Zolpidem impairs non-associative memory in mice

Zolpidem prejudica a memória não associativa em camundongos

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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/GM, 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 in 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). For 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 BZ1, benzodiazepine recognition site (also called ) – in the GABA receptors1. Such selectivity confers its mainly hypnotic properties with weaker anticonvulsant and myorelaxant effects, common symptoms following the administration of classical benzodiazepines2-5. 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 humans6-11.

Concerning animal studies, evidences had suggested that amnestic2,6,12,13, hypolocomotor2,14-17 and anxiolytic effects18-20 and...
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 task12,21 and inhibitory avoidance22,23.

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 task24,28. 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 reexposed 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.° 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 hours31,32 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
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 \[F(2,27)=0.50; p=0.002\] (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
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 \( t(9)=9.45; p<0.001 \) 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 \( t(9)=14.53; p<0.001 \). 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-
lecitivity by the BZ, site in the GABA<sub>A</sub> receptors<sup>33,34</sup>, it can present side effects concerning motor activity and memory, as the classical benzodiazepines do<sup>21,16,35,36</sup>. 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<sup>2,4,8,10,13,21-23,37,38</sup>. 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<sup>19</sup>. 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 <i>in vitro</i><sup>39</sup> and a reduction of binding for GABA<sub>A</sub> receptors in the cerebral cortex of mice at the dose of 10 mg/kg<sup>16</sup>. Also, this drug increases the oxidative metabolism in the hippocampus<sup>17</sup>, 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<sup>43</sup> and hippocampal neurotransmissions<sup>42</sup> and to hippocampal oxidative status.<sup>43</sup>

Our results strength the notion that although its well-accepted clinical use, Zolp should be prescribe 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<sup>44</sup>. 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.

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