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ORIGINAL INVESTIGATION

Ketamine differentially affects implicit and explicit memory processes in rats

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Abstract

Rationale Ketamine, a non-competitive NMDA receptor antagonist, produces antidepressant effects at subanesthetic doses. The therapeutic effect, however, is often accompanied by cognitive side effects, including memory impairments. Yet, the specific effects of ketamine on different processes of implicit and explicit memory remain to be elucidated.

Objectives We examined the effect of an antidepressant dose of ketamine (10 mg/kg, IP) on the encoding, retrieval, and modulation processes of fear memory and spatial memory in adult Wistar rats.

Methods Ketamine was administered before the fear acquisition, retrieval, or extinction procedures in a Pavlovian fear conditioning task. In another set of experiments, it was administered before the training, probe trial, or reversal training phases of the Morris Water Maze (MWM).

Results The antidepressant dose of ketamine partially impaired fear extinction when administered before the acquisition or retrieval. In contrast, it facilitated memory modulation and decreased the escape latency in the first day of reversal training in the MWM when administered before the training or reversal training sessions. Encoding or retrieval performance in either type of memory was not affected.

Conclusions These findings show that ketamine does not impair the acquisition or retrieval processes of cued fear or spatial memory; but exerts differential effects on memory modulation of these implicit and explicit memory paradigms, by disrupting fear extinction and facilitating reversal spatial learning.

Keywords Ketamine · Implicit memory · Explicit memory · Fear conditioning · Morris water maze

Introduction

Ketamine, initially recognized as a general anesthetic (Austin and Tamlyn 1972; Rogers et al. 2004), has proved to be a promising rapid-acting antidepressant at sub-anesthetic doses. The antidepressant effects of ketamine have been well observed in rodents at different doses and routes of administration, including intraperitoneal (IP) injections (da Silva et al. 2010; Garcia et al. 2008; Gokalp and Unal 2024; Yilmaz et al. 2002), oral consumption (Ecevitoglu et al. 2019; Kingir et al. 2023), and ointment preparations (Akan et al. 2023). While the therapeutic power of ketamine is well documented in preclinical (Autry et al. 2011; Brachman et al. 2016; Engin et al. 2009; Gass et al. 2019; Jett et al. 2015; Papp et al. 2017; Sofia and Harakal 1975) and clinical studies (Berman et al. 2000; Li et al. 2016; Murrough et al. 2013; Zarate et al. 2006), its cognitive side effects requires further exploration. Specifically, the effects of ketamine on implicit, or affective, memory have been mostly studied using fear conditioning paradigms, yet its comparative influence on different stages of fear memory remains unclear (Choi et al. 2020). Likewise, studies investigating the impact of antidepressant ketamine on explicit memory is also limited. In this study, we examined the impact of an antidepressant dose of ketamine (10 mg/kg; see Carrier and Kabbaj 2013; Choi et al. 2020) on the encoding, retrieval, and modulation processes in adult Wistar rats by utilizing cued fear conditioning and the Morris water maze (MWM), two widely employed rodent tests for assessing implicit and

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explicit memory (Morris 1981; Vorhees and Williams 2006; Zola-Morgan and Squire 1990).

The effects of ketamine on implicit learning and memory have been extensively studied (Calzavara et al. 2009; Mastrodonato et al. 2020; McGowan et al. 2017, 2018; Radford et al. 2020, 2022; Saur et al. 2017). However, the results are often conflicting (Silote et al. 2020), particularly in fear conditioning paradigms (Choi et al. 2020). Administration of a 10 mg/kg IP ketamine 15 min prior to the contextual fear acquisition reduced fear memory on the testing day (Calzavara et al. 2009). Conversely, an increase in fear memory was noted when the same dosage (IP) was applied 15 min before fear reactivation (Honsberger et al. 2015). A similar controversy was illustrated by two studies employing the identical higher dose (30 mg/kg, IP in mice) of ketamine. One study observed a reduction in fear response solely when administered before conditioning (McGowan et al. 2017), while the other noted this effect on fear generalization only with post-conditioning injection (Asim et al. 2020). Notably, when a 10 mg/kg IP ketamine was administered 24 h before extinction, it reduced fear memory and mitigated fear renewal (Girgenti et al. 2017). In contrast, administering it 24 h prior to a situational reminder resulted in an increase in fear memory (Saur et al. 2017), suggesting the significance of the stage of memory before which ketamine was applied. These results indicate that the variety of experimental designs, the dose of ketamine, as well as the route and timing of administration, determine the specific effects of ketamine on different phases of fear memory (Choi, 2020).

Similar to its effects on implicit paradigms, ketamine may produce a variety of side effects on cognitive processes (Enomoto and Floresco 2009; Goulart et al. 2010; Ide et al. 2019; Imre et al. 2006; Pitsikas et al. 2008; Riggs et al. 2021), including explicit memory (Alessandri et al. 1989; Li et al. 2017; Moosavi et al. 2012; Pitsikas et al. 2008; Sabbagh et al. 2012; Zhang et al. 2021). In particular, ketamine administration has yielded mixed results on spatial memory performance in the MWM. A single anesthetic dose of ketamine (100 mg/kg, IP) impaired spatial memory when administered 120 min prior to the training or the probe trial, as well as immediately after the training (Moosavi et al. 2012). Similarly, an 80 mg/kg subcutaneous (SC) ketamine injection 24 h before the probe trial impaired performance, while 20 mg/kg facilitated it, suggesting a dose-dependent effect (Shi et al. 2021). Conversely, a 30 mg/kg IP ketamine administration 90 min before the probe trial (Duan et al. 2013), and a 15 mg/kg dose 40 min prior to the training or probe trial (Moosavi et al. 2012) caused memory impairments. A single dose of 10 mg/kg IP ketamine given 3 days before the first training session had no effect on spatial memory (Holubova et al. 2016). However, no study has systematically evaluated the effects of a clinically relevant dose of 10 mg/kg IP ketamine on various spatial memory processes in the Morris water maze.

In this study, we evaluated the effects of ketamine on memory encoding, retrieval, and modulation processes in an implicit and an explicit learning paradigm using a comparable experimental design. We administered a clinically relevant dose of ketamine (10 mg/kg, IP) 30 min prior to either the acquisition, retrieval, or extinction phases of a cued fear conditioning paradigm. In another set of experiments, the same dose was administered before the training, probe trial, or reversal training phases of the MWM. This design enabled us to systematically compare the effects of ketamine on the encoding, retrieval, and modulation processes of both fear memory and spatial memory.

Materials and methods

Subjects

A total of 60 experimentally naïve adult male Wistar rats were used. Twenty-eight rats were utilized in the fear conditioning experiment (3–5 months old, $M = 311.71$ g, $SD = 311.72$ g, $n = 7$) and thirty-two rats were employed for the MWM experiment (3–5 months old, $M = 299.44$, $SD = 13.88$, $n = 8$). The animals were housed in cages of four under controlled laboratory conditions (21 ± 1 °C; 40–60% humidity; 12:12 day/night cycle, lights on at 08:00) with *ad libitum* access to food and water. All experimental procedures were approved by the Boğaziçi University Ethics Committee for the Use of Animals in Experiments.

