Sleep Science

ORIGINAL ARTICLE

Zolpidem impairs non-associative memory in mice

Zolpidem prejudica a memória não associativa em camundongos

Karina Agustini Zanin[1,2], Camilla de Lima Patti[1,2], Sérgio Tufik[1], Dalva Poyares[1], Roberto Frussa-Filho[2]

ABSTRACT

Objective: The aim of this study was to evaluate the effects of acute administration of zolpidem (Zolp) on mice's habituation response to the open-field (OF) apparatus, a model of non-associative memory. **Methods:** Three-month old male Swiss mice were assigned to one of the following groups: Sal/HC, Sal/OF and Zolp/OF. Mice received an intraperitoneal (i.p.) saline (Sal) injection and, 30 minutes later, they were returned to home cage (HC) (Sal/HC group) or exposed to the OF arena (Sal/OF). Still, a group of mice received an i.p. 10 mg/kg Zolp injection and 30 minutes later were exposed to OF. Animals were exposed to OF for 10 minutes and their general activity was quantified in the first 3 minutes. Ten days after the first exposure, all animals were submitted to a 3-minute OF exposure and their general activity was quantified. **Results:** In the first exposure, the Zolp/OF group presented a decreased peripheral, central and total locomotion frequencies and increased immobility duration in relation to the Sal/OF group. In the second exposure, only the Sal/OF group presented habituation behavior, demonstrated by a significant decrement of central and total locomotion frequencies and increased immobility duration when compared to Sal/HC group (exposed for the first time to OF). Conversely, Zolp/OF group did not differ from the Sal/HC group in any of the parameters analyzed, suggesting habituation impairment. **Conclusion:** Our results suggest that the acute administration of 10 mg/kg Zolp before the first exposure to OF promotes a reduction on motor activity and induces habituation deficits, reflecting a non-associative memory impairment.

Keywords: sleep; memory/drug effects; memory/physiology; locomotion; motor activity; pyridines/administration & dosage; hypnotics and sedatives/pharmacology; rats.

RESUMO

Objetivo: O objetivo do presente estudo foi avaliar os efeitos da administração aguda de zolpidem (Zolp) sobre a resposta de habituação ao modelo de campo aberto (CA), um modelo de memória não associativa, em camundongos. **Métodos:** Camundongos Swiss machos de 3 meses de idade foram distribuídos nos seguintes grupos: Sal/GM, Sal/CA e Zolp/CA. Os camundongos receberam uma injeção intraperitoneal (i.p.) de salina e, após 30 minutos, retornaram às suas gaiolas moradia (Sal/GM) ou foram expostos à arena de CA (Sal/CA). Ainda, um grupo de camundongos recebeu uma injeção i.p. de 10 mg/kg de Zolp e, após 30 minutos, foram expostos ao CA (Zolp/CA). Os animais foram expostos ao CA por 10 minutos e a atividade geral foi quantificada nos primeiros 3 minutos. Dez dias após a primeira exposição, todos os animais foram submetidos a uma exposição ao CA por 3 minutos e a atividade geral foi quantificada. **Resultados:** Na primeira exposição, o grupo Zolp/CA apresentou frequências de locomoção periférica, central e total diminuídas, e um aumento na duração da imobilidade em relação ao grupo Sal/CA. Na segunda exposição, apenas o grupo Sal/CA apresentou comportamento de habituação, demonstrado por uma diminuição significante das frequências de locomoção central e total, e um aumento da duração da imobilidade quando comparado ao grupo Sal/GM (exposto pela primeira vez ao CA). Por outro lado, o grupo Zolp/CA não diferiu do grupo Sal/GM em nenhum dos parâmetros analisados, sugerindo um prejuízo da habituação. **Conclusão:** Nossos resultados sugerem que a administração aguda de 10 mg/kg de Zolp antes da primeira exposição ao CA promove uma redução da atividade motora e induz déficits de habituação, refletindo um prejuízo de memória não associativa.

Palavras-chave: sono; memória/efeitos de drogas; memória/fisiologia; locomoção; atividade motora; piridinas/administração & dosagem; hipnóticos e sedativos/farmacologia; ratos.

INTRODUCTION

Zolpidem (Zolp) is an imidazopyridine agent which binds selectively to BZ_1 – benzodiazepine recognition site (also called ω_1) – in the $GABA_A$ receptors[1]. Such selectivity confers its mainly hypnotic properties with weaker anticonvulsant and myorelaxant effects, common symptoms following the administration of classical benzodiazepines[2-5]. In this way, although Zolp seems to exhibit fewer deleterious effects on cognitive processes, when compared to classical benzodiazepines, psychomotor and amnestic effects are still reported in humans[5-11].

