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Effect of block of α_1-adrenoceptors on overall motor activity but not on spatial cognition in the object-position recognition task

Short title: Prazosin reduced motor activity without affecting cognition

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Summary

Prazosin, an α_1-adrenoceptor antagonist, is well known for its depressant effect on motivation and motor activity, while it has no effect on retention of spatial behavior in several tasks, e.g. in the Morris water maze and radial arm maze. The role of α_1-adrenoceptors in operant tasks with stimulus-controlled behavior has not yet been tested. The present study investigated the effect of prazosin on the modulation of overall motor activity and on cognitive performance in a spatial operant task called object-position recognition task, where operant behavior (lever pressing) was controlled by spatial stimuli displayed on a computer screen. This task has been previously showed to be hippocampal-dependent. Pre-test injection of prazosin at the dose of 3 mg/kg decreased the responding rate, while it did not affect the recognition of object's position. In conclusion, we validated the new cognitive test with a drug with known pharmacological effects on behavior and confirmed the depressant effect of prazosin on motor activity and no effect on retrieval of spatial memory in the hippocampal-dependent operant task.

Keywords: prazosin; spatial cognition; operant behavior; motor activity; motivation

Introduction

Noradrenaline is an abundant neurotransmitter in the central nervous system. Fibers releasing this neurotransmitter originate almost exclusively in the brainstem nuclei, particularly in *locus coeruleus* (Berridge and Waterhouse 2003). Noradrenergic neurons project to many brain areas ranging from the spinal cord and cerebellum to the forebrain, including neocortex, hippocampus, amygdala and thalamus (Sirviö and MacDonald 1999). Noradrenaline acts also as a paracrine neuromodulator and therefore can affect other neuronal and non-neuronal cells via non-synaptic mechanisms (O'Donnel *et al.* 2012). There are three main subclasses of adrenoceptors: α_1-, α_2- and β-receptors. The present study focused on α_1-adrenoceptor antagonist prazosin. Alpha 1-adrenoceptors are members of G-protein coupled receptors superfamily and signal through the $G_{q/11}$ signaling pathway (Chen and Minneman 2005). At present, there are three recognized α_1- subtypes (α_{1A}-, α_{1B}- and α_{1D}-) and all these subtypes are expressed mainly postsynaptically. Prazosin crosses the blood-brain barrier and binds to all α_1-adrenoceptors subtypes. Prazosin binding sites are dense in the cerebral cortex, but very low or absent in the striatum (Trovero *et al.* 1992). Alpha 1-adrenoceptors are present also in the pyramidal neurons of the CA1-CA3 fields of the hippocampus, hilar and granular neurons of the dentate gyrus and in the thalamus (Pieribone *et al.* 1994).

Noradrenaline plays a crucial role in arousal, vigilance and attention (Sirviö and MacDonald 1999). The noradrenergic system affects learning and memory in some experimental configurations, however other neurotransmitter systems (glutamatergic, dopaminergic, GABAergic, cholinergic, serotonergic) appear to play more important role in these processes (for review see Myhrer 2003). It is well known that the activation of central α_1-adrenoceptors may facilitate motor activity. Administration of α_1-adrenoceptors agonists (e.g., methoxamine, phenylephrine) enhances locomotor activity in an open-field test and this

effect can be blocked by the administration of prazosin (Heal 1984). The activation of α_1-adrenoceptors promotes vigilance and exerts influences upon working memory, while has only a minor effect in the modulation of long-term memory (Sirviö and MacDonald 1999). Inhibition of α_1-adrenoceptors by prazosin did not affect spatial performance in the radial arm maze (Liao *et al.* 2002) nor in the Morris water maze (Riekkinen *et al.* 1996). However, the effect of prazosin on retention of the stimulus-controlled behavior in operant tasks is unclear.

