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The interaction between esketamine and cannabis in mice and its implications for the treatment of psychiatric disorders

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Abstract

Depression is a common psychiatric disorder that is often accompanied by severe functional impairment. In treatment-resistant depression (TRD), conventional antidepressants frequently fail to provide adequate relief of symptoms. For this reason, the prescription of other alternative therapies is increasing, including (S)-ketamine, a fast-acting NMDA receptor antagonist. Its robust clinical effects on a variety of related diseases and high safety profile additionally led to increased use for recreation and self-medication in the last decade. Cannabis is one of the most common drugs used in recreational polydrug events with ketamine, either as a harm reduction strategy or for the amplification of subjective effects, and its consumption, particularly its psychoactive component Δ9-tetrahydrocannabinol (THC), is increasing. Despite its complex effects on cognition, emotions, and motor behavior, THC is also frequently used for self-medication in disorders similar to those treated with (S)-ketamine. However, while the interaction between THC and medical (S)-ketamine is recognized, it remains poorly studied. This study aimed to investigate the acute behavioral and physiological effects of THC, (S)-ketamine, and their combination. Adult male C57BL/6J mice received the comparable recreational dose 5 mg/kg THC, the therapeutic dose of 10 mg/kg (S)-ketamine, or both. Locomotor activity was assessed via open field and rotarod tests, while core body temperature was measured to indicate successful drug effects. In the final experiment, brain, liver and blood samples were collected for future molecular analysis. While THC and (S)-ketamine alone had observable effects on body temperature and motor performance by themselves, their simultaneous presence led to a significant decrease in body temperature and a pronounced impairment of motor coordination, but only when THC was administered before (S)-ketamine, and not vice versa. Our results suggest that the sequential combination of THC and (S)-ketamine in healthy mice produce synergistic physiological and behavioral effects beyond those of either drug alone, underscoring the need to better understand their interactions in both clinical and recreational contexts.

Zusammenfassung

Depression gehört zu den häufigsten psychischen Erkrankungen und ist häufig mit erheblicher Beeinträchtigung der Lebensqualität verbunden. Bei therapieresistenten Verläufen zeigen klassische Antidepressiva oft unzureichende Wirkung. In solchen Fällen rückt (S)-Ketamin, ein schnell wirksamer NMDA-Rezeptor-Antagonist, zunehmend in den Fokus als mögliche Behandlungsoption.

Parallel greifen viele Betroffene zu Cannabis als Selbstmedikation zurück. Meist in der Form seines psychoaktiven Hauptbestandteiles Δ9-Tetrahydrocannabinol (THC), obwohl dessen Wirkung auf Kognition, Emotion und Motorik sehr vielschichtig und nicht vorhersehbar ist. Da THC ein häufiger Bestandteil von Freizeit-Polydrogenkonsum ist, stellt sich zunehmend die Frage nach möglichen Wechselwirkungen mit medizinisch eingesetztem (S)-Ketamin. Die Interaktion beider Substanzen ist bisher kaum erforscht worden.

Ziel unserer Studie war es daher, den Einfluss von akutem THC, (S)-Ketamin sowie deren Kombination im Tiermodell mittels Verhaltens und physiologischer Effekte zu eruieren.

Es wurden erwachsene männliche C57BL/6J-Mäuse entweder mit einer umgerechneten „Freizeitdosis“ THC (5 mg/kg), einer therapeutischen Dosis (S)-Ketamin (10 mg/kg) oder mit beiden Substanzen behandelt. Die Bewegung wurde mittels Open-Field und Rotarod-Test analysiert. Zusätzlich diente die Körperkerntemperatur als physiologischer Marker. Nach dem Versuch wurden Hirn-, Leber- und Blutproben für molekulare Analysen gesammelt.

Einzeln zeigen beide Substanzen einen Einfluss auf Bewegung und Temperatur. Bei der kombinierten Verabreichung, kam es zu verstärkten Ergebnissen, jedoch nur wenn THC vor (S)-Ketamin injiziert wurde. Dies zeichnete sich durch eine ausgeprägte Hypothermie sowie Einschränkung der Bewegung aus.