Concerning animal studies, evidences had suggested that amnestic[2,4,12,13], hypolocomotor[2,14-17] and anxiolytic effects[18-20]

Study carried out at Department of Psychobiology, Universidade Federal de São Paulo – UNIFESP, São Paulo (SP), Brazil.
[1]Department of Psychobiology, Universidade Federal de São Paulo – UNIFESP, São Paulo (SP), Brazil.
[2]Department of Pharmacology, Universidade Federal de São Paulo – UNIFESP, São Paulo (SP), Brazil.
Corresponding author: Roberto Frussa-Filho – Departamento de Farmacologia da Univerisdade Federal de São Paulo – Rua Botucatu, 862, Edifício Leal Prado, 1º andar – CEP: 04023062 – São Paulo (SP), Brazil – e-mail: frussa.farm@epm.br
Received: March 17, 2011; Accepted: June 24, 2011

could be induced by Zolp. Regarding the mnemonic processes, Zolp seems to have a deleterious effect in animals models of learning and memory. In this respect, the studies generally investigate associative memory tasks, as fear conditioning task[12,21] and inhibitory avoidance[22,23].

To our knowledge the effects of Zolp on non-associative memory task has not been investigated yet. In rodents, open-field (OF) habituation is considered the classical non-associative task[24-28]. The task consists in exposing the animals to the OF for a free exploration of the apparatus during a certain time. In the second exposure, the animals are rexposed to the arena and memory is evaluated by the habituation, measured by a progressively decrement on the exploration (represented by a decreased general motor activity within a session or between sessions)[26,29,30].

Within this context, the objectives of the present study were to evaluate the effects of the acute administration of a sedative dose of Zolp on mice general motor activity and also on the habituation to the OF arena.

METHODS

Subjects

Three-month-old Swiss EPM-M1 male mice (outbred, raised, and maintained in the Centre for Development of Experimental Models in Medicine and Biology of Universidade Federal de São Paulo – Unifesp) were used. Animals weighing 30-35 g were housed under conditions of controlled temperature (22-23°C) and lighting (12 hours light, 12 hours dark; lights on at 6:45 a.m.). Food and water were available *ad libitum* throughout the experiment. Animals used in this study were maintained in accordance with the National Institute of Health Guide for the care and use of laboratory animals (NIH publications n.º 80-23, revised 1996) and the experimental procedures were approved by the Ethics Committee under the protocol #1126/08.

Drugs

Zolp was diluted in saline 0.9% solution which was also the control solution, both administered intraperitoneally in the volume of 10 mL/kg body weight. The dose of Zolp (10 mg/kg) was chosen based on pilot experiments of our group, demonstrating a robust sedative effect in mice. Thus, this dose could be considered as a "therapeutic" dose.

Open-field evaluation

The OF apparatus used in the present study was a circular wooden arena (40 cm in diameter and 50 cm high) with an open top and floor divided into 19 squares. Hand-operated counters were used to score total locomotion (number of any floor unit entered), peripheral locomotion (number of entrances into the floor units close to the walls of the apparatus) and central locomotion (number of entrances into any floor unit not close to the walls of the apparatus) and stopwatches were used to quantify duration of immobility (total of seconds of lack of movement). The observer was always unaware of the experimental design.

Experimental procedures

Thirty animals were randomly assigned to one of the following groups: Sal/HC (10), Sal/OF (10) and Zolp/OF (10). Mice received an intraperitoneal (i.p.) saline injection and 30 minutes after, they were returned to home cage (HC) (Sal/HC group) or exposed to the OF arena (Sal/OF group). Still, a group of mice received an i.p. 10 mg/kg Zolp injection and 30 minutes after were exposed to OF. The Sal/OF and Zolp/OF groups were exposed to a 10-minute session in the OF and had their general activity quantified only during the first 3 minutes. Ten days after the first exposure, all animals were submitted to a 3-minute session and their general activity was quantified again.

The inclusion of the group Zolp/HC could raise the investigation of a residual effect of Zolp on motor activity in the second exposure. Although it is a possible rationale, the half-life of the drug is about 3 hours[31,32] and the second exposure occurred only 10 days the first exposure to the OF arena, discarding the investigation of a residual effect of Zolp. In this context, this group was not included for ethical reasons.

