH	VZD0.5	VZD1.0		
1	29.75	26.05	25.39	1	24.66	21.32	25.83		
2	23.32	26.39	33.95	2	27.34	29.49	29.05		
3	31.53	29.17	18.75	3	27.15	24.93	34.77		
4	27.78	28.70	26.83	4	28.44	34.66	30.83		
5	39.73	35.74	32.58	5	23.69	26.44	20.24		
6	28.85	28.30	29.82	6	28.66	23.67	25.40		
7	30.87	31.27	32.22	7	29.64	27.70	32.15		
8	30.54	36.16	46.85	8	29.73	23.00	23.53		
$F_{2,23} = 0.02315, P = 0.9771$				$F_{2,23} = 0.2486, P = 0.7821$					

d) Freezing time /min (s)									
	♀				♂				
	VEH	VZD0.5	VZD1.0		VEH	VZD0.5	VZD1.0		
1	15.18	10.24	7.43	1	10.09	7.14	8.15		
2	9.27	10.16	16.19	2	11.49	10.34	11.18		
3	17.23	14.40	6.27	3	12.16	11.09	18.13		
4	12.69	12.27	12.77	4	11.37	19.47	10.88		
5	22.93	19.23	12.46	5	10.44	11.40	5.24		
6	13.23	10.55	12.52	6	11.45	10.12	12.03		
7	14.31	15.71	18.51	7	16.46	10.96	14.32		
8	12.97	20.02	29.67	8	13.98	6.82	5.84		
$F_{2,23} = 0.03047, P = 0.9700$				$F_{2,23} = 0.3940, P = 0.6793$					

e) Immobility time /min (s)									
	♀				♂				
	VEH	VZD0.5	VZD1.0		VEH	VZD0.5	VZD1.0		
Active avoidance rate (s)	56.67	70.00	70.00	Active avoidance rate (s)	80.00	93.33	80.00		
	80.00	66.67	90.00		60.00	76.67	70.00		
	70.00	23.33	73.33		83.33	56.67	83.33		
	100.00	40.00	83.33		86.67	66.67	83.33		
	33.33	23.33	90.00		70.00	96.67	100.00		
	66.67	100.00	86.67		33.33	30.00	83.33		
	36.67	50.00	86.67		63.33	36.67	56.67		
	86.67	96.67	83.33		43.33	90.00	80.00		
	70.00	26.05	25.39		24.66	21.32	25.83		
	66.67	26.39	33.95		27.34	29.49	29.05		
	23.33	29.17	18.75		27.15	24.93	34.77		
	40.00	28.70	26.83		28.44	34.66	30.83		
	23.33	35.74	32.58		23.69	26.44	20.24		
	90.00	28.30	29.82		28.66	23.67	25.40		
	86.67	31.27	32.22		29.64	27.70	32.15		
	86.67	36.16	46.85		29.73	23.00	23.53		
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	90.00								
	86.67								
	86.67								
	83.33								

f) Freezing time /min (s)									
	♀				♂				
	VEH	VZD0.5	VZD1.0		VEH	VZD0.5	VZD1.0		
Active avoidance rate (s)	56.67	70.00	70.00	Active avoidance rate (s)	80.00	93.33	80.00		
	80.00	66.67	90.00		60.00	76.67	70.00		
	70.00	23.33	73.33		83.33	56.67	83.33		
	100.00	40.00	83.33		86.67	66.67	83.33		
	33.33	23.33	90.00		70.00	96.67	100.00		
	66.67	100.00	86.67		33.33	30.00	83.33		
	36.67	50.00	86.67		63.33	36.67	56.67		
	86.67	96.67	83.33		43.33	90.00	80.00		
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