Die Ergebnisse weisen auf eine synergistische Wechselwirkung der Substanzen hin, die über ihre Einzeleffekte hinausgeht. Durch den stetig steigenden Konsum beider Substanzen, steigt ebenso die Relevanz dieses Forschungsschwerpunkts.

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Abbreviations

BDNF	brain-derived neurotrophic factor
CB_1R	cannabinoid receptor type 1
EMA	European Medicines Agency
FDA	US Food and Drug Administration
MDD	Major Depressive Disorder
NMDA	N-methyl-D-aspartate
SNRI	serotonin-norepinephrine reuptake inhibitors
SSRI	selective serotonin reuptake inhibitors
THC	Δ^9-tetrahydrocannabinol
TRD	treatment-resistant depression

Introduction

Challenges in Treating Major Depressive Disorder

Depression is a common mental illness that not only significantly influences emotional and physical functioning but also challenges public health. In the years 1990 to 2021 there has been a 1.8-fold increase in cases for depressive disorder globally (X.-D. Chen et al., 2025). Among clinical forms, Major Depressive Disorder (MDD) is one of the most prevalent, impacting roughly 5% of the adult population globally in 2010 (Ferrari et al., 2013). There are many established treatments, such as selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs) and tricyclic antidepressants. However, those treatments often take several weeks to show results or even fail to produce an adequate response (Hillhouse & Porter, 2015).

The percentage of patients with MDD who are affected by treatment-resistant depression (TRD), ranges depending on how stringent the definition is. When defined by the US Food and Drug Administration (FDA) or European Medicines Agency (EMA) criteria, which is the insufficient response to at least two sequential antidepressant trials despite adequate treatment, approximately 55% of people with MDD are affected by TRD (McIntyre et al., 2023).

Both MDD and TRD can lead to severe limitations including job performance, social relationships and family dynamics. These impairments not only result in depressive symptoms, but also contribute to maintaining the disorder itself and even worsening it. For example, people with depression often experience increased work absences, reduced work productivity and strained interpersonal relationships (White et al., 2025). This highlights the importance of exploring novel and fast acting treatments that can address both the emotional and functional dimensions of depression, especially in cases where standard therapies often fail.

(S)-ketamine as a treatment

Originally known and used as a dissociative anesthetic, it is also favored as a sedative for minor procedures due to its rapid onset and short duration of action (Hashimoto et al., 2024). In recent years, (S)-ketamine gained attention as a promising treatment option for individuals with severe treatment-resistant depression. Contrasting conventional

antidepressants, which primarily target monoaminergic systems and often take several weeks to produce clinical effects, (S)-ketamine acts quickly, often within hours (Berman et al., 2000; Zarate et al., 2006). As has been shown, this rapid effect and improvement is particularly essential in patients with suicidal ideation, as this treatment leads to a reduction in these thoughts lasting from several days to weeks (Sajid et al., 2025).

By quickly crossing the blood-brain barrier, it acts as a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor (Johnston et al., 2024; Lord et al., 2013; Yamakura & Shimoji, 1999). (*S*)-ketamine blocks NMDA receptors on inhibitory neurons, which normally suppress excitatory neurons. By reducing this inhibition, excitatory pyramidal neurons temporarily become more active, releasing glutamate that then stimulates AMPA receptors and promotes changes that strengthen connections between neurons (Johnston et al., 2024). This triggers downstream processes that promote synaptic plasticity, including increased release of brain-derived neurotrophic factor (BDNF) and enhanced neuronal connectivity (Medeiros et al., 2024; Pardossi et al., 2024).