Experimental groups and design

Two sets of experiments were conducted to assess implicit (fear conditioning experiment) and explicit memory (Morris water maze experiment). The animals in each experiment were divided into four different groups based on the timing of ketamine administration (i.e., before a particular memory phase). Each group received an antidepressant dose (10 mg/kg) of IP ketamine injection prior to one of the phases of fear conditioning or MWM. For the fear conditioning experiment, Ket-Acq group received ketamine 30 min prior to the fear acquisition, Ket-Ret group prior to the fear retrieval, and Ket-Ext group prior to the first day of fear extinction. In the MWM experiment, Ket-Tra group received ketamine 30 min prior to the first day of training, Ket-Pro group prior to the probe trial, and Ket-Rev group prior to the first day of reversal training (Table 1). Ketamine hydrochloride (Keta-control, Doğa İlaç, 100 mg/ml, Istanbul, Turkey) in normal saline was administered at a 1 ml/kg volume. All

Table 1 IP injection timeline

Groups	Fear Conditioning		
	Acquisition (Day 7)	Retrieval (Day 8)	Extinction I (Day 9)
Ket-Acq	Ketamine	Saline	Saline
Ket-Ret	Saline	Ketamine	Saline
Ket-Ext	Saline	Saline	Ketamine
Vehicle	Saline	Saline	Saline
	Morris Water Maze		
	Training Day 1 (Day 6)	Probe Trial (Day 10)	Reversal Training Day 1 (Day 11)
Ket-Tra	Ketamine	Saline	Saline
Ket-Pro	Saline	Ketamine	Saline
Ket-Rev	Saline	Saline	Ketamine
Vehicle	Saline	Saline	Saline

animals were injected with saline (vehicle) when they did not receive ketamine (Table 1).

Both the fear conditioning and the MWM experiments started with a 5-day habituation and handling period in order to reduce handling stress during IP injections and behavioral testing (Fig. 1). On the third day of handling, all animals were subjected to an open field test (OFT) to assess their baseline levels of locomotor activity and anxiety-like behavior (Prut and Belzung 2003). This was followed by either the fear conditioning or the MWM. All behavioral procedures were carried out between 09.00 and 19.00 in the same test room. The animals underwent transcardial perfusion-fixation with 4% depolymerized paraformaldehyde at the end of behavioral testing.

Open field test

The test apparatus consisted of a square arena (70 × 70 × 45 cm) enclosed with opaque walls. The center of the maze was illuminated to a brightness of 90 ± 5 lx, making it brighter than the periphery, which was illuminated to 55 ± 5 lx. The test duration was 5 min, during which overall mobility, total travel distance, and the time spent in the periphery and center of the arena were analyzed. To prevent the presence of olfactory cues from previous animals, the maze was cleaned with 70% ethanol between each test session. Behavioral analyses were conducted using ezTrack software (Pennington et al. 2019).

Fear conditioning

The cued fear conditioning procedure consisted of habituation, fear acquisition, fear retrieval, two days of fear extinction in a different context, and fear renewal following a two-day gap (Fig. 1A). The conditioning chamber (21 × 45 × 27 cm) was a rectangular cage with glass walls and a floor made up of 32 metal grids (Context A). The

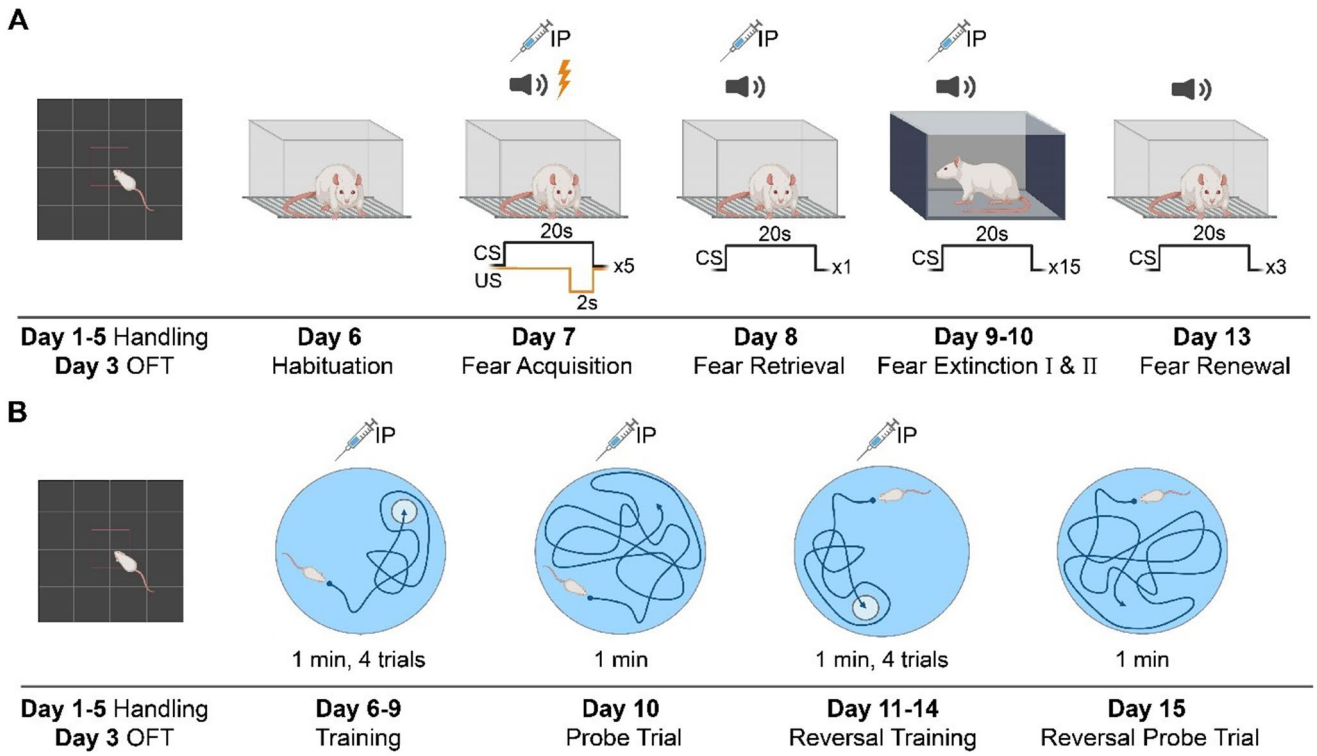

Fig. 1 Experimental timeline of **(A)** the fear conditioning experiment and **(B)** the Morris water maze experiment. IP, intraperitoneal injections; CS, conditioned stimulus; US, unconditioned stimulus; OFT, open field test

procedure started on Day 6 by placing the animals in the conditioning chamber for 10 min to allow them to habituate to the context. On subsequent days, animals were given 3 min to adjust to the test environment prior to the session and 1 min afterward before being gently transferred to their home cages. The test cage was cleaned with 70% ethanol between each session.

