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ORIGINAL PAPER

Effects of Anesthetic Ketamine on Anxiety-Like Behaviour in Rats

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Abstract
There is scarce information regarding the effects of anesthetic doses of the non-competitive N-methyl-D-aspartate receptor antagonist ketamine on anxiety. The current study evaluated the acute effects of intraperitoneally (i.p.) administered anesthetic ketamine (100 mg/kg) i.p. on anxiety in rats. For this purpose, the light/dark and the open field tests were utilized. The effects of anesthetic ketamine on motility were also examined using a motility cage. In the light/dark test, anesthetic ketamine, administered 24 h before testing reduced the number of transitions between the light and dark compartments and the time spent in the light compartment in the rats compared with their control cohorts. In addition, ketamine was found to exert a depressive effect on rats' motility. In the open field test, animals treated with anesthetic ketamine 24 h before testing spent essentially no time in the central area of the apparatus, decreased horizontal ambulatory activity, and preserved to a certain extent their exploratory behaviour compared to their control counterparts. The results suggest that, in spite of its hypokinetic effect, a single anesthetic ketamine administration apparently induces an anxiety-like state, while largely preserving exploratory behaviour in the rat. These effects were time-dependent they since they were extinguished when testing was carried out 48 h after anesthetic ketamine administration.

Keywords Anesthetic ketamine · Anxiety · Rat

Introduction

The non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor ketamine is a dissociative anesthetic known for its strong psychotomimetic effects in humans and animals [1, 2]. Ketamine is commonly utilized in clinical and veterinary medicine in anesthesia and perioperative analgesia [3]. Anesthetic ketamine does not exert a depressant action on cardiovascular and respiratory system [4]. By contrast, it can induce undesired effects such as dysphoria, agitation and cognitive impairments [5].

Conflicting information is available, however, regarding the role exerted by this NMDA antagonist on anxiety. Specifically, both anxiolytic [6–8] and anxiogenic [6, 7, 9, 10] effects of treatment with sub-anesthetic doses of ketamine have been reported. Interestingly, these effects on anxiety of sub-anesthetic ketamine cannot be attributed to changes in locomotor activity. Alternatively, sub-anesthetic doses of ketamine were reported being ineffective in influencing anxiety and rodents' motor functions [11, 12].

At the moment, there is scarce information concerning the role of anesthetic ketamine in anxiety. In a previous study aiming to assess the effects on anxiety of anesthetic ketamine, performed 48 h after its administration, no effect of ketamine was observed, either in the elevated plus maze or in the new object exploration test, two behavioural procedures utilized to evaluate anxiety in rodents [13]. In addition, the authors did not show differences in terms of locomotor activity between vehicle and anesthetic ketamine-treated animals [13]. Importantly, the short-term effects of anesthetic ketamine on anxiety have not been studied up to date.

Taking into consideration the above evidence, the current experiment was designed in order to examine the short-term effects of the anesthetic ketamine on anxiety in rats. Subsequently, we thought it would be of interest to detect when the effects of anesthetic ketamine on anxiety were extinguished.

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For these studies, the light/dark box and the open field tests were used. The light/dark box test is a behavioural paradigm that is based on the natural aversion of rodents to stay in brightly illuminated areas and the conflicting tendency of animals to explore new spaces [14]. The open field test involves an encounter of the rodent with new open spaces and trigger behavioural and physiological reactions related to anxiety [15]. In addition, motor activity was also tested as an independent parameter of the potential effects of ketamine on locomotion that might affected rats' performance in the light/dark test [16].

Materials and Methods

Animals

Male 3-month-old albino Wistar rats (Hellenic Pasteur Institute, Athens, Greece) that weighed 250–300 g were utilized in this experimentation. Rats were housed in Makrolon cages (47.5 cm length × 20.5 cm height × 27 cm width) three per cage, in a climate-regulated environment (21 ± 1 °C; 50–55% relative humidity) under a 12 h/12 h (lights on at 7:00 AM) light/dark cycle with free access to food and water.

The procedures that involved animals and their care were conducted in accordance with international guidelines and national and international laws and policies (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *NIH Guide for Care and Use of Laboratory Animals*, NIH publication no. 85-23, 1985).

Drugs

Ketamine hydrochloride (Sigma, St. Louis, MO, USA) was dissolved in saline (NaCl 0.9%) and injected intraperitoneally (i.p.) at the anesthetic dose of 100 mg/kg, in a volume of 1 ml/kg. Control animals received isovolumetric amounts of the vehicle solution (saline).