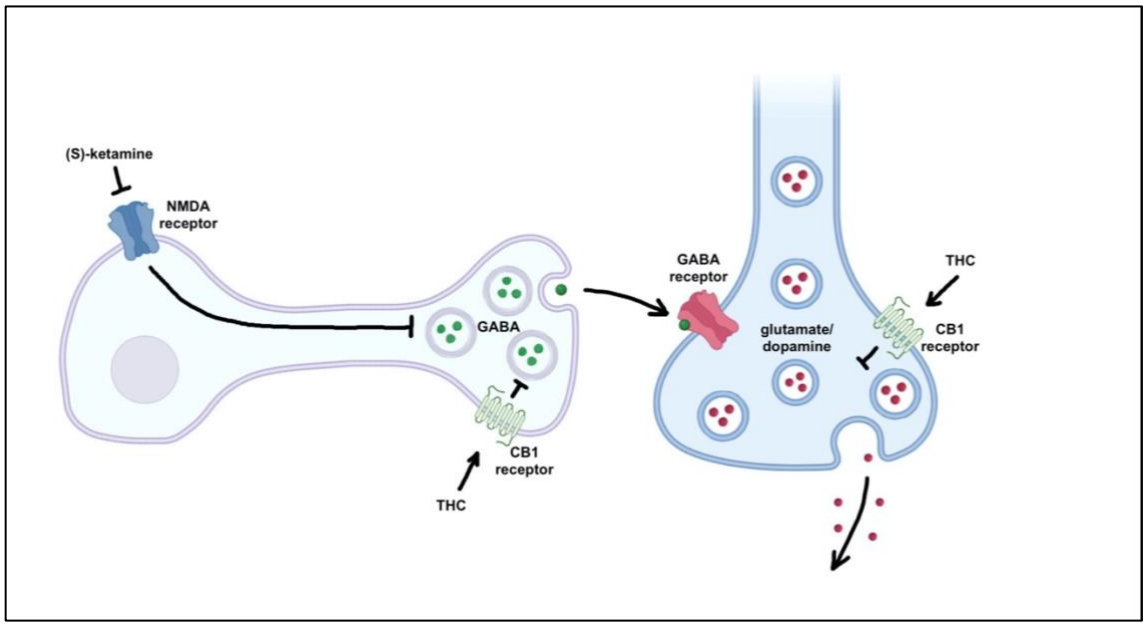

Figure 1 The figure shows the interaction between GABAergic and glutamatergic neurons modulated by (S)-ketamine and Δ9-tetrahydrocannabinol (THC). (S)-ketamine acts as an NMDA receptor antagonist on GABAergic interneurons, leading to reduced GABA release. Similarly, THC binds to CB1 receptors located on GABAergic terminals, further decreasing GABA release. The resulting inhibition of GABAergic transmission leads to a disinhibition of excitatory glutamatergic neurons, therefore increasing network excitation. However, since CB1 receptors are also present on excitatory (glutamatergic) terminals, THC can at the same time inhibit glutamate release and thus regulate network activity on another level. Figure adapted from Watson et al., 2025 and Ghosal et al., 2020. .

Numerous clinical studies and meta-analyses have shown that both racemic ketamine and its more powerful (S)-enantiomer (S)-ketamine exhibit rapid and robust antidepressant effects, particularly in patients with TRD (Medeiros et al., 2024). Accordingly, (S)-ketamine has been approved by the FDA as an intranasal formulation for the treatment of TRD (*SPRAVATO® (Esketamine)*, 2025).

However, despite its therapeutic efficacy, (S)-ketamine can cause acute side effects such as dizziness, dissociation, increased blood pressure and nausea (Medeiros et al., 2024). Therefore, it is necessary to evaluate the long-term safety profile and potential interactions with other psychoactive substances, such as cannabinoids. Further research needs to be done to reveal the risks and mechanisms of co-use.

Cannabis Use and Neuropsychiatric Effects

Cannabis remains the most widely used illicit substance worldwide. In Europe, an estimated 8.4% of adults, approximately 24 million individuals aged 15 to 64, reported cannabis use in 2024 (EUDA - European Union Drugs Agency, 2025). In addition to medical use, the legalization of cannabis has also increased, most recently in Germany since 2024 (Bundesministerium für Gesundheit, 2025). Studies show that the use of cannabis, especially a lifetime history of heavy cannabis use (≥1000 times), is still associated with a range of psychiatric illnesses (Gowin et al., 2025). It has been shown, that the brain activity during working memory tasks is lower in frequent cannabis users (Crean et al., 2011; Gowin et al., 2025; Hoch et al., 2025).