Fear conditioning (Day 7) consisted of 5 pairings of a conditioned stimulus (CS) and an unconditioned stimulus (US) presented with 90–120 s of randomly generated intertrial intervals (ITI). The CS was a neutral tone (75 dB, 2 kHz) lasting for 20 s, and the US was a mild foot shock (0.7 mA) lasting for 2 s, co-terminating with the CS. The fear retrieval procedure was carried out on the next day by presenting a single CS (without US) in the same context. On the next two days (Day 9 and 10), fear extinction procedure was applied in a different chamber (41 × 43 × 31) with transparent acrylic walls and flat ground, placed in a different location of the test room (Context B). The illumination of the test room was darker, and the cage was cleaned with 1% peppermint oil to provide a different olfactory cue compared to Context A. In this phase, 15 CSs were presented in each day with the same ITI range, followed by a two-day gap. On Day 13, the fear renewal phase was carried out in Context A by presentation of 3 CSs. Freezing levels were analyzed with ezTrack (Pennington et al. 2019).

Morris water maze

The MWM design in this study consisted of 4 days of training, a probe trial, 4 days of reversal training, and a reversal probe trial (Fig. 1B). The maze (diameter = 120 cm, water temperature 27 ± 1 °C) had four distinct visual cues that were attached to the walls, 90° apart from each other. In both the training and reversal training days, there was a hidden escape platform (diameter = 10 cm) located 2.5 cm below the water surface, which were removed in the probe and reversal probe trials.

Training days (Day 6–9) consisted of four consecutive trials lasting 2 min each. In each trial, animals began from a different starting point that was counterbalanced across the training days. Animals were given 60 s to find the platform (Platform A) in the target quarter (Quarter A). If they were unable to find the platform within this time frame, they were gently guided to it by their tails. In either case, animals were allowed to stay on the platform for 15 s. For reversal training days (Day 11–14), the same procedure was applied, but the platform (Platform B) was located in the opposite quarter (Quarter B).

In the probe trials (Day 10–15), animals were placed from the opposite direction of where the platform was located in the corresponding training session. There was a single trial lasting for 60 s. All parameters were analyzed for Quarter A and Quarter B, as well as Platform A and Platform B. Escape latencies, quarter durations, quarter entries, and platform crossings were analyzed using EthoVision XT 17 (Noldus, Netherlands).

Statistical analysis

To assess the effect of ketamine on different processes of implicit or explicit memory, each experimental group was compared with the vehicle group in both experiments. This approach was selected to maintain statistical power since the groups transitioned into experimental groups across the test sessions. The Ket-Acq group in the fear conditioning experiment and the Ket-Tra group in the MWM were compared with their corresponding vehicle groups to assess the effects of ketamine on encoding. The Ket-Ret and Ket-Pro groups were used to evaluate the impact of ketamine on memory retrieval. Lastly, the Ket-Ext and Ket-Rev groups were compared with the vehicle groups to assess the potential impact of ketamine on the modulation of an acquired memory.

Sessions involving multiple cues (i.e., conditional stimuli) during fear conditioning, comparisons across extinction sessions, as well as both training and reversal training in the MWM, were analyzed using two-way repeated measures Analysis of Variance (ANOVA). Paired or independent samples *t*-tests were employed to analyze the freezing levels in the fear retrieval session, which involved a single cue, as well as MWM probe trials. During the extinction phase, three blocks were created by averaging the freezing response to every five cues. Post hoc comparisons were applied with Bonferroni correction following ANOVA. Differences were considered statistically significant if $p < 0.05$. All analyses were conducted using GraphPad Prism 10.02.

Results

Locomotor activity and anxiety-like behavior

Fear conditioning groups did not differ in terms of the total distance traveled ($F(3, 24) = 0.278$, $p = 0.841$, one-way ANOVA; Fig. 2A), and the time spent in the center of the open field ($F(3, 24) = 0.614$, $p = 0.612$, one-way ANOVA; Fig. 2B). Similarly, there were no differences between the MWM groups in their travel distance ($F(3, 28) = 1.306$, $p = 0.292$, one-way ANOVA; Fig. 2C) and the time spent in the center of the maze ($F(3, 28) = 0.371$, $p = 0.774$, one-way ANOVA; Fig. 2D). These results show that the baseline locomotor activity and anxiety levels of the experimental groups did not differ from each other.

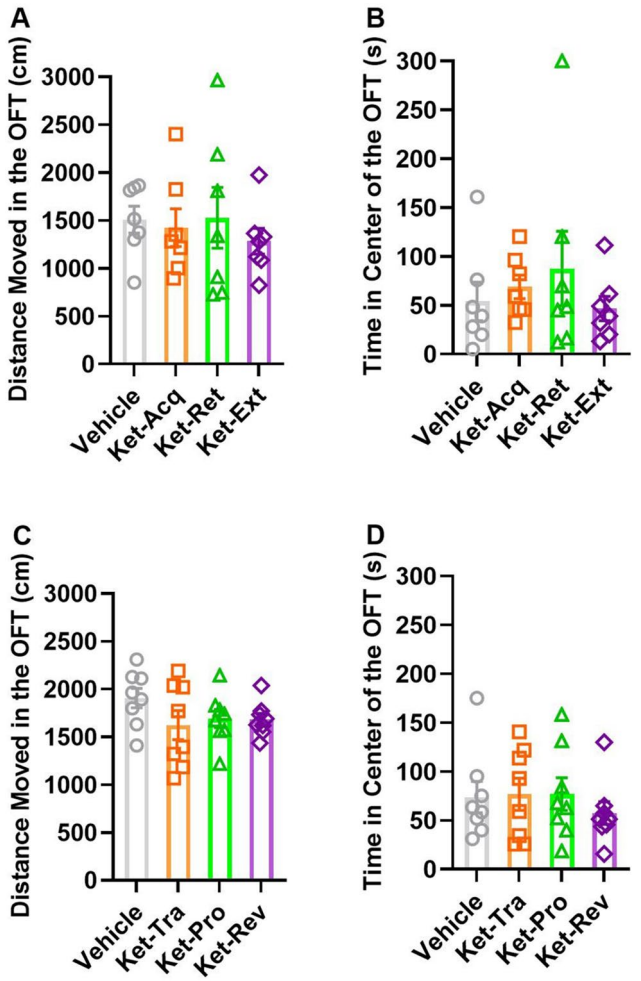

Fig. 2 Locomotion and anxiety-like behavior in the OFT. **(A)** Locomotor activity levels in terms of distance moved (cm) in the fear conditioning experiment ($n=7$). **(B)** The time spent in the center of the OFT in the fear conditioning experiment. **(C)** Locomotor activity levels in terms of distance moved (cm) in the MWM experiment ($n=8$). **(D)** The time spent in the center of the OFT in the MWM experiment

The effects of ketamine on fear memory

During the acquisition phase, the freezing response of all groups increased across cues, indicating successful auditory conditioning. Additionally, all groups showed more freezing during the baseline period of the retrieval phase compared to the acquisition phase, highlighting the effects of contextual conditioning. During the renewal phase, the freezing responses did not change across the cues (refer to Table 2 for statistical results).