Light/Dark Test

The light/dark box apparatus consisted of a wooden box (48 cm length × 24 cm height × 27 cm width) divided into two equal-size compartments by a barrier that contained a doorway (10 cm height × 10 cm width). One of the chambers was painted black and was covered with a lid and the other chamber was painted white and illuminated with a 60-W light bulb positioned 40 cm above the upper edge of the box. The test was carried out as described previously [17]. On the test day, the animals were transported to the darkened test room and remained undisturbed in their home cages for 2 h. Then the rats were placed in the middle of the lit chamber, facing away from the dark compartment. The rats were allowed to freely explore the apparatus for 5 min. The latency to enter (with all four paws) the dark compartment, number of transitions and the amount of time spent in the light and dark chambers were recorded.

Locomotor Activity Test

Spontaneous locomotor activity was assessed in an activity cage (Ugo Basile, Varese, Italy). The device consisted of a box made of Plexiglas (41 cm length × 33 cm height × 41 cm width). Every movement of the animal produced a signal caused by variations in the inductance and capacitance of resonance circuitry of the apparatus. The signals were then automatically converted into numbers that reflected motor activity counts. Changes in motor activity counts represent a standard behavioural assay for evaluating the effects of drugs on motility. The procedure used was described previously [17]. On test day, naive animals were transported to the darkened test room and left in their home cages for 2 h. Thereafter, each rat was placed into the locomotor activity cage and spontaneous locomotion was recorded for 5 min.

Open Field Test

Anxiety and motor behaviour were recorded with computerized activity monitoring (ENV515, Activity Monitor, v. 5; Med. Associates) in a transparent open activity box, as previously described [18–20].

The open activity box (40 cm length × 40 cm height × 40 cm width) was equipped with three 16-beam infrared arrays, two located on the X and Y axes for positional tracking and one on the Z axis for rearing detection. On the test day, the rats were transported to the testing room and remained undisturbed in their home cages for 2 h. Each rat was then placed in the same corner of the open field arena and its behaviour was recorded for 1 h. The parameters recorded were horizontal activity (ambulatory distance, expressed in centimeters), vertical activity (number of vertical counts) and time spent in the central area of the apparatus (expressed in seconds). Ambulatory distance reflects the animal's overall motor activity that corresponds to walking. The number of vertical counts (rearings) is a measure of the animal's reactivity to a novel environment [21] and the amount of the time spent in the central area of the open field arena is regarded as a measure of anxiety [15].

To avoid the presence of olfactory cues, all the testing apparatuses (light/dark box, open field arena and motility cage) were thoroughly cleaned with 20% ethanol and then wiped with dry paper after each trial.

Experimental Protocol

Experiments were conducted between 9:00 AM and 3:30 PM during the light phase of the light/dark cycle. Behavioural testing was performed 24 or 48 h following treatment [22]. Anesthetic state was defined as loss of righting reflex and movement. Time to gain the righting reflex was the parameter recorded for evaluating recovery from anesthesia. Data evaluation was performed by two experimenters who were unaware of the pharmacological treatment of each animal.

Experiment 1: Short-Term Effects of Anesthetic Ketamine on Rats' Performance in the Light/Dark Test

Naive rats were randomly divided into two experimental groups (10 rats per group), as follows: vehicle and ketamine 100 mg/kg. Ketamine and vehicle were administered 24 h before testing.

Experiment 2: Short-Term Effects of Anesthetic Ketamine on Rats' Performance in the Locomotor Activity Test

Naive rats were randomly divided into two experimental groups (10 rats per group), as follows: vehicle and ketamine 100 mg/kg. Ketamine and vehicle were administered 24 h before testing.

Experiment 3: Short-Term Effects of Anesthetic Ketamine on Rats' Performance in the Open Field Test

Naive rats were randomly divided into two experimental groups (7 rats per group), as follows: vehicle and ketamine 100 mg/kg. Ketamine and vehicle were administered 24 h before testing.

Experiment 4: Long-Term Effects of Anesthetic Ketamine on Rats' Performance in the Light/Dark Test

Naive rats were randomly divided into two experimental groups (8 rats per group), as follows: vehicle and ketamine 100 mg/kg. Ketamine and vehicle were administered 48 h before testing.

Experiment 5: Long-Term Effects of Anesthetic Ketamine on Rats' Performance in the Locomotor Activity Test

Naive rats were randomly divided into two experimental groups (8 rats per group), as follows: vehicle and ketamine 100 mg/kg. Ketamine and vehicle were administered 48 h before testing.

Experiment 6: Long-Term Effects of Anesthetic Ketamine on Rats' Performance in the Open Field Test

Naive rats were randomly divided into two experimental groups (9–10 rats per group), as follows: vehicle and ketamine 100 mg/kg. Ketamine and vehicle were administered 48 h before testing.