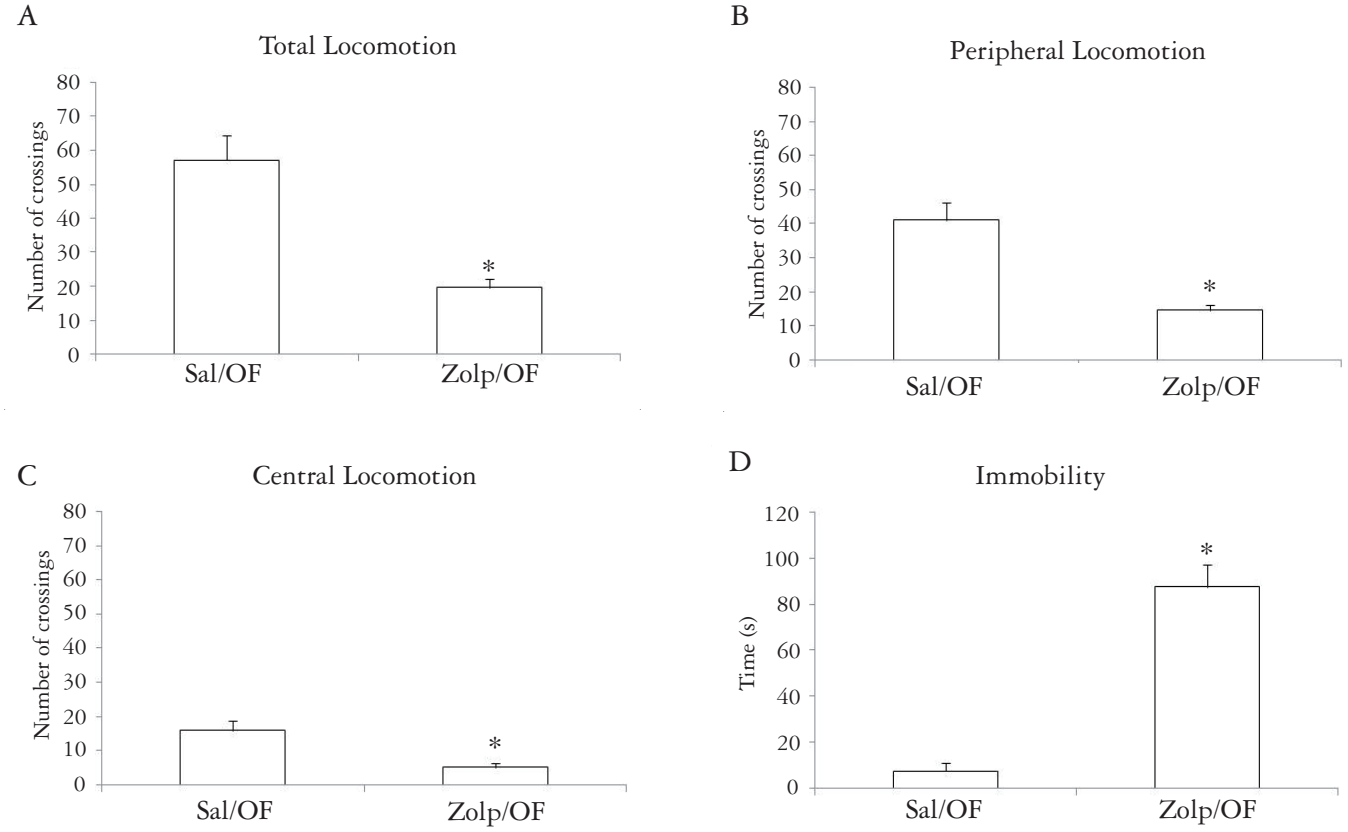
Statistical analysis

On the first exposure, all parameters were compared using *t*-test for independent samples and on the second exposure, one-way ANOVA followed by Duncan's test was used. In order to compare the parameters between the first and second sessions within the groups Sal/OF and Zolp/OF a paired samples *t*-test was applied. A probability of $p<0.05$ was considered to show significant differences for all comparisons made.

RESULTS

In the first exposure to the OF arena, there was a remarkable effect of Zolp on mice's general motor activity as revealed the *t*-test for independent samples. In fact, Zolp promoted a significant decrement in total [$t(18)=4.84$; $p=0.001$] (Figure 1A), peripheral [$t(18)=5.08$; $p<0.001$] (Figure 1B) and central [$t(18)=3.54$; $p=0.002$] (Figure 1C) locomotion frequencies and a significant increase in immobility [$t(18)=7.82$; $p<0.001$] (Figure 1D).