Fear memory acquisition

The freezing response to the cues did not differ between Ket-Acq and the vehicle groups during the acquisition phase, as indicated by the lack of group effects ($F(1, 12)=1.154$, $p=0.304$, two-way repeated measures ANOVA; Fig. 3A) and interaction effects ($F(4, 48)=1.675$, $p=0.170$). This pattern was also observed in the retrieval phase ($t(12)=0.548$, $p=0.594$, independent samples t-test; Fig. 3B). In the extinction, there was a significant effect of blocks on both the first day ($F(2, 24)=5.295$, $p=0.012$, $\eta 2=0.119$, two-way repeated measures ANOVA; Fig. 3C) and the second day ($F(2, 24)=4.666$, $p=0.019$, $\eta 2=0.123$; Fig. 3D); even though there was no group (Extinction I: $F(1, 12)=0.573$, $p=0.464$; Extinction II: $F(1, 12)=0.418$, $p=0.530$) or interaction effect in these sessions (Extinction I: $F(2, 24)=1.410$, $p=0.264$; Extinction II: $F(2, 24)=1.532$, $p=0.237$).

Post-hoc tests revealed that the freezing response in the vehicle group decreased from the first block ($M=59.674$, $SD=23.433$) to the last block on the second day of extinction ($M=36.760$, $SD=11.941$; $t(24)=3.103$, $p=0.015$, Cohen's $d=1.232$, Bonferroni corrected), whereas this difference was not observed in Ket-Acq group (first block: $M=59.520$, $SD=20.631$; last block: $M=52.217$, $SD=17.621$; $t(24)=0.989$, $p=0.998$, Bonferroni corrected). This indicates that ketamine partially impaired fear extinction when administered before acquisition. Notably, baseline freezing levels increased across the extinction sessions (Table 2; Fig. 3C, D), suggesting a contextual generalization of the fear response. This increase was statistically significant in the vehicle group (Extinction I: $M=5.683$, $SD=7.014$; Extinction II: $M=27.840$, $SD=19.595$; $t(12)=3.391$, $p=0.011$, Cohen's $d=1.506$, Bonferroni corrected); but not in the Ket-Acq group (Extinction I: $M=13.003$, $SD=10.511$; Extinction II: $M=22.210$, $SD=14.272$; $t(12)=1.409$, $p=0.369$, Cohen's $d=0.735$, Bonferroni corrected). The groups did not differ in terms of the freezing response during the renewal phase ($F(1, 12)=0.003$, $p=0.955$, two-way repeated measures ANOVA, Fig. 3E), and no group-cue interaction was observed in this session ($F(2, 24)=0.290$, $p=0.751$).

Fear memory retrieval

No difference was observed between Ket-Ret and vehicle groups in their freezing response to the cue during the acquisition phase (Table 2; Fig. 3A), as expected since the Ket-Ret group had not yet received the ketamine injection. However, the groups also did not differ in the fear retrieval phase ($t(12)=0.468$, $p=0.648$, independent samples t-test; Fig. 3B). During the extinction phase, freezing levels decreased across the blocks on both the first ($F(2, 24)=4.283$, $p=0.026$, $\eta 2=0.079$, two-way repeated measures ANOVA; Fig. 3C) and the second day ($F(2, 24)=6.580$, $p=0.005$, $\eta 2=0.110$, two-way repeated measures ANOVA; Fig. 3D). However, no group-level effects or interactions

Table 2 Statistical comparisons

Group(s)	Phase(s)	Measurement		Statistical Test	Comparison	F/t	df	*p*	η2/d
Vehicle	Acquisition & Retrieval	Freezing	Baseline	Paired t-test (one-tailed)	Phase	2.374	6	0.028	0.704
Ket-Acq	Acquisition & Retrieval	Freezing	Baseline	Paired t-test (one-tailed)	Phase	3.778	6	0.005	1.813
Ket-Ret	Acquisition & Retrieval	Freezing	Baseline	Paired t-test (one-tailed)	Phase	2.017	6	0.045	0.555
Ket-Ext	Acquisition & Retrieval	Freezing	Baseline	Paired t-test (one-tailed)	Phase	2.102	6	0.040	1.186
Ket-Acq vs. Vehicle	Acquisition	Freezing	5 Cues	RMANOVA	Cues	12.000	4,48	< 0.0001	0.414
Ket-Acq vs. Vehicle	Extinction I&II	Freezing	Baseline	RMANOVA	Phase	11.520	1,12	0.005	0.265
					Group	0.022	1,12	0.884	0.007
					Interaction	1.964	1,12	0.186	0.045
Ket-Acq vs. Vehicle	Renewal	Freezing	Cues	RMANOVA	Cues	0.244	2,24	0.786	0.009
Ket-Ret vs. Vehicle	Acquisition	Freezing	5 Cues	RMANOVA	Cues	10.600	4,48	< 0.0001	0.367
					Group	0.013	1,12	0.909	0.000
					Interaction	0.340	4,48	0.850	0.012
Ket-Ret vs. Vehicle	Extinction I&II	Freezing	Baseline	RMANOVA	Phase	35.330	1,12	< 0.001	0.241
					Group	1.379	1,12	0.263	0.069
					Interaction	0.590	1,12	0.457	0.004
Ket-Ret vs. Vehicle	Renewal	Freezing	Cues	RMANOVA	Cues	0.399	2,24	0.676	0.013
Ket-Ext vs. Vehicle	Acquisition	Freezing	5 Cues	RMANOVA	Cues	17.020	4,48	< 0.0001	0.486
					Groups	0.770	1,12	0.398	0.009
					Interaction	0.681	4,48	0.608	0.019
Ket-Ext vs. Vehicle	Retrieval	Freezing	Cue	Ind. t-test (two-tailed)	Groups	1.086	12	0.299	0.580
Ket-Ext vs. Vehicle	Extinction I&II	Freezing	Baseline	RMANOVA	Phase	12.010	1,12	0.005	0.257
					Group	0.217	1,12	0.649	0.007
					Interaction	3.508	1,12	0.086	0.075
Ket-Ext vs. Vehicle	Renewal	Freezing	Cues	RMANOVA	Cues	0.070	2,24	0.945	0.002
Ket-Tra vs. Vehicle	Training	Escape Latency	Days	RMANOVA	Days	28.050	3,42	< 0.0001	0.560
Ket-Tra vs. Vehicle	Reversal Training	Escape Latency	Days	RMANOVA	Days	23.140	3,42	< 0.0001	0.474
Ket-Tra vs. Vehicle	Reversal Probe	Entry	Quarter A	Ind. t-test (two-tailed)	Groups	0.306	14	0.765	0.153
Ket-Tra vs. Vehicle	Reversal Probe	Duration	Quarter A	Ind. t-test (two-tailed)	Groups	0.899	14	0.384	0.449
Ket-Tra vs. Vehicle	Reversal Probe	Crossing	Platform A	Ind. t-test (two-tailed)	Groups	0.475	14	0.642	0.238
Ket-Pro vs. Vehicle	Training	Escape Latency	Days	RMANOVA	Days	46.040	3,42	< 0.0001	0.601
					Groups	0.040	1,14	0.844	0.001
					Interaction	0.385	3,42	0.764	0.005
Ket-Pro vs. Vehicle	Reversal Training	Escape Latency	Days	RMANOVA	Days	15.290	3,42	< 0.0001	0.413
Ket-Pro vs. Vehicle	Reversal Probe	Entry	Quarter A	Ind. t-test (two-tailed)	Groups	0.404	14	0.693	0.202
Ket-Pro vs. Vehicle	Reversal Probe	Duration	Quarter A	Ind. t-test (two-tailed)	Groups	0.346	14	0.734	0.173
Ket-Pro vs. Vehicle	Reversal Probe	Crossing	Platform A	Ind. t-test (two-tailed)	Groups	0.424	14	0.678	0.212
Ket-Rev vs. Vehicle	Training	Escape Latency	Days	RMANOVA	Days	39.180	3,42	< 0.0001	0.555
					Groups	0.434	1,14	0.521	0.007
					Interaction	0.517	3,42	0.673	0.007
Ket-Rev vs. Vehicle	Reversal Training	Escape Latency	Days	RMANOVA	Days	40.630	3,42	< 0.0001	0.586
					Groups	0.001	1,14	0.979	0.000
Ket-Rev vs. Vehicle	Probe	Entry	Quarter A	Ind. t-test (two-tailed)	Groups	1.457	14	0.167	0.729
Ket-Rev vs. Vehicle	Probe	Duration	Quarter A	Ind. t-test (two-tailed)	Groups	0.461	14	0.652	0.230
Ket-Rev vs. Vehicle	Probe	Crossing	Platform A	Ind. t-test (two-tailed)	Groups	1.571	14	0.139	0.785
Ket-Rev vs. Vehicle	Reversal Probe	Entry	Quarter A	Ind. t-test (two-tailed)	Groups	1.063	14	0.306	0.531
Ket-Rev vs. Vehicle	Reversal Probe	Duration	Quarter A	Ind. t-test (two-tailed)	Groups	1.081	14	0.298	0.540