Statistical Analysis

Data were expressed as the mean ± SEM. Light/dark and motility data were analyzed using the Student independent *t* test. This statistical test is utilized for analyzing the significance of the difference between two means. Overall effects of ketamine (0–60 min) on open field data were analyzed using the Student's independent *t* test. Data were also analyzed with two-way repeated measures analysis of variance (ANOVA) with the treatment (ketamine) as between-subjects factor and time (10 min time bins) as within-subjects factor. Post-hoc comparisons between treatment means were made using Tukey's test.

Values of $p < 0.05$ were considered statistically significant [23].

Results

The righting reflex disappeared in rats that received 100 mg/kg ketamine within 8 min after treatment. Ketamine-treated animals recovered from their anesthetic effects (time to gain their righting reflex) within 30 min following drug administration.

Experiment 1: Short-Term Effects of Anesthetic Ketamine on Rats' Performance in the Light/Dark Test

The effects of anesthetic ketamine on animals' performance in the light/dark test are illustrated in Fig. 1. Anesthetic ketamine did not influence the latency to enter the dark compartment ($p > 0.05$, not significant (n.s.)) (Fig. 1A). Analysis of number of transitions results (Fig. 1B) revealed an effect of treatment. Anesthetic ketamine-treated rats made fewer transitions compared with their control counterparts ($p < 0.05$). Total time spent in the light compartment by ketamine-treated animals was significantly lower as compared to that spent by the vehicle-treated animals ($p < 0.05$, Fig. 1C).