In addition to recreational use, cannabis is increasingly reported as a form of self-medication for anxiety, depression and sleep problems (Sarvet et al., 2018). Many users attempt to alleviate their symptoms through cannabis use, often without medical supervision. When cannabis use is stopped, withdrawal symptoms such as insomnia/interrupted sleep, irritability, anxiety, and vivid dreams may reoccur, which often overlap with or even exacerbate the reasons for self-medication (Wallis et al., 2022).

This trend underscores the importance of understanding the complex effects of THC on the central nervous system and its potential therapeutic and adverse effects. Its main psychoactive component, Δ^9-tetrahydrocannabinol (THC) activates cannabinoid receptor

type 1 (CB_1) (Pertwee, 2006; Thorsen et al., 2025). CB_1 receptors, one of the most abundant G protein-coupled receptors, are widely expressed in the central nervous system (Kendall & Yudowski, 2017). Activation of CB_1 receptors by THC leads to a reduction in neurotransmitter release, including dopamine, glutamate and GABA, which contribute to its diverse behavioral and physiological effects (Szabo & Schlicker, 2005).

While low to moderate doses of THC may have anxiolytic effects and can elevate one's mood, higher or chronic exposure has been associated with the opposite. This is then associated with cognitive harm, altered emotional coping and increased risk of psychiatric illnesses (Ahrens et al., 2025; Lichenstein, 2022). The interplay of dosage and context-specific effects, which can range from potentially beneficial outcomes at low concentrations to harmful effects at higher concentrations (Keimpema et al., 2021), highlight the complexity of THC's effects on the brain.

The Tetrad Test in Cannabinoid Research

To investigate the main effects of THC in preclinical models, the tetrad test has been widely used as a standardized behavioral test in rodents. This test evaluates four characteristic effects of cannabinoid receptor activation: hypolocomotion, catalepsy, hypothermia and analgesia (Table 1). These parameters show the CB_1-mediated pharmacological effect and are considered a reliable in vivo model for cannabinoid research (Fride et al., 2006; Metna-Laurent et al., 2017).

The tetrad test has contributed to our understanding of the behavioral and physiological responses to THC, especially as its subjective effects are difficult to quantify in animal models. Additionally, it serves as a valuable tool for assessing the potency and efficacy of cannabinoid compounds.

However, the outcomes of behavioral experiments are influenced by numerous factors, including the testing environment, the mouse strain used, sex and age of the animals (Lulek et al., 2023), and even the gender of the experimenter (Chapman et al., 2018), which can lead to variability in results. Moreover, THC concentrations reported in the literature often differ and may not be relevant to our specific research objectives. Therefore, it was essential for us to determine an appropriate THC concentration under

our own experimental conditions and mouse strain, in order to establish a reproducible baseline for future studies.

Table 1 Overview of Tetrad test parameters (De Giacomo et al., 2020)

Hypolocomotion	Decrease in spontaneous movement or activity, reflecting reduced motor function.
Catalepsy	Maintenance of an imposed posture for an extended time, indicating motor rigidity.
Hypothermia	Reduction in core body temperature following drug administration.
Analgesia	Decreased sensitivity to pain, typically measured by latency in pain response tests.

Physiological and Behavioral Effects of (S)-ketamine

(S)-Ketamine, the S-enantiomer of ketamine, is a fast-acting non-competitive NMDA receptor antagonist widely studied for its antidepressant effect (Jelen et al., 2021; Kraus et al., 2017). In a recent study with rodents published in 2023 it was shown, that in comparison of the same dose of 15 mg/kg (R)-ketamine and (S)-ketamine, only (S)-ketamine showed an antidepressant-like activity.

In preclinical research, (S)-ketamine has been shown to produce a variety of physiological and behavioral effects, such as changes in thermoregulation, locomotion and some stereotypic behaviors (Acevedo et al., 2023; W.-C. Chen et al., 2023; Lin et al., 1978). These effects are known to be dose-dependent and influenced by factors including strain, age, sex, housing conditions, and experimental design. This study demonstrated that when administering the same dose, the locomotor response of adult BALB/c mice versus C57BL/6 was significantly different (W.-C. Chen et al., 2023).