The present object-position recognition task is an operant task, which was designed to study spatially-driven behavior in non-locomoting rats (Nekovarova and Klement 2006, Klement *et al.* 2010). The rats have to recognize position of the object located in an inaccessible part of the environment in this task. Our recent study showed that rats use hippocampus in the task (Levcik *et al.* 2013) similarly as in the tasks with approachable objects that can be explored by the animals (Mumby *et al.* 2002, Gilbert and Kesner 2004). Therefore, this is the first study, in which the effect of prazosin on spatial cognition has been studied in a hippocampal-dependent operant task.

The aim of the present study is to test the cognitive performance in the previously designed object-position recognition task with a treatment with known pharmacological actions on behavior. The main question was whether α_1-adrenoceptor antagonist prazosin will exert an effect on the measures of cognition due to the expected effect on the motor responding.

Methods

Subjects and apparatus

The subjects (n = 16) were male Long-Evans rats (3-months old at the beginning of the experiment). The rats were obtained from the breeding colony of the Institute of

Physiology, Academy of Sciences of the Czech Republic, and housed in groups of two or three per cage in a temperature-controlled room (21 ºC) with a regular 12/12 light/dark cycle. Water was freely available but access to food was restricted to maintain the rats at 90% of their free feeding weight (380 – 450 g). All procedures were in accordance with Animal Protection Code of Czech Republic, EU directive 86/609/EEC and National Institute of Health guidelines. The rats were trained in the same apparatus as in our previous study (Levcik *et al.* 2013)(Fig. 1A).

Pretraining

The time scheme of the experiment is shown in Fig. 1C. Food-deprived rats were trained to press the lever in the operant chamber for food reward under the continuous reinforcement schedule. The food reward was one to three 20 mg pasta pellets. The rats required from three to nine sessions lasting approximately 30 min to learn the operant behavior. During the training a white rectangle (width × height: 40 × 300 pxl) was displayed at position 380 pxl (Fig. 1B). We refer to this 2-dimensional rectangle as to an object. Each rat was randomly assigned to one of the two apparatuses and it was trained there only.

Object-position recognition task

The white rectangle was displayed on the screen during the whole session. The rats were conditioned to press the lever for food reward when the rectangle was displayed in the reward position and not to press when it was displayed in the two non-reward positions (Fig. 1B). The rectangle was displayed in the reward position at the beginning of the training session. It changed its position every 35 s. The order of presented positions was pseudorandom. The rats were trained for 34 sessions. The duration of the presentation of the rectangle in one position changed during the training but it was fixed to the 35 s mentioned above in the last 16 training sessions. Each apparatus had its own pseudorandom sequence as

in our previous study (Levcik *et al.* 2013). The sequence was repeated three times during the training sessions, thus, the sessions lasted 31.5 min. Initially, the rats were rewarded for each correct response. Later, when they preferentially responded to the reward stimulus, the continuous reinforcement schedule was replaced by the variable ratio schedule with geometric distribution of the number of presses necessary for getting the reward. The average number of responses necessary for activating the feeder was gradually increased to four. The first response after the change of the stimulus was never rewarded.

After 34 standard sessions, when all the rats had reached an asymptotic performance, they were assigned to the 2 mg/kg and 3 mg/kg groups to match their cognitive performance. Thereafter, the rats received a habituation intraperitoneal injection of 2 mg/kg (n = 8) or 3 mg/kg of prazosin (n = 8) and were left in their homecages until the next day. Then the rats underwent the control session (saline application) after two standard sessions following the habituation infusion of prazosin and the test session (2 mg/kg or 3 mg/kg prazosin application) the next day.

Drug application

Prazosin (Sigma-Aldrich, Czech Republic) was dissolved in distilled water at a concentration of 0.5 mg/ml and injected intraperitoneally 20 min prior to behavioral testing at the dose of 2 mg/kg or 3 mg/kg in the test session. The same volume of saline (0.9% solution of NaCl) was injected in the same way in the control session. The doses of prazosin were chosen on the basis of previous experiments in our laboratory, which were done in the active place avoidance task (Stuchlik and Vales 2008).