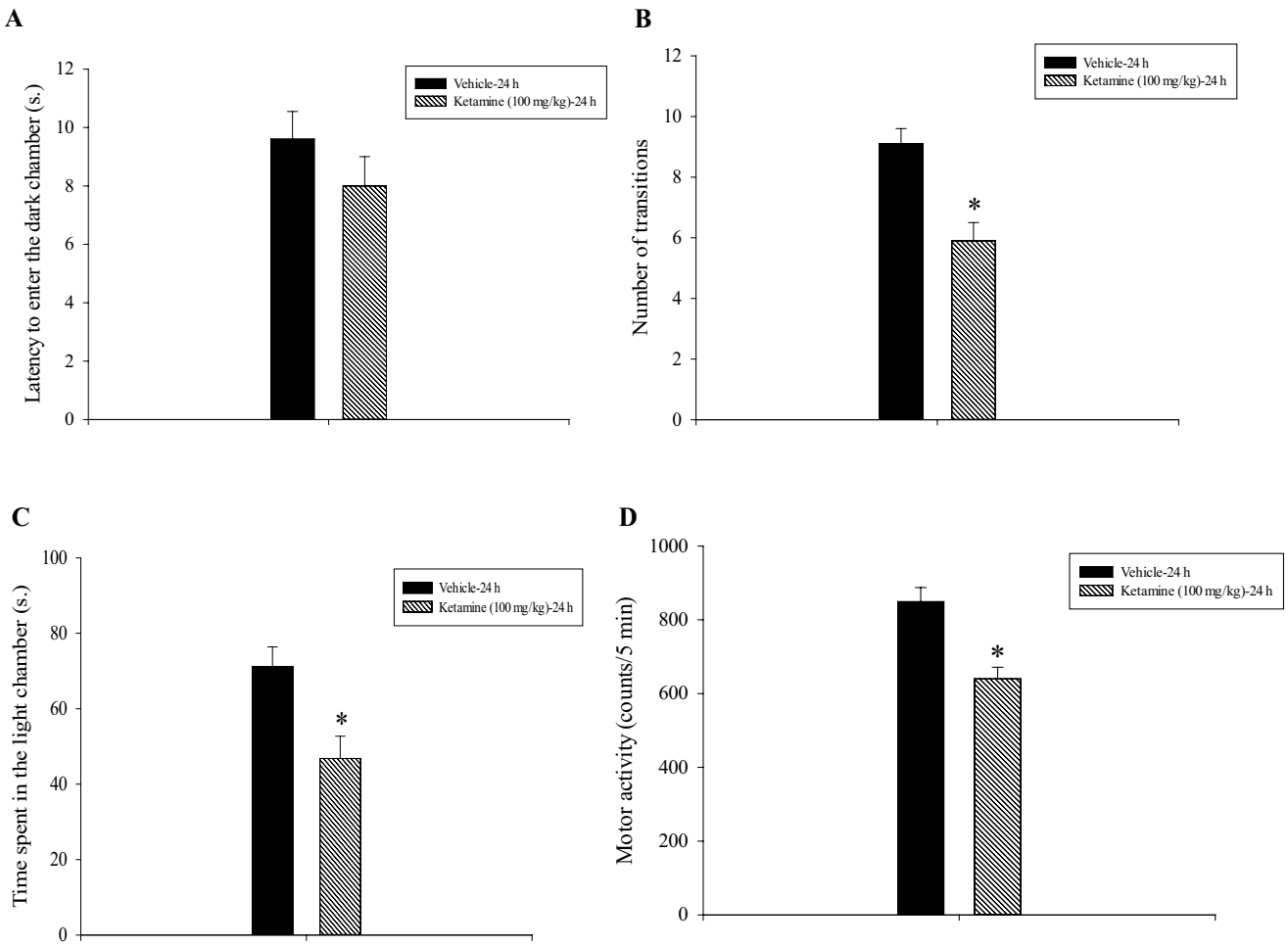

Fig. 1 Light/dark test. Vehicle and ketamine were injected intraperitoneally 24 h before testing. Results are expressed as mean ± SEM. **A** Latency to enter the dark chamber. **B** Number of transitions. **C** Time spent in the light chamber. **D** Locomotor activity counts. *p < 0.05 compared to the vehicle-treated group

Experiment 2: Short-Term Effects of Anesthetic Ketamine on Rats' Performance in the Locomotor Activity Test

Anesthetic ketamine-treated animals expressed a significantly lower locomotor activity as compared to the vehicle-treated animals ($p < 0.05$, Fig. 1D).

Experiment 3: Short-Term Effects of Anesthetic Ketamine on Rats' Performance in the Open Field Test

The results of the open field test are shown in Fig. 2 and Table 1. Animals that received anesthetic ketamine exhibited increased anxiety compared with their vehicle-treated counterparts, when tested 24 h post-injection, that was accompanied by lower levels of horizontal ambulatory and vertical activity.

Specifically, analysis of 24 h open field data in 10 min bins (Fig. 2) showed a significant treatment x time interaction for the center time [$F_{(5,60)} = 10.2$, $p < 0.001$] and for horizontal ambulatory activity [$F_{(5,60)} = 10.6$, $p < 0.001$]. Main effects of treatment [$F_{(1,12)} = 6.3$, $p = 0.03$] and time [$F_{(5,60)} = 47.5$, $p < 0.001$] were observed for vertical counts.

During the open field test, ketamine-treated rats essentially spent no time in the central zone of the test arena, at any time point recorded, with the exception of the first 10 min period, when they were present in the center for approximately 3 s. In contrast, their control counterparts spent 10x more time in the center and maintained this behaviour throughout the first 40 min of the test ($p < 0.01$, Fig. 2A).

During the test, both treatment groups gradually decreased their horizontal ambulatory activity. Ketamine-treated rats had approximately 50% lower ambulatory distance in the beginning of the test, compared with vehicle,