Previous studies in mice have described a biphasic locomotor response to ketamine, with lower doses inducing hyperactivity, antidepressant effects (Ouyang et al., 2021; Zaytseva et al., 2023) while higher doses lead to hypolocomotion, sedation (W.-C. Chen et al., 2023) or even induce schizophrenia-like behavior in rats (Brakatselos et al., 2024).

Similar patterns have been reported for changes in body temperature (Lin et al., 1978). However, many of these studies have used high doses, ranging from 25 to 50 mg/kg (W.-

C. Chen et al., 2023), which may not accurately reflect the effects observed with lower, clinically relevant doses used in humans for therapeutic purposes.

Aim of the Study

Since THC and (S)-ketamine are used to treat similar psychiatric symptoms, their behavioral and physiological effects are alike, and both drugs are increasingly used for recreational and self-medication purposes, it is important to understand the possible interactions of these two substances. While both substances have been investigated individually in clinical and preclinical studies, there is a lack of scientific data on their combined effects.

The aim of this study was therefore to investigate the acute effects of THC and (S)-ketamine, administered alone or in combination. We focused on body temperature and motor coordination in adult healthy C57BL/6JRj mice. By identifying specific interaction effects and evaluating whether the combination leads to additive or synergistic outcomes, this work provides a basis for future molecular analyses. The goal was to contribute to a more comprehensive understanding of the potential risks and mechanisms associated with the use of multiple drugs, especially in clinical settings.

Materials and Methods

Animals

For the following experiments C57BL/6JRj mice purchased from Janvier Labs were used. The male mice, aged 8-10 weeks, had a bodyweight ranging from 25 to 28 grams. Prior to the experiment, the animals were acclimatized to the laboratory environment. Mice were housed under standard laboratory conditions, with an artificial 12-hour light/dark cycle (lights off at 10:00 am) in a temperature and humidity-controlled room. The experiments were conducted during the dark phase of the cycle to suit the natural activity periods of the mice.

In the Experiment Aim 1, animals were group housed, with a maximum of 6 mice per standardized cage. For all other experiments carried out, the mice were single housed for 3 or 5 days prior to the experiment. Mice had *ad libitum* access to food and tap water throughout the experiment.

Solutions

Preparation of THC and Vehicle solution

A stock solution of THC was prepared at a concentration of 62.5 mg/mL by dissolving 250 mg of Dronabinol (Gatt-Koller) in Dimethyl sulfoxide (DMSO; Sigma-Aldrich). The solution was aliquoted and stored at −20 °C until use to ensure stability and prevent degradation.

For injections, working solutions were freshly prepared by combining 2% of the THC stock solution with 10% Cremophor (Fluka Analytica) and 88% sterile 0.9% sodium chloride solution (NaCl; Braun). For the 5 mg/kg dose in a 25 g mouse, a total injection volume of 100 µL was administered. To achieve lower or higher concentrations (1 mg/kg, 3 mg/kg, and 10 mg/kg), the volume of THC stock and saline was adjusted accordingly, while the amount of Cremophor was kept constant at 10% of the total injection volume. The vehicle solution was prepared using the same composition as the THC injection solution, excluding the active compound. Specifically, the vehicle consisted of 10% Cremophor (Fluka Analytica), 2% DMSO (Sigma-Aldrich, Methylsulfinylmethane,

Molecular Biology Grade), and 88% sterile 0.9% sodium chloride solution (NaCl; Braun). This formulation ensured that all treatment groups received the same injection components, controlling for potential effects of the solvents.

Preparation of (*S*)-Ketamine Solutions

For the following experiment, various concentrations of (S)-ketamine were prepared using Eskelan (Gerot Lannach, 25 mg/ml). On each experimental day, a new, unopened 2 ml vial was used to ensure solution integrity. Mice received doses of 5 mg/kg, 10 mg/kg, or 15 mg/kg, with all ketamine solutions diluted in sterile 0.9% NaCl. A volume of 100 µl was administered per 25 g mouse. Saline alone was used as the control treatment.

Injections

For all injections, Omnifix-F Luer Lock Solo syringes (0.01–1 mL; B. Braun) were used in combination with Sterican needles (0.30 × 12 mm; B. Braun).