Data analysis

We analyzed the overall responding rate (number of presses per second; expressed in Hz) and the cognitive efficiency (ratio of reward and non-reward presses) of rats. The

responding rate was analyzed during the whole session. However, the data analysis of the cognitive efficiency was restricted to those periods of stimuli presentation which were preceded by the non-reward periods and only to the first 15 seconds of these periods. This restriction was introduced in order to decrease the effect of the reaction of the feeder on behavior. For example, an animal may keep responding not because it sees the reward stimulus on the screen but because its immediately preceding responses were reinforced (for detailed information see Levcik *et al.* 2013). One rat was excluded from the analysis of the cognitive efficiency because it pressed the lever only once in the test session (2 mg/kg of prazosin) and this response was not made in the first 15 seconds of the stimulus presentation. The results are reported as means ± S.E.M. Statistical tests were done with R software. Group means were compared by the Wilcoxon signed rank tests. The level of significance was set to 0.05. Holm–Bonferroni correction was used to keep the level of significance of the multiple comparisons 0.05 (Holm 1979).

Results

The assignment of the rats to the 2 mg/kg and 3 mg/kg groups was done to match their cognitive efficiency in the last standard session before the habituation infusion (2 mg/kg group: 0.83 ± 0.05; 3 mg/kg group: 0.79 ± 0.04; *Wilcoxon rank sum test*: $W = 36.5$, $P = 0.6742$). The overall responding rate tent to be lower in the 2 mg/kg group, although the difference was not significant (2 mg/kg group: 0.11 ± 0.04 Hz; 3 mg/kg group: 0.14 ± 0.02; *Wilcoxon rank sum test*: $W = 15$, $P = 0.083$).

The analysis of the overall responding rate showed no effect of the dose of 2 mg/kg of prazosin on motor activity. The overall responding rate of rats was 0.10 ± 0.03 Hz in the control session and 0.07 ± 0.03 Hz after the application of 2 mg/kg of prazosin (*Wilcoxon signed rank* test: $V = 6$, *P-adjusted* = 0.1094). The dose of 3 mg/kg decreased the responding

rate to 55 ± 5 % of control. The overall responding rate of rats was 0.14 ± 0.02 Hz in the control session and 0.08 ± 0.01 Hz after the application of 3 mg/kg of prazosin (*Wilcoxon signed rank* test: $V = 6$, *P-adjusted* = 0.0156). The reduction of the lever-pressing activity was observed in all rats in the test session with the dose of 3 mg/kg.

The dose of 2 mg/kg had no effect on cognitive performance in the object-position recognition task. The ratio of reward and non-reward presses was 0.73 ± 0.01 in the control session and 0.77 ± 0.01 after the application of 2 mg/kg of prazosin (*Wilcoxon signed rank* test: $V = 17$, *P-adjusted* = 0.6875). Injection of the dose of 3 mg/kg also did not alter the cognitive efficiency. The ratio of reward and non-reward presses was 0.81 ± 0.03 in the control session and 0.87 ± 0.03 after the application of 3 mg/kg of prazosin (*Wilcoxon signed rank* test: $V = 6$, *P-adjusted* = 0.2968).

Discussion

We have demonstrated that α_1-adrenoceptor antagonist prazosin (3 mg/kg, i.p.) decreased the overall motor activity without affecting the cognitive performance in the object-position recognition task. The lower dose (2 mg/kg, i.p.) had no effect on the responding rate nor on the cognitive efficiency.

The absence of the effect of prazosin on the responding rate at the 2 mg/kg dose might be due to the low responding rate in the corresponding control session. The rats assigned to this 2 mg/kg group tent to in general respond at lower rate than the rats assigned to the 3 mg/kg group.