Table 2 (continued)

Group(s)	Phase(s)	Measurement		Statistical Test	Comparison	F/t	df	*p*	η2/d
Ket-Rev vs. Vehicle	Reversal Probe	Crossing	Platform A	Ind. t-test (two-tailed)	Groups	0.261	14	0.798	0.130

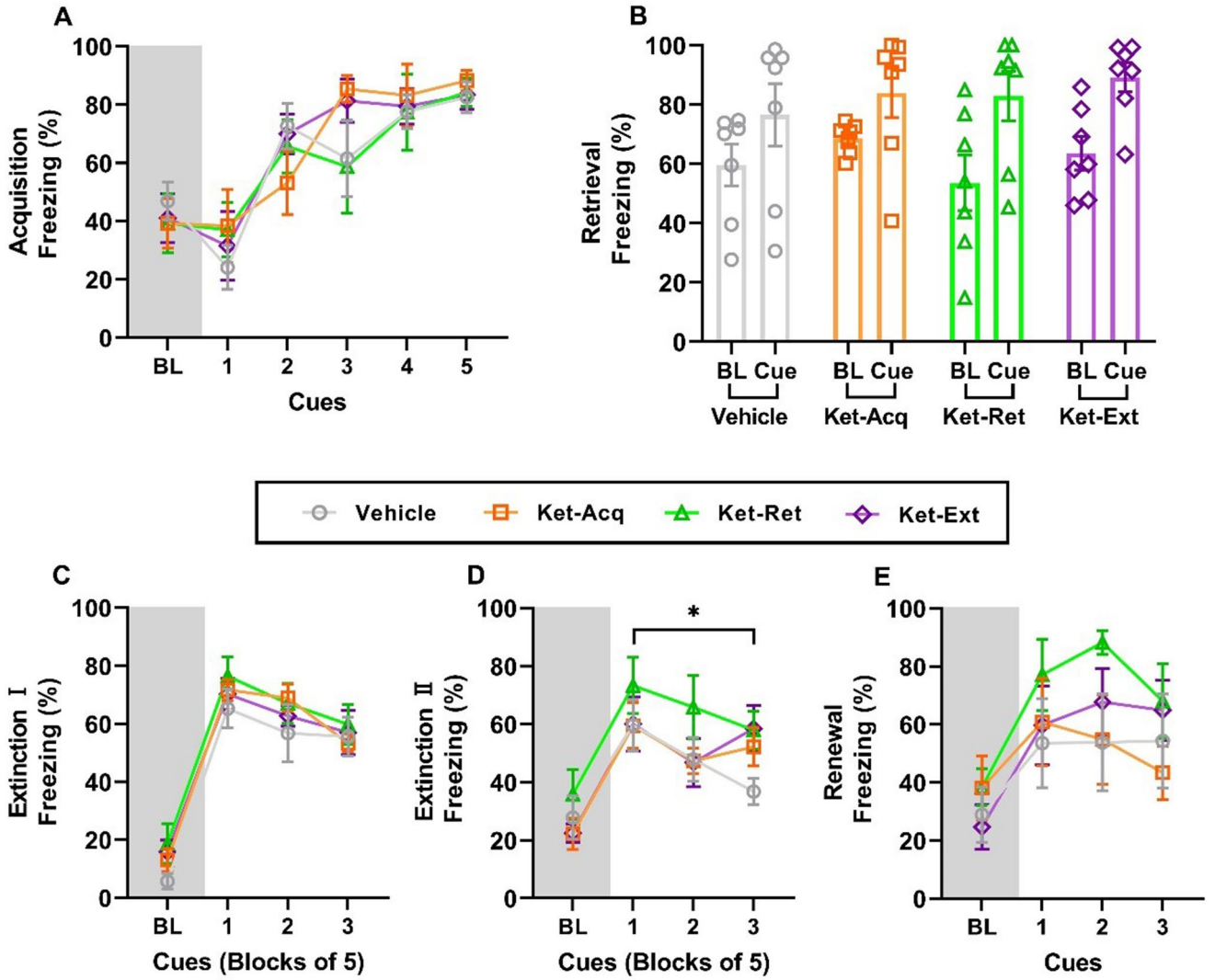

Fig. 3 Comparison of the freezing percentages ($n=7$ for each phase), in response to the conditioned stimulus in **(A)** Fear Acquisition, **(B)** Fear Retrieval, **(C)** Extinction I, **(D)** Extinction II, and **(E)** Fear Renewal. Gray areas show baseline (BL) freezing during the 3-minute acclimation period before the first cue. Error bars represent SEM. The asterisk (*) depicts $p<0.050$

were observed in either session (Extinction I: Group: $F(1, 12)=0.900$, $p=0.362$; GroupxBlocks: $F(2, 24)=0.340$, $p=0.715$; Extinction II: Group: $F(1, 12)=3.072$, $p=0.105$; GroupxBlocks: $F(2, 24)=0.256$, $p=0.776$).