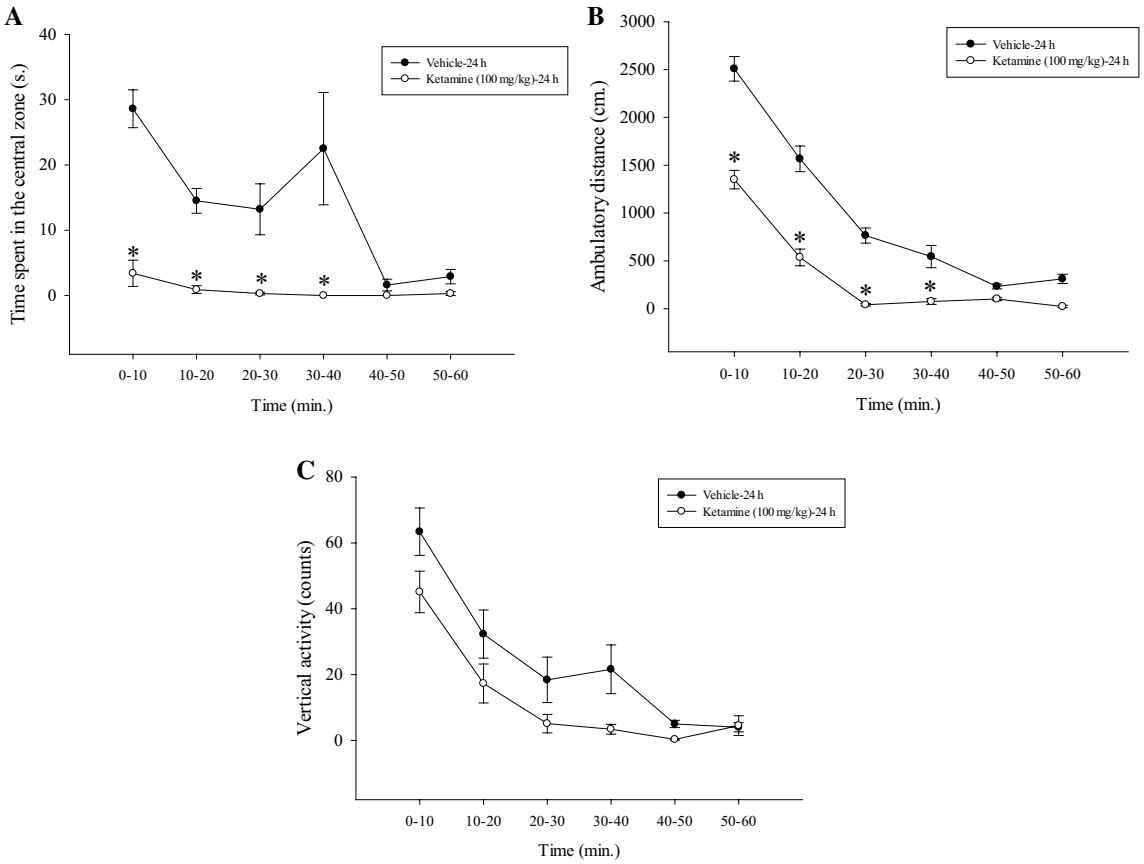

Fig. 2 Open field test. Vehicle and ketamine were injected intraperitoneally 24 h before testing. Results are expressed as mean ± SEM. **A** Time spent in the central zone. **B** Ambulatory distance. **C** Vertical activity counts. *p < 0.05 compared to the vehicle-treated group

Table 1 Open field activity recorded 24 h or 48 h after one anesthetic ketamine injection in rats

Time frame	Vehicle-24 h	Ketamine-24 h	Vehicle-48 h	Ketamine-48 h
		Time spent in the central zone (s)		
0–60 min	83.3 ± 9.9	4.8 ± 2.0 *	62.7 ± 11.0	63.2 ± 18.0
		Ambulatory distance (cm)		
0–60 min	5929.6 ± 115.3	2036.1 ± 277.8*	5009.3 ± 394.1	5405.5 ± 758.1
		Vertical counts (number of rearings)		
0–60 min	144.7 ± 26.1	75.6 ± 19.8*	120.1 ± 18.2	133.8 ± 19.7

The values are mean ± SEM. * p < 0.01 vs. vehicle

and exhibited very little ambulation thereafter. Ketamine-treated rats had significantly lower ambulatory activity compared with vehicle, during the first four time bins ($p < 0.01$, Fig. 2B).

Finally, vertical activity gradually decreased in both treatment groups and was lower in ketamine-treated rats, but the difference did not reach statistical significance at any time point (Fig. 2C).

Cumulative behavioural measures such as total time spent in the center of the open field, total horizontal ambulatory activity and total vertical activity were significantly lower in ketamine-treated rats compared with their vehicle-treated cohorts ($p < 0.001$, $p < 0.001$ and $p = 0.03$ respectively) (Table 1).

Experiment 4: Long-Term Effects of Anesthetic Ketamine on Rats' Performance in the Light/Dark Test

Treatment with anesthetic ketamine had no effect on animals' performance in the light/dark test assessed 48 h following its administration. Specifically, anesthetic ketamine

did not affect the first entry into to the dark chamber (Fig. 3A), the number of transitions between the two compartments (Fig. 3B) or the time spent in the light chamber of the apparatus (Fig. 3C) ($p > 0.05$).

Experiment 5: Long-Term Effects of Anesthetic Ketamine on Rats' Performance in the Locomotor Activity Test

The statistical results of motor activity data revealed that anesthetic ketamine injected 48 h before testing did not influence rats' motility ($p > 0.05$, Fig. 3D).

Experiment 6: Long-Term Effects of Anesthetic Ketamine on Rats' Performance in the Open Field Test

The results of the open field test carried out in rats 48 after the administration of anesthetic ketamine are reported in Fig. 4 and Table 1. The findings indicate that treatment with ketamine did not influence rats' performance in this test.

Discussion

The light/dark test is a behavioural paradigm designed to evaluate the anxiolytic- and anxiogenic-like action of drugs in rodents [15]. It is a rapid procedure, is easy to use and does not require training of animals. In addition, to not requiring training, the light/dark test does not require food or water deprivation [24]. Transitions between the light and dark chambers are considered an index of activity/exploration because habituation over time is observed with this measure, while the time spent in each chamber of the apparatus is considered an index of aversion or attraction [25].