Other studies investigated effects of prazosin in operant tasks. Overwhelming majority of these tasks assessed its effect on the responding rate and motivation. For instance, prazosin (0.5 mg/kg, i.p.) decreased responding rate (lever-pressing) in food self-administration operant tasks (Dwoskin and Sparber 1983, Zhang and Kosten 2005). However, the application

of this drug at similar or higher doses (0.25 – 2 mg/kg, i.p.) did not reduce food self-administration in other studies (Forget *et al.* 2010, Lê *et al.* 2011). These dissimilar results could be explained by different schedules of reinforcement used in the studies mentioned above. The effect of prazosin on lever-pressing in operant food self-administration tasks was distinguishable only in experiments that applied higher fixed ratio (e.g. FR-15) in their experimental protocol. Prazosin also affects the rewarding effects of several drugs, e.g. nicotine, alcohol, cocaine and heroin (Zhang and Kosten 2005, Wee *et al.* 2008, Greenwell *et al.* 2009, Forget *et al.* 2010, Lê *et al.* 2011, Verplaetse *et al.* 2012). Although the motivational processes for food-seeking and drug-seeking are not the same, the effect of prazosin on motivation is evident. In the present study, prazosin (3 mg/kg, i.p.) decreased the responding rate (to 55 ± 5 % of control), which is in agreement with the general depressant effect of this drug on motivation and/or motor activity.

Several studies showed that prazosin do not alter spatial cognition in common behavioral tasks. Prazosin (0.5 mg/kg or 5 mg/kg, i.p.) did not impair cognitive performance in place and/or cue version of the radial arm maze, while the high dose increased the time to complete the cue task (Liao *et al.* 2002). This drug (at doses 0.1, 0.3, 1 and 2 mg/kg, i.p.) also did not induce cognitive deficit in retention of the hidden platform version of the Morris water maze, although the highest dose decreased swimming speed (Riekkinen *et al.* 1996). In agreement, we showed that prazosin had no effect on spatially-driven cognition although it decreased the motor activity in the object-position recognition task.

The effect of prazosin on the stimulus-controlled behavior in operant tasks has not been extensively studied. To our knowledge, there are only two studies which assessed the role of prazosin on performance in this kind of operant tasks and both of them were focused on attention (Puumala *et al.* 1997, Berridge *et al.* 2012). Prazosin (0.3 mg/kg, subcutaneous; s.c.) slightly reduced the choice accuracy in the 5-CSRTT (five-choice serial reaction time

task) (Puumala *et al.* 1997), while the dose 0.5 mg/kg (i.p.) had no effect on sustained attention (Berridge *et al.* 2012). In contrast, we assessed the effect of prazosin on the stimulus-controlled behavior in the hippocampal-dependent operant task studying spatial cognition.

In a few studies, a non-specific effect of prazosin on performance in behavioral tasks was observed. Hahn and Stolerman (2005) reported that prazosin (1 mg/kg, s.c.) facilitated improvement in response accuracy induced by nicotine in the 5-CSRTT. This could indicate positive effect of prazosin on visuospatial attention. However, the same dose decreased anticipatory responding (criterion that appears to be modulated by motivational processes) in this task. The authors explained this observation as an example of response-depressant effects of a pharmacological manipulation causing an "artificial" increase in accuracy. Therefore, better performance in the 5-CSRTT after the application of prazosin in the presence of nicotine was caused by the negative effect on motivation and it cannot be assigned to the enhancement of visuospatial attention. Prazosin also impaired performance in the active place avoidance task (Stuchlik and Vales 2008). The drug at the dose 4 mg/kg (i.p.) decreased locomotion of the rats as well as all behavioral measures of spatial cognition. The authors proposed that the impairment of cognitive performance was caused by altered motor activity rather than by impaired spatial cognition.

According to these findings, we could expect altered cognitive efficiency after the application of the dose of prazosin that affects responding rate in the object-position recognition task. However, the spatial performance of the rats in the present task was not significantly influenced by decreased motor activity induced by prazosin.

In conclusion, our results show that prazosin (3 mg/kg, i.p.) had a depressant effect on responding rate but no effect on recognition in the spatial-operant object-position recognition task. We validated the task with a drug with known pharmacological effects on spatial

behavior and showed that prazosin has no effect on cognitive performance also in the present hippocampal-dependent operant task.