During the second session, performed 10 days after the first one, animals of the 3 groups were observed for 3 minutes and only the Sal/OF group displayed habituation to the OF. In fact, although ANOVA followed by Duncan's test



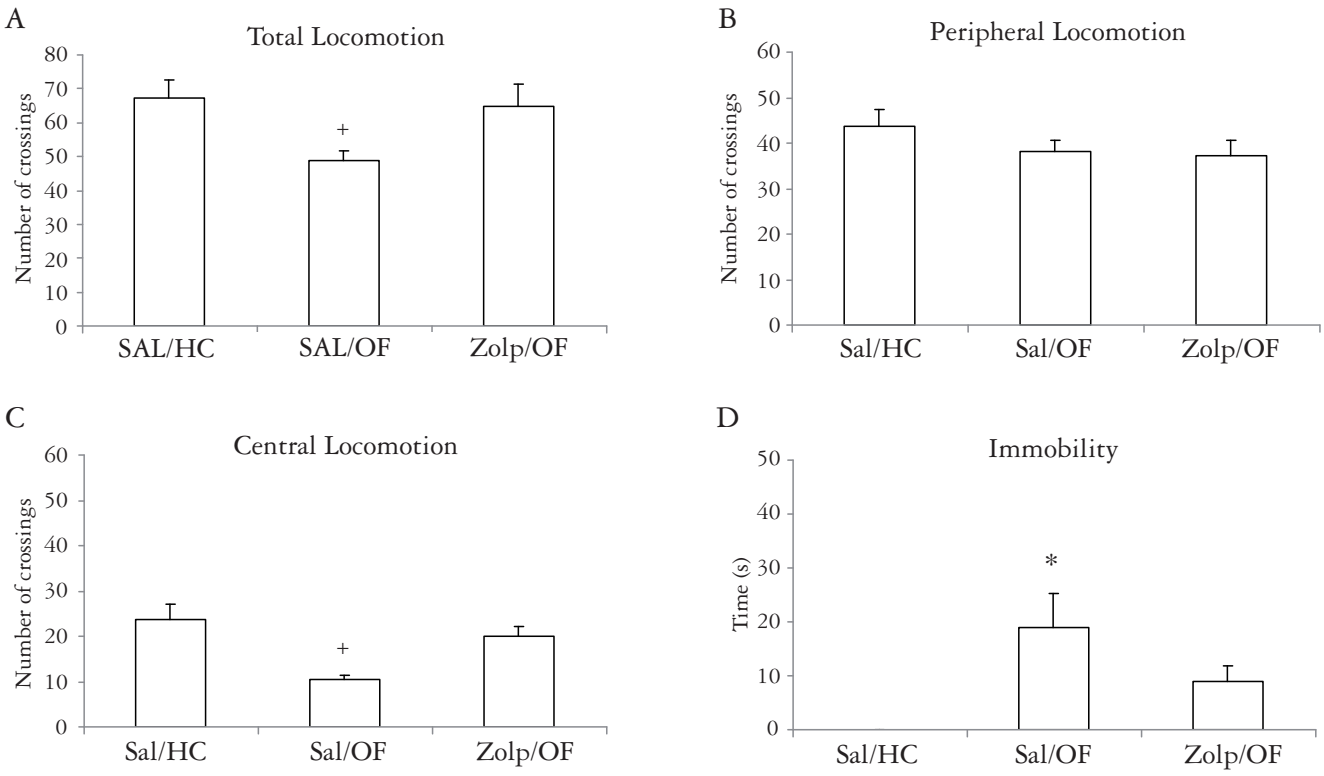
*p<0.05 compared to the Sal/OF group (t-test for independent samples).

Figure 1. Effects of acute administration of 10 mg/kg of zolpidem on a non-associative memory task in mice. Mice received saline (Sal) and were returned to their home cages (HC) on the first exposure (Sal/HC) or received saline (Sal/OF) or zolpidem 10 mg/kg (Zolp/OF) and were exposed for the first time to the open-field (OF) for 10 minutes and general activity was quantified for the first 3 minutes. Results are presented as mean±standard error of number of crossings trough all quadrants (A), the peripheral quadrants (B), the central quadrants (C) and duration of immobility (s) (D) in the open-field apparatus during the first exposure.

revealed that there were no significant differences between groups in peripheral locomotion [$F(2,27)=1.21$; $p=0.31$] (Figure 2B). The groups Sal/OF presented a decrement in central locomotion [$F(2,27)=7.58$; $p=0.002$] (Figure 2C) and also in total locomotion [$F(2,27)=3.94$; $p=0.03$] (Figure 2A) frequencies in relation to the other groups (Sal/HC and Zolp/OF). Concerning duration of immobility, the group Sal/OF presented an increased time of immobility when compared to the Sal/HC group [$F(2,27)=5.44$; $p=0.01$] (Figure 2D), as shown by ANOVA followed by Duncan's test. Thus, the acute administration of Zolp before the first OF exposure abolished the OF habituation in mice.