Post-hoc analyses showed that the freezing levels of the vehicle group decreased significantly from the first block ($M=59.674$, $SD=23.433$) to the last block ($M=36.760$, $SD=11.941$) in Extinction II ($t(24)=3.069$, $p=0.016$, Cohen's $d=1.232$, Bonferroni corrected). However, no such difference was observed in the Ket-Ret group (first block: $M=73.325$, $SD=25.695$; last block: $M=57.937$, $SD=17.233$; $t(24)=2.061$, $p=0.151$, Bonferroni corrected), suggesting that ketamine impaired fear extinction when administered before fear retrieval. In addition, an increase in freezing levels was observed between the baseline periods of extinction sessions (Table 2; Fig. 3C, D) in both the vehicle (Extinction I: $M=5.683$, $SD=7.014$; Extinction II: $M=27.840$, $SD=19.595$; $t(12)=4.746$, $p<0.001$, Cohen's $d=1.506$, Bonferroni corrected) and Ket-Ret groups (Extinction I: $M=18.738$, $SD=18.121$; Extinction II: $M=35.825$, $SD=22.693$; $t(12)=3.660$, $p=0.007$, Cohen's $d=0.832$, Bonferroni corrected). The freezing response of

the groups did not show a difference in the renewal phase either as group effect ($F(1, 12) = 2.784$, $p = 0.121$, two-way repeated measures ANOVA; Fig. 3E) or through an interaction with the cues ($F(2, 24) = 0.404$, $p = 0.653$).

Fear memory extinction

There was no difference in freezing response between the Ket-Ext and vehicle groups during both the acquisition and retrieval phases (Table 2; Fig. 3A, B). During both days of fear extinction, freezing levels of the animals did not change across blocks (Extinction I: $F(2, 24) = 2.358$, $p = 0.116$; Extinction II: $F(2, 24) = 2.095$, $p = 0.145$, two-way repeated measures ANOVA; Fig. 3C, D). These results differ from those of the previous comparisons (Ket-Acq vs. Vehicle and Ket-Ret vs. Vehicle). Additionally, there were no significant differences in freezing response between groups in each extinction session (Extinction I: $F(1, 12) = 0.300$, $p = 0.594$; Extinction II: $F(1, 12) = 0.814$, $p = 0.385$; Fig. 3C, D), and no interaction was observed between the groups and the cue blocks (Extinction I: $F(2, 24) = 0.097$, $p = 0.908$; Extinction II: $F(2,24) = 1.673$, $p = 0.209$). However, the increase in baseline freezing responses to the extinction context between the two sessions (Table 2; Fig. 3C, D) was observed only in the vehicle group (Extinction I: $M = 5.683$, $SD = 7.014$; Extinction II: $M = 27.840$, $SD = 19.595$; $t(12) = 3.775$, $p = 0.005$, Cohen's $d = 1.506$, Bonferroni corrected), but not in the Ket-Ext group (Extinction I: $M = 15.883$, $SD = 10.833$; Extinction II: $M = 22.492$, $SD = 8.354$; $t(12) = 1.126$, $p = 0.564$, Cohen's $d = 0.683$, Bonferroni corrected), suggesting a ketamine-induced decrease in fear generalization to Context B. In fear renewal session, there were no group-level differences ($F(1, 12) = 0.460$, $p = 0.510$, two-way repeated measures ANOVA; Fig. 3E), nor was there an interaction effect ($F(2, 24) = 0.057$, $p = 0.653$).

The effects of ketamine on spatial memory

The escape latency scores of all groups decreased across training days, indicating successful learning of Platform A's location. A similar pattern was also observed during the reversal training for Platform B (refer to Table 2 for statistical results).

Spatial memory acquisition

Ket-Tra and vehicle groups performed similarly across training days ($F(1, 14) = 0.547$, $p = 0.472$, two-way repeated measures ANOVA; Fig. 4A), with no interaction observed between days and groups ($F(3, 42) = 0.032$, $p = 0.992$). In the probe trial, Ket-Tra animals displayed fewer Quarter A entries ($M = 4.750$, $SD = 2.188$) compared to the vehicle group ($M = 6.750$, $SD = 1.282$; $t(14) = 2.231$, $p = 0.043$, Cohen's $d = 1.116$, independent samples t-test; Fig. 4B). There were no group differences in the time spent in Quarter A ($t(14) = 1.439$, $p = 0.172$, independent samples t-test; Fig. 4C), and the number of Platform A crossings ($t(14) = 0.357$, $p = 0.727$, independent samples t-test; Fig. 4D).

In the reversal training, both groups performed equally well ($F(1, 14) = 0.957$, $p = 0.344$, two-way repeated measures ANOVA; Fig. 4E), however an interaction between the test days and the experimental groups was observed ($F(3, 42) = 4.572$, $p = 0.007$, $\eta 2 = 0.094$). Ket-Tra group ($M = 17.126$, $SD = 9.039$) spent less time locating the platform in the first day, as compared to the vehicle group ($M = 28.542$, $SD = 12.093$; $t(56) = 3.428$, $p = 0.001$, Cohen's $d = 1.069$, Bonferroni corrected; Fig. 4E, I). In the reversal probe day, no group-level differences were found in Quarter A entries and durations, and Platform A crossings (all $ps > 0.05$, Table 2); as well as Quarter B entries ($t(14) = 1.671$, $p = 0.117$, independent samples t-test; Fig. 4F), Quarter B durations ($t(14) = 0.\ 060$, $p = 0.953$, independent samples t-test; Fig. 4G), and Platform B crossings ($t(14) = 1.160$, $p = 0.266$, independent samples t-test; Fig. 4H).

Spatial memory retrieval

There were no differences between the escape latencies of Ket-Pro and the vehicle groups during MWM training (Table 2; Fig. 4A), as Ket-Pro group have not received ketamine yet. In the probe trials, Quarter A entries of the vehicle group ($M = 6.750$, $SD = 1.282$) were higher than Ket-Pro group ($M = 5.000$, $SD = 1.852$; $t(14) = 2.198$, $p = 0.045$, Cohen's d = -1.099, independent samples t-test; Fig. 4B). However, the groups did not differ in the time spent in Quarter A ($t(14) = 1.526$, $p = 0.149$, independent samples t-test; Fig. 4C) or in Platform A crossings ($t(14) = 0.000$, $p = 1.000$, independent samples t-test; Fig. 4D). In the reversal training, both groups performed equally well ($F(1, 14) = 0.024$, $p = 0.879$, two-way repeated measures ANOVA; Fig. 4E, I), with no interaction found between groups and days ($F(3, 42) = 1.584$, $p = 0.208$), unlike the observations in the Ket-Tra group. This did not change during the reversal probe day, where the groups had no difference in Quarter A entries, durations, or Platform A crossings (all $ps > 0.05$; Table 2); as well as Quarter B entries, ($t(14) = 0.000$, $p = 1.000$, independent samples t-test; Fig. 4F), durations ($t(14) = 0.831$, $p = 0.420$, independent samples t-test; Fig. 4G), and Platform B crossings ($t(14) = 1.809$, $p = 0.092$, independent samples t-test; Fig. 4H).