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References

Berridge CW, Shumsky JS, Andrzejewski ME, McGaughy JA, Spencer RC, Devilbiss DM, Waterhouse BD: Differential sensitivity to psychostimulants across prefrontal cognitive tasks: differential involvement of noradrenergic α_1 - and α_2-receptors. *Biol Psychiatry* **71**: 467-473, 2012.

Berridge CW, Waterhouse BD: The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev* **42**: 33-84, 2003.

Chen ZJ, Minneman KP: Recent progress in alpha1-adrenergic receptor research. *Acta Pharmacol Sin* **26**: 1281-1287, 2005.

Dwoskin LP, Sparber SB: Comparison of yohimbine, mianserin, chlorpromazine and prazosin as antagonists of the suppressant effect of clonidine on operant behavior. *J Pharmacol Exp Ther* **226**: 57-64, 1983.

Forget B, Wertheim C, Mascia P, Pushparaj A, Goldberg SR, Le Foll B: Noradrenergic alpha1 receptors as a novel target for the treatment of nicotine addiction. *Neuropsychopharmacology* **35**: 1751-1760, 2010.

Gilbert PE, Kesner RP: Memory for objects and their locations: the role of the hippocampus in retention of object-place associations. *Neurobiol Learn Mem* **81**: 39-45, 2004.

Greenwell TN, Walker BM, Cottone P, Zorrilla EP, Koob GF: The alpha1 adrenergic receptor antagonist prazosin reduces heroin self-administration in rats with extended access to heroin administration. *Pharmacol Biochem Behav* **91**: 295-302, 2009.

Hahn B, Stolerman IP: Modulation of nicotine-induced attentional enhancement in rats by adrenoceptor antagonists. *Psychopharmacology (Berl)* **177**: 438-447, 2005.

Heal DJ: Phenylephrine-induced activity in mice as a model of central alpha 1-adrenoceptor function. Effects of acute and repeated administration of antidepressant drugs and electroconvulsive shock. *Neuropharmacology* **23**: 1241-1251, 1984.

Holm S: A simple sequentially rejective multiple test procedure. *Scand J Statist* **6**: 65-70, 1979.

Klement D, Levcik D, Duskova L, Nekovarova T: Spatial task for rats testing position recognition of an object displayed on a computer screen. *Behav Brain Res* **207**: 480-489, 2010.

Lê AD, Funk D, Juzytsch W, Coen K, Navarre BM, Cifani C, Shaham Y: Effect of prazosin and guanfacine on stress-induced reinstatement of alcohol and food seeking in rats. *Psychopharmacology (Berl)* **218**: 89-99, 2011.

Levcik D, Nekovarova T, Stuchlik A, Klement D: Rats use hippocampus to recognize positions of objects located in an inaccessible space. *Hippocampus* **23**: 153-161, 2013.

Liao RM, Lai WS, Lin JY: The role of catecholamines in retention performance of a partially baited radial eight-arm maze for rats. *Chin J Physiol* **45**: 177-185, 2002.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H: Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn Mem* **9**: 49-57, 2002.

Myhrer T: Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. *Brain Res Brain Res Rev* **41**: 268-287, 2003.

Nekovarova T, Klement D: Operant behavior of the rat can be controlled by the configuration of objects in an animated scene displayed on a computer screen. *Physiol Res* **55:** 105-113, 2006.

O'Donnell J, Zeppenfeld D, McConnell E, Pena S, Nedergaard M: Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance. *Neurochem Res* **37**: 2496-2512, 2012.

Pieribone VA, Nicholas AP, Dagerlind A, Hökfelt T: Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. *J Neurosci* **14**: 4252-4268, 1994.

Puumala T, Riekkinen PSr, Sirviö J: Modulation of vigilance and behavioral activation by alpha-1 adrenoceptors in the rat. *Pharmacol Biochem Behav* **56**: 705-712, 1997.

Riekkinen M, Stefanski R, Kuitunen J, Riekkinen PJr: Effects of combined block of alpha 1-adrenoceptors and NMDA receptors on spatial and passive avoidance behavior in rats. *Eur J Pharmacol* **300**: 9-16, 1996.