An inter-session (first *versus* second exposure) analysis was performed by ANOVA with repeated measures (MANOVA) and by paired samples *t*-test for Sal/OF and Zolp/OF groups. In this respect, ANOVA with repeated measures with exposure (first *versus* second) as a within-subject and treatment (Sal *versus* Zolp) as a between-subject factor was performed for the OF parameters. Concerning total locomotion, MANOVA revealed significant exposure [$F(1,18)=13.94$; $p=0.002$], exposure *versus* treatment interaction [$F(1,18)=29.72$; $p<0.001$] and a trend toward treatment factor [$F(1,18)=3.70$; $p=0.07$] effects. When peripheral locomotion was evaluated, significant exposure [$F(1,18)=11.20$; $p=0.004$], treatment [$F(1,18)=15.04$; $p=0.001$] and exposure *versus* treatment interaction [$F(1,18)=18.89$; $p<0.001$] effects were revealed by MANOVA. When central locomotion was analyzed, MANOVA revealed significant exposure [$F(1,18)=5.04$; $p=0.03$] and exposure *versus* treatment interaction [$F(1,18)=24.03$; $p<0.001$], but not treatment [$F(1,18)=0.11$; $p=0.74$] effects. Finally, MANOVA revealed significant exposure [$F(1,18)=33.92$; $p<0.001$], treatment [$F(1,18)=28.15$; $p<0.001$] and exposure *versus* treatment interaction [$F(1,18)=61.04$; $p<0.001$] effects when duration of immobility was analyzed.

Paired-samples *t*-test (Table 1) revealed that the Sal/OF groups displayed a decrement in central locomotion frequency [$t(9)=3.94$; $p<0.05$] in the second exposure to the



*p<0.05 compared to the Sal/HC group and +p<0.05 compared to all groups (one-way ANOVA and Duncan's test).

Figure 2. Effects of acute administration of 10 mg/kg of zolpidem (Zolp) on a non-associative memory task in mice. Mice received saline (Sal) and were returned to their home cages (HC) on the first exposure (Sal/HC) or received saline (Sal/OF) or 10 mg/kg zolpidem (Zolp/OF) and were exposed for the first time to the open-field (OF). Ten days later, all groups were exposed to the open-field apparatus for 3 minutes. Results are presented as mean±standard error of number of crossings trough all quadrants (A), the peripheral quadrants (B), the central quadrants (C) and duration of immobility (s) (D) in the open-field apparatus during the second exposure.

Table 1. Effects of acute administration of 10 mg/kg of zolpidem (Zolp) on a non-associative memory task in mice. Mice received saline (Sal/OF) or 10 mg/kg zolpidem (Zolp/OF), were exposed to the open field (OF) for 10 minutes and general activity was quantified during the first 3 minutes. Ten days later, the groups were exposed to the open field apparatus for 3 minutes. Results are presented as mean±standard error of number of crossings trough the peripheral and the central quadrants, total number of crossings in all quadrants and duration of immobility (s).

	First exposure	Second exposure
Sal/OF		
Peripheral locomotion	39.67±2.11	38.83±1.45
Central locomotion	15.00±0.91	10.17±0.75*
Total locomotion	54.00±2.86	49.5±1.98
Immobility	3.00±1.03	15.17±4.17*
Zolp/OF		
Peripheral locomotion	16.33±0.38	35.83±1.98#
Central locomotion	4.17±0.31	18.83±0.93#
Total locomotion	20.83±0.75	57.5±2.63#
Immobility	86.67±4.97	8.00±2.13#

*p<0.05 and #p<0.001 compared to the same group in the first exposure (paired samples t-test).

OF when compared to the first. As expected, these animals also displayed an increment in the duration of immobility [t(9)=2.95; p<0.05] in the second OF exposure. Regarding the Zolp/OF group performance, animals presented an increase in all the locomotion parameters (central [t(9)=15.21; p<0.001], peripheral [t(9)=9.47; p<0.001] and total [t(9)14.35; p<0.001]), as well as a decrease in duration of immobility when the second exposure was compared to the first [t(9)=14.57; p<0.001]. This within-group comparisons also supported the notion that only the Sal/OF group (and not the Zolp/OF group) presented habituation to the OF apparatus.

DISCUSSION

In the current study, we have demonstrated the effects of Zolp, a non-benzodiazepine hypnotic, on the general motor activity and habituation in the OF, an animal model of non-associative memory. In this way, our study is in line with many studies that demonstrated that although Zolp has se-

lectivity by the BZ_1 site in the $GABA_A$ receptors[33,34], it can present side effects concerning motor activity and memory, as the classical benzodiazepines do[2,15,16,35,36]. Indeed, in the present study Zolp promoted a marked decrement on locomotor activity during the first exposure to the OF arena. Importantly, we also demonstrated that acute administration of this sedative dose of Zolp induced a markedly impairment in the habituation to OF, an animal model of non-associative memory.