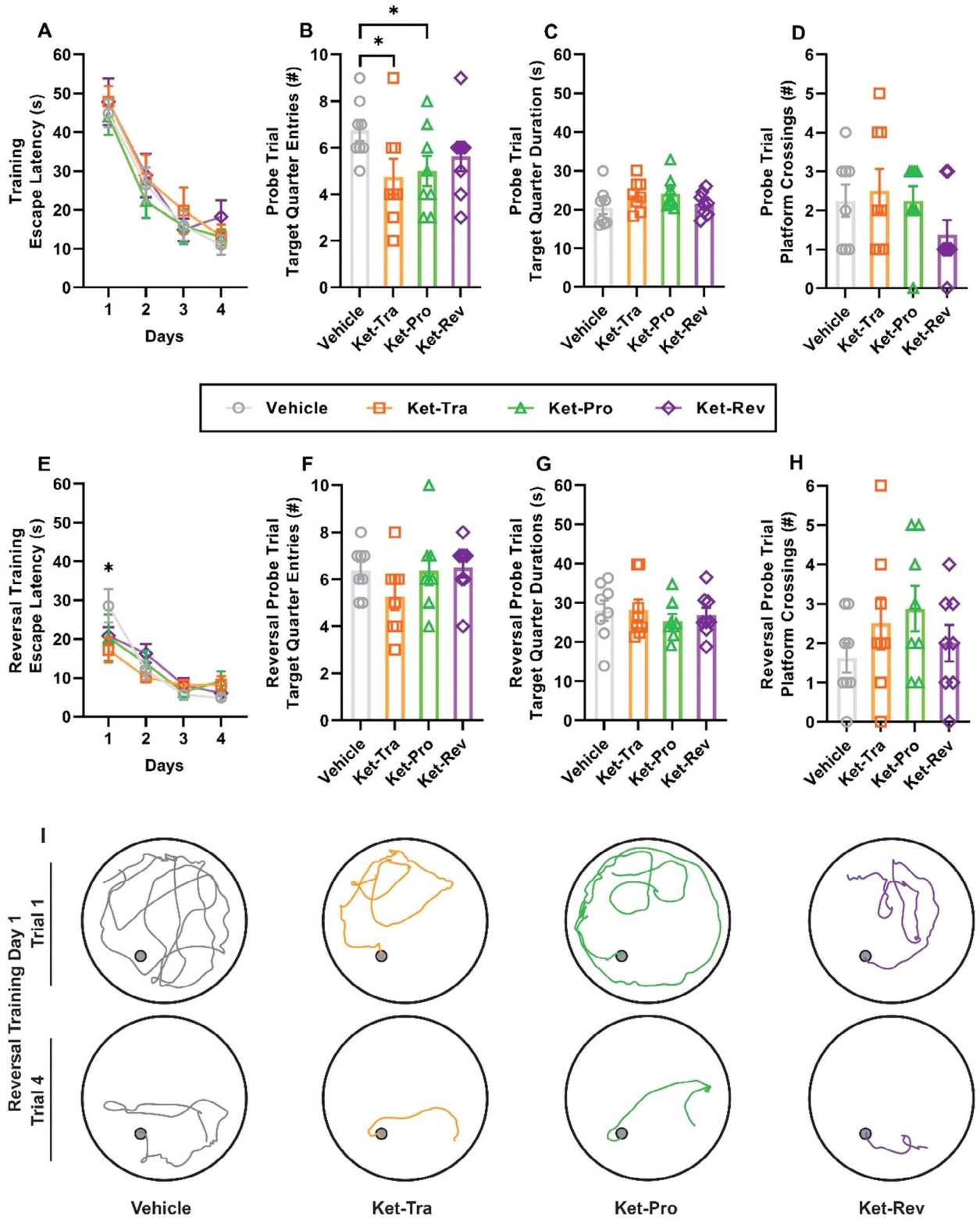

Fig. 4 Comparison of the Morris water maze performance ($n = 8$ for each phase). **(A)** The latency to escape across training days. **(B)** The number of Quarter A entries in the probe trial. **(C)** The time spent in Quarter A in the probe trial. **(D)** The number of Platform A crossings in the probe trial. **(E)** The escape latency scores across reversal training. **(F)** The number of Quarter B entries in the reversal probe trial. **(G)** The time spent in Quarter B in the reversal probe trial. **(H)** The number of Platform B crossings in the reversal probe trial. **(I)** Path trajectories of representative animals for trials 1 and 4 during the first day of reversal training. Gray circles indicate Platform B. Error bars represent SEM. The asterisks (*) depict $p < 0.050$

Spatial memory reversal

There was no difference in the escape latency scores of Ket-Rev and the vehicle groups on the training, reversal training or probe days (Table 2; Fig. 4A-H), while a significant interaction between the reversal training days and the groups was observed ($F(3, 42) = 3.753$, $p = 0.018$, $\eta 2 = 0.540$; Fig. 4E, I). Post-hoc comparisons indicated that the Ket-Rev group found the platform faster ($M = 20.813$, $SD = 6.274$) than the vehicle group ($M = 28.542$, $SD = 12.093$) in the first day ($t(56) = 2.422$, $p = 0.019$, Cohen's $d = 0.802$, Bonferroni corrected). In the reversal probe day, the groups did not differ in terms of Quarter A entries, duration, and Platform A crossings (all $ps > 0.05$; Table 2); as well as Quarter B entries ($t(14) = 0.221$, $p = 0.828$, independent samples t-test; Fig. 4F), duration ($t(14) = 0.363$, $p = 0.722$, independent samples t-test; Fig. 4G), and Platform B crossings ($t(14) = 0.630$, $p = 0.539$, independent samples t-test; Fig. 4H).

Discussion

We examined how different implicit and explicit memory processes are affected by an antidepressant dose of ketamine (10 mg/kg) by IP administration before memory acquisition, retrieval and modulation stages in cued fear conditioning and the MWM. In the implicit memory task, ketamine impaired fear memory extinction in the second session when it was administered before fear acquisition or retrieval. Additionally, ketamine administration 30 min before fear acquisition or extinction decreased fear generalization to the novel context. In contrast, animals showed better performance on the first day of the MWM reversal training when ketamine was injected before training or reversal training sessions, indicating easier modulation of the previously acquired spatial memory. Furthermore, ketamine administration before the first day of training or the probe trial increased the target quarter entries without altering the duration spent in the target quadrant or the number of platform crossing, suggesting altered search strategies.

Ketamine administration before encoding and retrieval of cued fear memory increased freezing rates in the second extinction session, while it did not have an effect when administered before the extinction. This finding contrasts with the previous studies where 10 mg/kg IP ketamine decreased freezing and enhanced fear extinction when administered before and immediately after CS-US pairing (Calzavara et al. 2009; Girgenti et al. 2017; Radford et al. 2018). These studies, however, did not have a retrieval session. In the present study, the retrieval session carried out in the same context as the fear acquisition may have facilitated access to the original memory (Agren 2014; Lee et al. 2017). Therefore, retrieval may have induced reconsolidation, which can strengthen or weaken the original memory through reactivation, as shown in previous studies (Misanin et al. 1968; Lewis 1979; Nader et al. 2000; Duvarci and Nader 2004). Ketamine administration before and after fear memory reactivation increases freezing rates in the subsequent test sessions in rats (Honsberger et al. 2015) as well as the fear response in humans when the original fear memory is activated under ketamine exposure in a Pavlovian conditioning paradigm (Corlett et al. 2013). In line with these observations, Ket-Acq and Ket-Ret groups, exhibiting higher freezing response in the extinction, were under the influence of ketamine during the retrieval session. Consequently, the impaired fear extinction observed in this study may arise from the strengthened fear memory following the retrieval procedure under ketamine exposure.