Sirviö J, MacDonald E: Central alpha1-adrenoceptors: their role in the modulation of attention and memory formation. *Pharmacol Ther* **83**: 49-65, 1999.

Stuchlik A, Vales K: Role of alpha1- and alpha2-adrenoceptors in the regulation of locomotion and spatial behavior in the active place avoidance task: a dose-response study. *Neurosci Lett* **433**: 235-240, 2008.

Trovero F, Blanc G, Hervé D, Vézina P, Glowinski J, Tassin JP: Contribution of an alpha 1-adrenergic receptor subtype to the expression of the "ventral tegmental area syndrome". *Neuroscience* **47**: 69-76, 1992.

Verplaetse TL, Rasmussen DD, Froehlich JC, Czachowski CL: Effects of prazosin, an α1-adrenergic receptor antagonist, on the seeking and intake of alcohol and sucrose in alcohol-preferring (P) rats. *Alcohol Clin Exp Res* **36**: 881-886, 2012.

Wee S, Mandyam SD, Lekic DM, Koob GF: Alpha 1-noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. *Eur Neuropsychopharmacol* **18**: 303-311, 2008.

Zhang XY, Kosten TA: Prazosin, an alpha-1 adrenergic antagonist, reduces cocaine-induced reinstatement of drug-seeking. *Biol Psychiatry* **57**: 1202-1204, 2005.

Figures

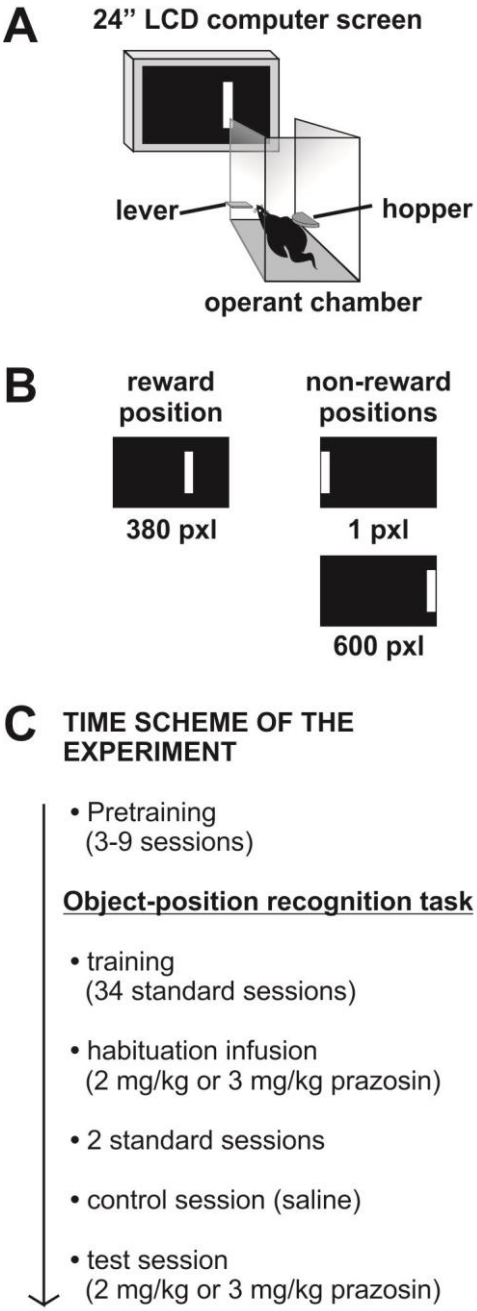

Figure 1. A: Experimental apparatus. B: Stimuli presented on the computer screen in the object-position recognition task. C: Time scheme of the experiment.