Concerning memory, it has been shown that Zolp could induce deleterious effects likely classical benzodiazepines[2,4,8,10-13,21-23,37,38]. However, these studies generally evaluated associative memories. Thus, as far as we know, this is the first study demonstrating that the amnestic effect of Zolp can be extended to non-associative memory.

Regarding anxiety-like behavior, it could be argued that similar to benzodiazepines, Zolp could exert an anxiolytic effect. In this sense, the central locomotion frequency could be an anxiety index, namely, the higher exploration of the central quadrants the lowest anxiety level[39]. Conversely, no differences were found specifically concerning the exploration of the central quadrants of the OF, suggesting no effects of Zolp on anxiety under our experimental conditions. In this scenario, we investigated the effects of the acute administration of 2.5 or 10 mg/kg Zolp in mice submitted to the plus-maze discriminative avoidance task, an animal model which evaluates learning, memory, anxiety and motor activity. In this study, we did not verify effects of any dose of Zolp on anxiety-like behavior (Zanin et al., non-published data).

In relation to the Sal/OF group, we can assure their habituation to the OF as long as they displayed generalized decrement in exploration during the second exposure when compared to Sal/HC group, which was exposed to OF arena only once. In addition, when performing a within-subject analysis (paired samples *t*-test), these animals had their exploration diminished in the second exposure when compared to the first. Concerning Zolp effects, the hypolocomotor effect observed in the first session cannot be discarded, since it could have interfered with the within-subject analysis, impairing a satisfactory comparison. Conversely, the amnestic effect of acute administration of Zolp can be detected by comparing the performance of previously OF-exposed Zolp-treated animals (Zolp/OF group) to that exhibited by saline-treated animals exposed to OF arena only in the second session (Sal/HC). In fact, both Zolp/OF and Sal/HC groups explored the OF in a similar manner, demonstrating, thus, the impairing effects of Zolp on a non-associative memory.

Studies have already reported that the acute administration of Zolp produced a slight decrement in the LTP phenomenon *in vitro*[40] and a reduction of binding for $GABA_A$ receptors in the cerebral cortex of mice at the dose of 10 mg/kg[16]. Also, this drug increases the oxidative metabolism in the hippocampus[17], known as an important structure for memories formation. Accordingly, taken together the facts cited above, we could hypothesize that the acute administration of Zolp before the first exposure to the OF arena promoted a metabolic alteration on the structures involved with non-associative memory formation, which resulted in the memory deficit observed. In this respect, spatial memory habituation tasks are critically related to both cortical[41] and hippocampal neurotransmissions[42] and to hippocampal oxidative status[43].

Our results strength the notion that although its well-accepted clinical use, Zolp should be prescript cautiously. In this regard, in humans non-associative (or implicit) memory is the one that allows the execution of cognitive and motor habits, i.e., the memory of the most common day-life tasks[44]. Thus, it could be suggested that Zolp could impair day-life tasks as driving, talking, walking and others unconscious memories. In conclusion, the present study demonstrates that Zolp promoted markedly effects on a non-associative memory model, OF habituation. Importantly, this memory impairment occurred concomitantly to a sedative "therapeutic" effect.

ACKNOWLEDGEMENTS

This research was supported by fellowships from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and from Associação Fundo de Amparo à Pesquisa (AFIP). The authors would like to thank Ms. Teotila R. R. Amaral, Ms. Claudenice M. Santos, Mr. Cleomar S. Ferreira and Mr. Antonio Rodrigues dos Santos Ferreira for capable technical assistance. D.P., S.T, and R.F.F. are recipients of CNPq fellowship.

REFERENCES

1. Sanger DJ, Benavides J, Perrault G, Morel E, Cohen C, Joly D, et al. Recent developments in the behavioral pharmacology of benzodiazepine (omega) receptors: evidence for the functional significance of receptor subtype. Neurosci Biobehav Rev. 1994;18(3):335-72.
2. Depoortere HB, Zivkovic B, Lloyd KG, Sanger D, Perrault G, Langer SZ, et al. Zolpidem, a novel nonbenzodiazepine hypnotic. I. Neuropharmacological and behavioral effects. J Pharmacol Exp Ther. 1986;237(2):649-57.
3. Lloyd KG, Zivkovic B. Specificity within the GABA receptor supramolecular complex: a consideration of the new omega 1 receptor selective imidazopyridine hypnotic zolpidem. Pharmacol Biochem Behav. 1988;129(4):781-3.
4. Zivkovic B, Perrault G, Morel E, Sanger DJ. Comparative pharmacology of zolpidem and other hypnotics and sleep inducers. In: Sauvanet JP, Langer SZ, Morselli PL, editors. Imidazopyridines in