Acute treatment with anesthetic ketamine, 24 h before testing, produced an anxiogenic-like effect in rats in the light/dark test. In this context, it has been observed that anesthetic ketamine did not influence the latency of the first

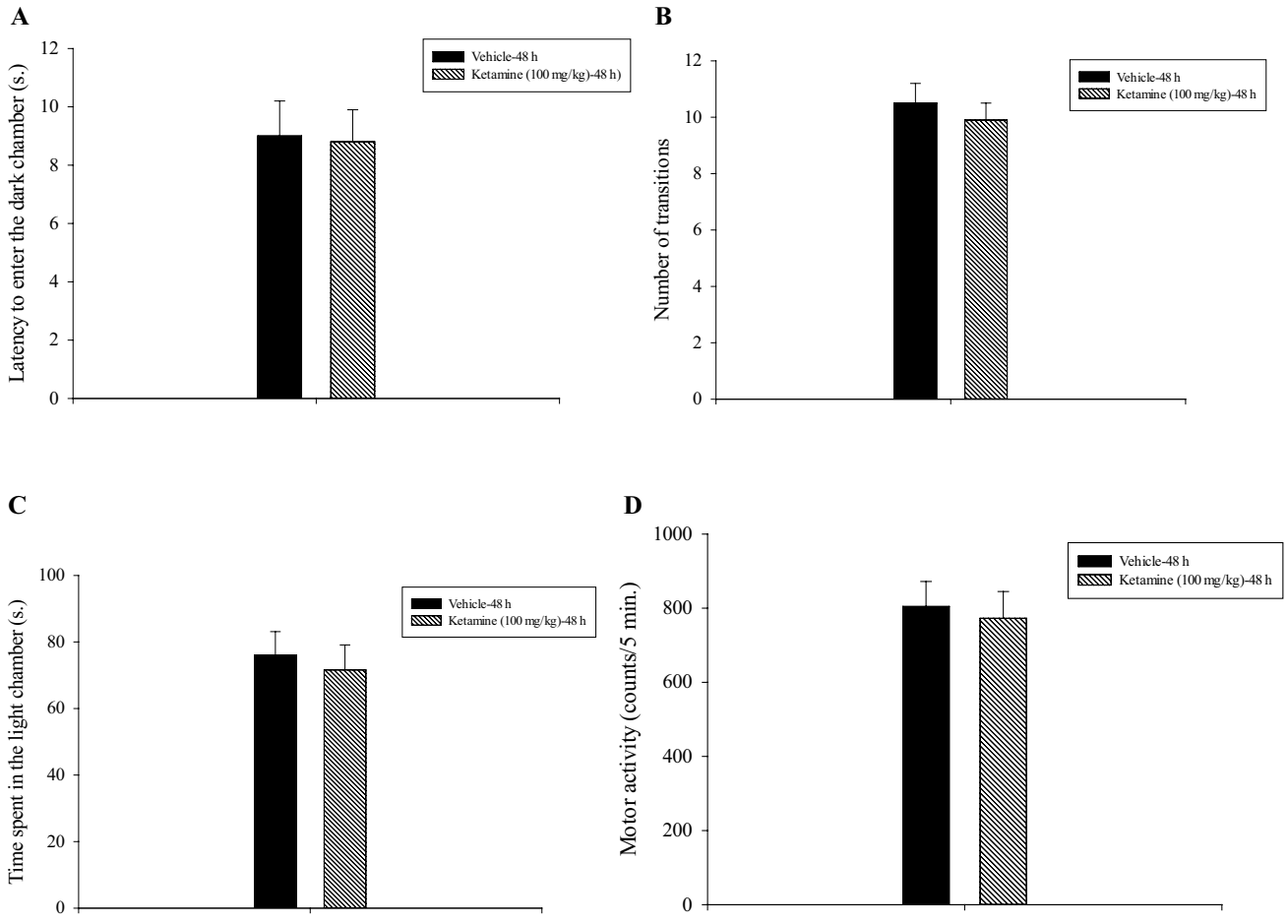

Fig. 3 Light/dark test. Vehicle and ketamine were injected intraperitoneally 48 h before testing. Results are expressed as mean ± SEM. **A** Latency to enter the dark chamber. **B** Number of transitions. **C** Time spent in the light chamber. **D** Locomotor activity counts