In our study, we demonstrated that ketamine administered before fear acquisition (Context A) or extinction (Context B) reduced fear generalization to the extinction context on the second day, as assessed via baseline freezing levels. The difference observed in the Ket-Ext group aligns with previous findings that ketamine (30 mg/kg, IP) decreased fear generalization in a novel context when administered 2 h prior to the test, but not before or after fear conditioning (Asim et al. 2020). Notably, we also observed a decrease in baseline freezing when ketamine was administered before conditioning. However, the aforementioned study only included one test day in a novel context, while we identified this difference on the second extinction day of our experiment. Additionally, a recent study showed that prophylactic ketamine, administered 1 week before fear acquisition, reduced freezing to a stimulus not associated with the shock during subsequent days of an extinction session in a novel environment (Ryan et al. 2022). Our results are consistent with these findings, as they suggest that ketamine administered before fear acquisition decreases contextual generalization. Furthermore, cues that predict and do not predict the unconditioned stimulus (US) may be influenced differentially, supporting our observation that the Ket-Acq group exhibited decreased baseline freezing on the second day of extinction while displaying an impaired extinction pattern to the conditioned stimulus.

In contrast to the deteriorating effect of ketamine on the extinction of fear memory, ketamine enhanced spatial memory reversal in the MWM. This effect was observed in the first day of reversal training and abolished in subsequent days. It must be noted that these animals have already undergone four days of training prior to reversal training and became well acquainted with the maze and the task, leading to concept learning (Whishaw 1985), which may have restricted the observed effect to the first day of reversal

training. The only study using ketamine in reversal training (Lalonde and Joyal 1991) reported that the same dose of ketamine decreased training and reversal training performance in the MWM, in contrast to the findings of the present study. However, Lalonde and Joyal (1991) administered ketamine on each day of training, which may underlie the observed memory impairment. Chronic administration of ketamine generally produces a decrease in spatial memory performance (Cao et al. 2014, 2021; Li et al. 2020; Sabbagh et al. 2012; Shi et al. 2021; Trofimiuk et al. 2019), unlike single low-dose administration (Holubova et al. 2016; Teng et al. 2024; Wesierska et al. 1990). Here, we show that a single antidepressant dose of ketamine enhances spatial memory modulation in the MWM.

Previous studies have shown that low doses of acute ketamine do not impact spatial memory performance in the training and probe trials (Holubova et al. 2016; Wesierska et al. 1990). However, an interesting observation emerged in our study: administering ketamine prior to the training or probe trial led to a decrease in the number of quarter entries during the probe session, despite the unchanged duration in the quarter and the platform crossings, which reflect overall performance. This discrepancy suggests a difference in path directionality, as fewer entries indicate a more direct path to the platform, implying a change in search strategy (Lalonde and Joyal 1991; Morris et al. 1986). Therefore, this finding complements the conclusion that an antidepressant dose of ketamine enhances spatial memory modulation, as it also supports retrieval when administered before training or the probe trial.

The novel experimental design used in this study, which allows for the assessment of distinct implicit and explicit memory processes across two common paradigms, presents some challenges for interpreting the results. In the fear conditioning experiment, for instance, we conducted retrieval in the same context as fear acquisition to assess fear retrieval for both the tone and the context. This approach may explain the lack of a significant increase in the freezing response to the cue delivery, likely due to relatively high baseline freezing levels to the context (i.e., 3-minute baseline freezing), which may have masked conditioned fear responses (Jacobs et al. 2010). Additionally, we attributed the group-level differences in extinction to retrieval-induced reconsolidation. Future studies could benefit from separating the retrieval of context and cue-based fear, providing clearer insights into how these factors are influenced by previous ketamine administration and how they affect subsequent fear extinction. Conducting retrieval in a different context or introducing a time gap between context exposure and cue delivery may further help isolate these effects.

Moreover, we did not find any group differences in fear renewal (Context A) following the impaired fear extinction (Context B) observed when ketamine was administered before fear acquisition or retrieval. Previous research has shown that conducting fear renewal in a different context than the extinction context can eliminate the attenuation of fear (Orsini et al. 2011), and extinction is often encoded in association with its contextual cues (Bouton 1993). In our study, fear renewal was carried out in the same context as acquisition and retrieval, which may have introduced non-extinguished contextual fear responses, complicating the interpretation of the results. Additionally, the ketamine-induced impairment in fear extinction within these groups, as revealed by indirect follow-up tests, may not have been strong enough to overcome these factors and yield significant group differences in fear renewal.

Another limitation of our study is the use of a single antidepressant dose of ketamine. Therapeutic effects have been reported for doses ranging from 3 to 30 mg/kg in rodents (Polis et al. 2019), while memory impairment is generally observed with doses starting at 8–10 mg/kg (IP) for both implicit (Choi et al. 2020) and explicit memory paradigms (Wasierska, 1990; Moosavi et al. 2012; de Souza et al. 2019; Shi et al. 2021). Similar variability in dosing, therapeutic outcomes (Andrade 2017) and memory impairments (Morgan and Curran 2006; Zhornitsky et al. 2022) has been noted in human studies, which makes it challenging to generalize the findings of the present study to a broader context. Nonetheless, our findings provide important insights into how ketamine differentially affects various implicit and explicit memory processes.

It is noteworthy that the same memory phase was affected in both the implicit and explicit memory tasks used in this study. We observed that the modulation of a previously formed memory is more sensitive to the effects of ketamine compared to encoding and retrieval. To our knowledge, this is the first study to demonstrate that the reactivation of fear memory under ketamine exposure complicates subsequent extinction, in contrast to reactivation without ketamine. However, ketamine enhanced spatial memory reversal only when administered before training or reversal training, not before the probe trial in which the escape platform is removed. Unlike probe trials, the training and reversal training sessions provide actual opportunities to learn the maze by repeatedly locating the escape platform. Thus, the results indicate that ketamine exerts a more robust effect when the animal is engaged in an active explicit learning experience.

These results collectively suggest that ketamine exerts differential effects on the modulation of implicit and explicit memories by engaging distinct behavioral mechanisms. In the context of fear memory, the reactivation period during retrieval is crucial, emphasizing the role of reconsolidation processes. Conversely, in spatial memory, the effects of ketamine are linked to the encoding of new learning

experiences and cognitive flexibility. Understanding these distinct mechanisms is essential for grasping the varied cognitive side effects associated with the antidepressant use of ketamine.

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Declarations

Ethics approval All experimental procedures were approved by the Boğaziçi University Ethics Committee for the Use of Animals in Experiments (permission no: 2023–013).

Conflict of interest The authors declare no conflict of interest.

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