sleep disorders: a novel experimental and therapeutic approach. New York: Raven Press; 1988. p. 97-109.
5. Salvà P, Costa J. Clinical pharmacokinetics and pharmacodynamics of zolpidem. Therapeutic implications. Clin Pharmacokinet. 1995;29(3):142-53.
6. Cashman JN, Power SJ, Jones RM. Assessment of a new hypnotic imidazo-pyridine (zolpidem) as oral premedication. Br J Clin Pharmac. 1987;24(1):85-92.
7. Langtry HD, Benfield P. Zolpidem. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential. Drugs. 1990;40(2):291-313.
8. Evans SM, Funderburk FR, Griffiths RR. Zolpidem and triazolam in humans: behavioral and subjective effects and abuse liability. J Pharmacol Exp Ther. 1990;255(3):1246-55.
9. Diagnostic Classification Steering Committee of the American Sleep Disorders Association. International classification of sleep disorders – diagnostic and coding manual. Rochester (MN): American Sleep Disorders Association; 1990.
10. Danjou P, Paty I, Fruncillo R, Worthington P, Unruh M, Cevallos W, et al. A comparison of the residual effects of zaleplon and zolpidem following administration 5 to 2 h before awakening. J Clin Pharmacol. 1999;48(3):367-74.
11. Dingemanse J, Bury M, Hussain Y, Van Giersbergen P. Comparative tolerability, pharmacodynamics, and pharmacokinetics of a metabolite of a quinolizinone hypnotic and zolpidem in healthy subjects. Drug Metab Dispos. 2000;28(12):1411-6.
12. Sanger DJ, Joly D, Zivkovic B. Effects of zolpidem, a new imidazopyridine hypnotic, on the acquisition of conditioned fear in mice. Comparison with triazolam and CL 218,872. Psychopharmacology (Berl). 1986;90(2):207-10.
13. Perrault G, Morel E, Sanger DJ, Zivkovic B. Differences in pharmacological profiles of a new generation of benzodiazepine and nonbenzodiazepine hypnotics. Eur J Pharmacol. 1990;187(3): 487-94.
14. Sanger DJ, Morel E, Perrault G. Comparison of the pharmacological profiles of the hypnotic drugs zaleplon and zolpidem. Eur Pharmacol. 1996;313(1-2):35-42.
15. Siemiatkowski M, Sienkiewicz-Jarosz H, Członkowska AI, Bidziński A, Płaźnik A. Effects of buspirone, diazepam, and zolpidem on open field behavior, and brain [3H]muscimol binding after buspirone pretreatment. Pharmacol Biochem Behav. 2000;66(3):645-51.
16. Fahey JM, Grassi JM, Reddi JM, Greenblatt DJ. Acute zolpidem administration produces pharmacodynamic and receptor occupancy changes at similar doses. Pharmacol Biochem Behav. 2006;83(1):21-7.
17. González-Pardo H, Conejo NM, Arias JL. Oxidative metabolism of limbic structures after acute administration of diazepam, alprazolam and zolpidem. Prog Neuropsychopharmacol Biol Psychiatry. 2006;30(6):1020-6.
18. Savić MM, Obradović DI, Ugresić ND, Cook JM, Yin W, Bokonjić DR. Bidirectional effects of benzodiazepine binding site ligands in the elevated plus-maze: differential antagonism by flumazenil and beta-CCt. Pharmacol Biochem Behav. 2004;79(2):279-90.
19. Cui XY, Zhao X, Chu QP, Chen BQ, Zhang YH. Influence of diltiazem on the behavior of zolpidem-treated mice in the elevated-plus maze test. J Neural Transm. 2007;114(2):155-60.
20. Kumar A, Goyal R. Possible GABAergic modulation in the protective effect of zolpidem in acute hypoxic stress-induced behavior alterations and oxidative damage. Neurochem Res. 2008;33(3):370-7.
21. Chodera A, Nowakowska E, Bartczak G. Tolerance to a new class of non-benzodiazepine anxiolytics. Pol J Pharmacol. 1994;46(5):479-81.
22. Tang AH, Smith MW, Carter DB, Im WB, Von Voigtlander PF. U-90042, a sedative/hypnotic compound that interacts differentially with the GABA-A receptor subtypes. J Pharmacol Exp Ther. 1995;275(2):761-7.
23. Edgar DM, Seidel WF, Gee KW, Lan NC, Field G, Xia H, et al. CCd-3693: an orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. J Pharmacol Exp. Ther. 1997;282(1):420-9.
24. Conceição IM, Maioline Júnior M, Mattia NF, Chang YH, Smaili S, Frussa-Filho R. Effect of withdrawal from long-term nifedipine administration on open-field habituation in the rat. Braz J Med Biol Res. 1994;27(6):1363-7.
25. Silva RH, Felicio LF, Nasello AG, Vital MA, Frussa-Filho R. Effect of ganglioside (GM1) on memory in senescent rats. Neurobiol Aging. 1996;17(4):583-6.
26. Vianna MR, Alonso M, Viola H, Quevedo J, de Paris F, Furman M, et al. Role of hippocampal signaling pathways in long-term memory formation of a nonassociative learning task in the rat. Learn Mem. 2000;7(5):333-40.
27. Thiemann G, Fletcher BC, Ledent C, Molleman A, Hasenöhrl RU. The genetic versus pharmacological invalidation of the cannabinoid CB(1) receptor results in differential effects on 'non-associative' memory and forebrain monoamine concentrations in mice. Neurobiol Learn Mem. 2007;88(4):416-23.
28. Réus GZ, Valvassori SS, Machado RA, Martins MR, Gavioli EC, Quevedo J. Acute treatment with low doses of memantine does not impair aversive, non-associative and recognition memory in rats. Naunyn Schmiedebergs Arch Pharmacol. 2008;376(5):295-300.
29. Izquierdo I, Schröder N, Netto CA, Medina JH. Novelty causes time-dependent retrograde amnesia for one-trial avoidance in rats through NMDA receptor- and CaMKII-dependent mechanisms in the hippocampus. Eur J Neurosci. 1999;11(9):3323-8.
30. Patti CL, Frussa-Filho R, Silva RH, Carvalho RC, Kameda SR, Takatsu-Coleman AL, et al. Behavioral characterization of morphine effects on motor activity in mice. Pharmacol Biochem Behav. 2005;81(4):923-7.
31. Thenot JP, Hermann P, Durand A, Burke JT, Allen J, Garrigou D, et al. Pharmacokinetics and metabolism of zolpidem in various animal species and in humans. In: Sauvanet JP, Langer SZ, Morselli PL; editors. Imidazopyridines in sleep disorders. New York: Raven Press; 1988. p. 139-53.
32. Garrigou-Gadenne D, Burke JT, Durand A, Depoortere H, Thenot JP, Morseli PL. Pharmacokinetics, brain distribution and pharmaco-eletrocorticographic profile of zolpidem, a new hypnotic, in the rat. J Pharmacol Exp Therapeut. 1989;248(3):1283-8.
33. Puia G, Vicini S, Seeburg PH, Costa E. Influence of recombinant gamma-aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of gamma-aminobutyric acid-gated Cl- currents. Mol Pharmacol. 1991;39(6):691-6.
34. Sancar F, Ericksen SS, Kucken AM, Teissére JA, Czajkowski C. Structural determinants for high-affinity zolpidem binding to GABA-A receptors. Mol Pharmacol. 2007;71(1):38-46.