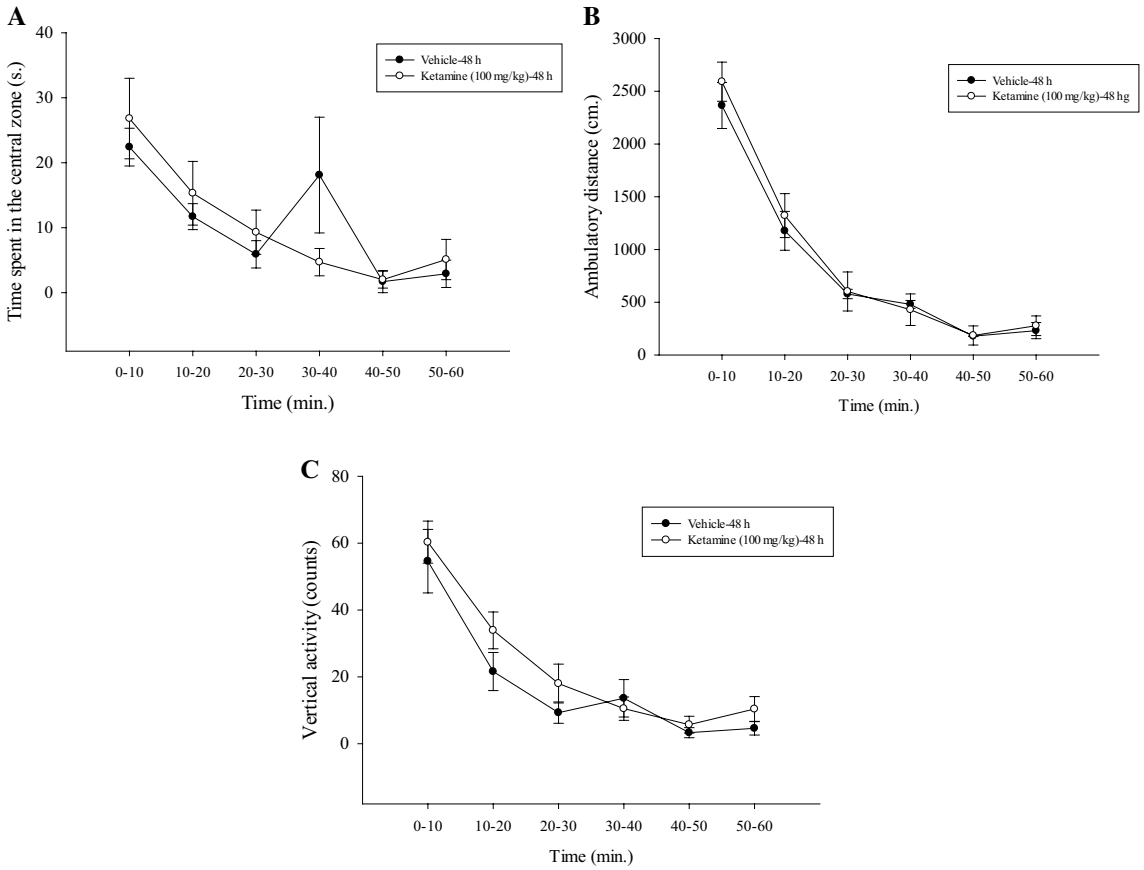

Fig. 4 Open field test. Vehicle and ketamine were injected intraperitoneally 48 h before testing. Results are expressed mean ± SEM. **A** Time spent in the central zone. **B** Ambulatory distance. **C** Vertical activity counts

entry into the dark chamber but decreased both the number of transitions between the dark and lit chamber and the time spent by rats in the light chamber of the apparatus compared to their control cohorts. These results indicate that the reduced time spent in the lit chamber of the light/dark box by the rats that received ketamine was partially due to a drug-induced consistent reduction of locomotor activity.

Drugs that affect general motor function may affect performance in anxiety tests because of changes in motoric activity that are unrelated to any anxiogenic- or anxiolytic-like effects of the compound. Thus, the assessment of motor activity in rodents after administration of a test drug is required to evaluate the possibility of nonspecific motoric effect that may confound the interpretation of the results from the anxiety tests. The present locomotor activity findings clearly propose that anesthetic ketamine depressed animals' motility in the motor activity test when injected 24 before testing. These motility results are in agreement with previous findings in which administration of anesthetic doses of ketamine (100–150 mg/kg) reduced rats' motor activity 24 h following treatment [26, 27]. On the other hand, anesthetic ketamine (150 mg/kg) assessed at different time frames (24, 48 and 72 h) after its administration did not alter mouse locomotor activity [28]. The species utilized (mouse vs. rat used in the other studies) and the age of animals (7 months-old mice vs. 3-month-old rats utilized in our studies) may underlie this discrepancy.

Interestingly, acute administration of anesthetic ketamine 48 h before testing had no effect on any measure in the light/dark box test and did not influence animals' motor abilities assessed in the motor activity cage.

The open field test is a neophobic test of anxiety and is based on rodent's natural tendency to avoid open spaces. Therefore, the amount of time spent in the central area of the apparatus reflects the anxiety-like state of rodents [15]. This procedure also evaluates responses to novelty and alert exploratory behaviour by measuring vertical activity (rearings) [18, 21], as well as overall locomotor activity by measuring ambulation (walking). Further, if the rats have not been previously placed in the open field chamber, as is the case for the current study, this test also assesses animal response to novelty [18]. Exploratory behaviour, mostly corresponding to vertical activity but also to ambulatory activity, is more prominent during the first half of

the test and gradually declines, as the animal habituates to the environment. Vertical activity in response to novelty has been previously used as a differentiating factor when characterizing drug-induced effects on behaviour [18].