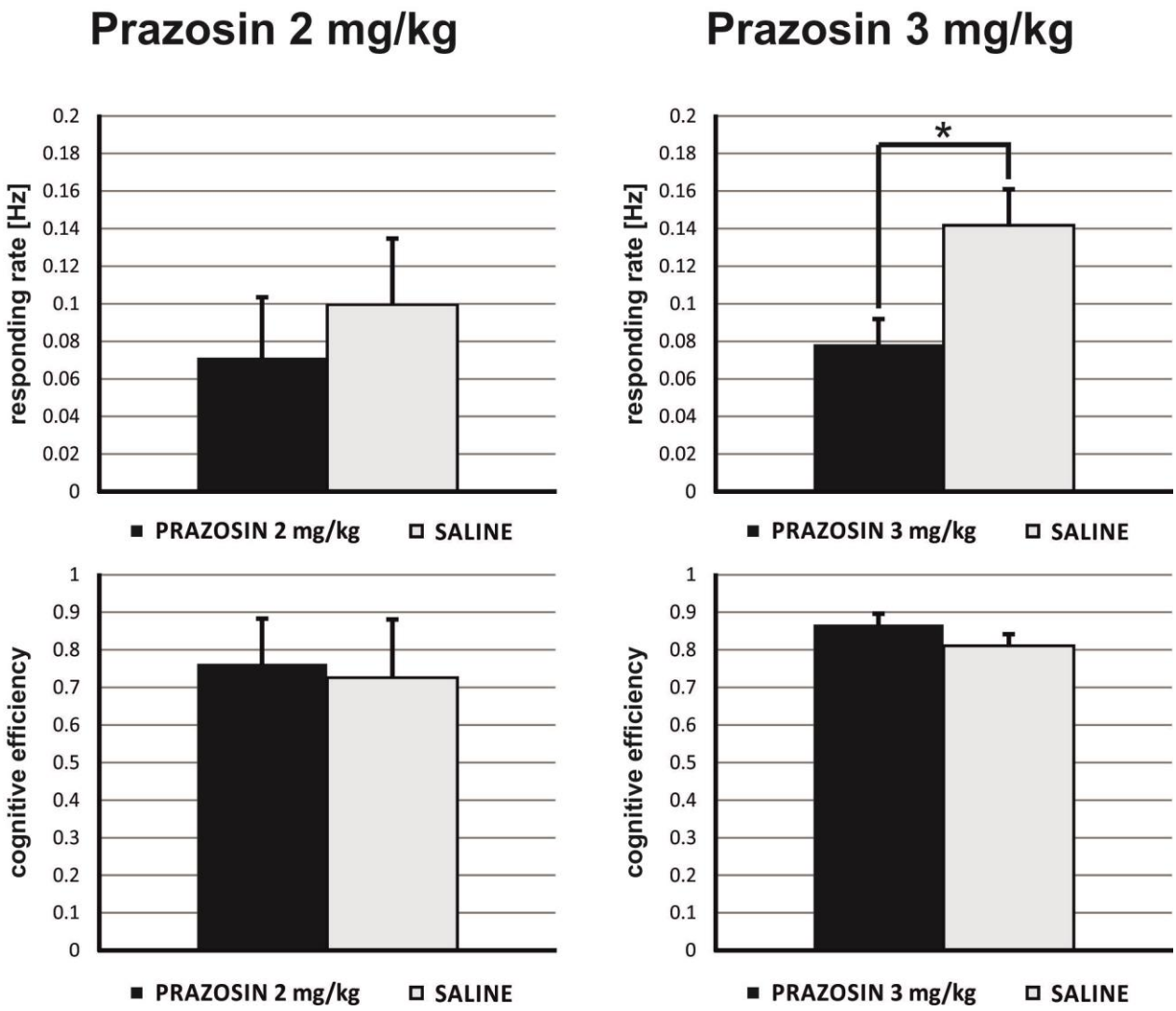

Figure 2. Overall responding rate of lever presses (upper graphs) and cognitive efficiency (lower graphs) in the test sessions and in the control sessions. Cognitive efficiency represents the ratio of reward and non-reward presses emitted during the first 15 s after the onset of stimuli presentation (for further details see Methods/Data analysis). The black color indicates the application of prazosin (2 mg/kg or 3 mg/kg, i.p.) and the grey color indicates the application of saline. Data are mean ± SEM. The one star indicates significant difference at the level of 0.05.