35. Crestani F, Martin JR, Möhler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol. 2000;131(7):1251-4.
36. Rush CR. Behavioral pharmacology of zolpidem relative to benzodiazepines: a review. Pharmacol Biochem Behav. 1998;61(3):253-69.
37. Cashman JN, Power SJ, Jones RM. Assessment of a new hypnotic imidazo-pyridine (zolpidem) as oral premedication. Br J Clin Pharmacol. 1987;24(1):85-9.
38. Herzog CD, Gandhi C, Bhattacharya P, Walsh TJ. Effects of intraseptal zolpidem and chlordiazapoxide on spatial working memory and high-affinity choline uptake in the hippocampus. Neurobiol Learn Mem. 2000;73(2):168-79.
39. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003;463(1-3):3-33.
40. Higashima M, Kinoshita H, Koshino Y. Differences in the effects of zolpidem and diazepam on recurrent inhibition and long-term potentiation in rat hippocampal slices. Neurosci Lett. 1998;245(2):77-80.
41. Rinaldi A, Mandillo S, Oliverio A, Mele A. D1 and D2 receptor antagonist injections in the prefrontal cortex selectively impair spatial learning in mice. Neuropsychopharmacol. 2007;32(2):309-19.
42. Izquierdo LA, Barros DM, Vianna MR, Coitinho A, de David e Silva T, Choi H, et al. Molecular pharmacological dissection of short-and long-term memory. Cell Mol Neurobiol. 2002;22(3):269-87.
43. Caceres LG, Aon Bertolino L, Saraceno GE, Zorrilla Zubilete MA, Uran SL, Capani F, et al. Hippocampal-related memory deficits and histological damage induced by neonatal ionizing radiation exposure. Role of oxidative status. Brain Res. 2010;1312:67-78.
44. Morgado I. [The psychobiology of learning and memory: fundamentals and recent advances]. Rev Neurol. 2005;40(5):289-97. Spanish.