Anesthetic ketamine induced an anxiogenic-like effect in the open field test, 24 h after its administration. In particular, rats that received ketamine essentially did not spend any time in the central zone of the open field at any time point recorded, as compared to the vehicle-treated rats.

On the other hand, anesthetic ketamine administration decreased but did not block ambulation compared with the vehicle. Ambulatory distance expressed by ketamine-treated rats was approximately 50% lower, respect to that expressed by vehicle-treated animals, during the first 20 min of the test, and dropped to very low levels thereafter. This suggests, that in spite of their overall lower ambulation, ketamine-treated rats exert their motor profile during the first phase of the test, when they encountered a novel environment. Finally, alert exploratory behaviour and response to novelty, as measured by the number of vertical counts, was overall lower in ketamine-treated rats, compared with their vehicle-treated counterparts, but the difference did not yield a statistical significance at any time point of the test.

Overall, these findings show that a single anesthetic ketamine administration is followed, at 24 h post-treatment, by an anxiety-like behavioural profile that is accompanied by decreased ambulation, while exploratory behaviour is sustained. Thus, we can postulate that the avoidance of the open field center was not merely a side-effect of the ketamine-induced hypokinetic phenotype but may be a specific manifestation of an anxiety-like state.

Ketamine-treated rats tested 48 h after treatment did not show any difference as compared to their control cohorts in all variables of the open field test.

As a whole, results here presented suggest that the behavioural effects of anesthetic ketamine were dependent on the time between the injection and testing since the effects were present at 24 h but were absent 48 h after treatment. The anxiogenic effect observed 24 h after treatment could be considered prominent, given that ambulatory and exploratory activities were preserved to a certain degree. Thus, it is reasonable to suggest that the anxiety-like state, observed in the open field and the light/dark tests, was not a residual effect of the decreased ambulation but an independent behavioural response.

Moreover, our results of the experiments performed 48 h after treatment with anesthetic ketamine are in agreement with a previous study in which anesthetic ketamine was also found ineffective to influence rats' anxiety levels, as determined with the elevated plus maze test, and to influence movement time in an arena, when it was administered 48 h before testing [13].

As we have already mentioned the impact of anesthetic ketamine on anxiety remains largely unexplored. Our results suggest that the behavioural effects of ketamine persist for 24 h (but disappeared after 48 h) indicating that this time point of its administration is crucial for its action.

The mechanism(s) by which anesthetic ketamine produces its unwanted behavioural effects has yet to be clarified. It has been reported that anesthetic ketamine produces its unwanted behavioural effects by acting, at least in part, as antagonist to the NMDA receptor [29]. Based on consistent experimental findings it cannot be ruled out that target sites other than NMDA receptors might mediate the actions of anesthetic ketamine such as the $GABA_A$ receptor [30] and/or the nicotinic cholinergic receptor [3].

Additionally, experimental evidence suggests that anxiety is related to oxidative stress [31] and *c-fos* expression in the limbic system [32]. Interestingly, ketamine at anesthetic doses was found to increase oxidative stress [33] and to induce marked *c-fos* expression in rodents [34].

The exact role of anesthetic ketamine in anxiety is difficult to interpret based on the above reported data. It is important to emphasize that the effects of anesthetic ketamine on anxiety were evaluated in the elevated plus maze, the new object exploration [13], the light/dark box and the open field test (present study). These paradigms are unconditioned exploration-driven anxiety-related tests that are based on the conflict between the desire to explore and avoidance of new spaces [35]. To our knowledge, the effects of anesthetic ketamine on anxiety were not tested by utilizing conditioned non-exploration-driven anxiety-related tests, such as ultrasonic vocalization and stress-induced hypothermia or conditioned operant conflict paradigms such as the Geller-Seifter and Vogel conflict tests [35].

Finally, the effects of anesthetic ketamine on anxiety were observed in behavioural studies conducted in male rats [13, present study]. Of note, no study has addressed the effects of anesthetic ketamine on anxiety-like behaviour in female animals up to date. Importantly, is well documented that the prevalence of anxiety for women is approximately twice that for men [36]. Exploring the effects of anesthetic ketamine on anxiety by utilizing female rodents might be of high translational relevance. Further research, therefore, is needed to definitively clarify the role of this dissociative anesthetic in anxiety disorders.

Conclusions

In summary, studies herein presented indicate a time-dependent anxiogenic-like action of anesthetic ketamine revealed in the light/dark box and the open field paradigms in rats which however was accompanied by a decrease but not blockade of motor activity.

Compliance with Ethical Standards

Conflict of interest The authors declare no potential conflicts of interest with respect to authorship and/or publication of this article.

Research Involving Animal Rights All applicable international and national guidelines for the care and use of animals were followed.

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