Behavior

Body temperature

Body temperature was measured using a rectal thermoprobe (Physitemp BAT-10) specifically designed for small laboratory animals. To ensure consistent and reliable readings across all animals, the vaseline coated probe was inserted approximately 1 cm into the rectum. Measurements were performed gently and swiftly to minimize handling stress, and care was taken to avoid injury or discomfort. All temperature assessments were conducted at room temperature under standardized environmental conditions.

Open Field Test

Before starting the experiment, the weight of the mice was measured and the mice were allowed to acclimatize in their home cages for 30-60 minutes. The animals were randomly assigned to the different groups.

The open field arena (42 x 42 x 21 cm, white background) was placed in the behavior room (with artificial light and 21°C ± 1. The light intensity in the center of the arena was 210-220 LUX (Voltcraft LX-10, Voltcraft GmbH, Germany) for all of the experiments. Before starting the experiment and after each animal, the arena was cleaned with 70% ethanol solution and paper towels.

The mouse was placed in the center of the arena and behavior was recorded for 30 minutes.

After the experiments were finished, the videos were analyzed by using Ethovision (Noldus Information Technology, the Netherlands) to measure parameters like time spent in the center (%), distance moved (cm), grooming (%), unsupported rearing (%), supported rearing (%), and rotations.

Catalepsy Test

The catalepsy cage was constructed as described in the published protocol of the research group Pier Vincenzo Piazza using a standard-sized plastic cage with a horizontal plastic bar installed across its width at a height of approximately 3.5 cm from the floor. Following the open field test and body temperature measurement, each mouse was gently lifted by the tail and positioned so that its forepaws rested on the horizontal bar, while the hind paws remained on the floor of the cage. This posture was used to assess cataleptic behavior. Each mouse was tested three times in succession.

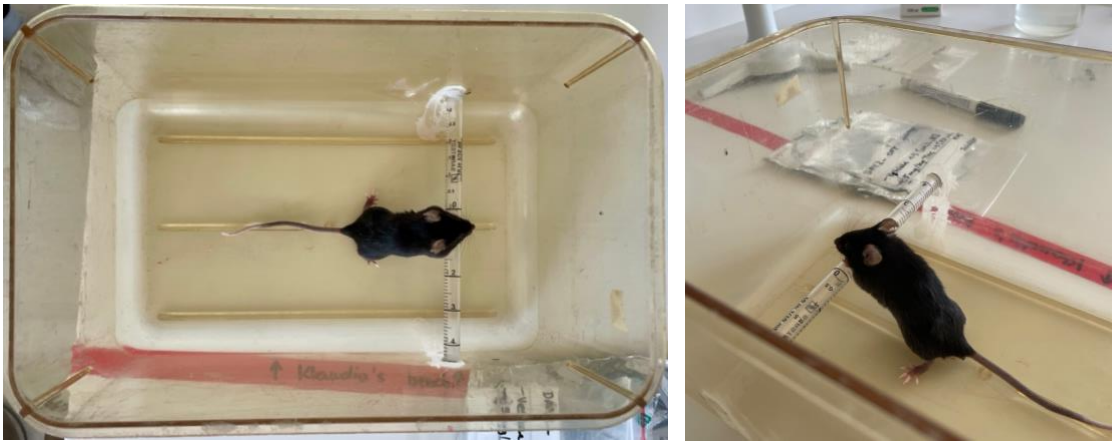

Figure 2 Catalepsy cage with plastic horizontal bar.

Rotarod

Motor coordination and balance were assessed using a rotarod device (Ugo Basile, Accelerating Rota-Rod, Jones & Roberts model for mice, 7650). Prior to the start of the experiment, mice were allowed to perform a series of test trials to familiarize themselves with the apparatus and reduce novelty-induced stress. During the testing phase, mice were gently placed on the rotating cylinder, and the acceleration mode was used for all trials. Each animal completed three consecutive trials. A trial was considered complete when the mouse either fell off the rod or completed one passive rotation (i.e., clinging to the rod without active locomotion). The latency to fall was recorded for each trial, and the mean of the three trials was used for further analysis as a measure of motor performance.

Experimental design

Aim 1: acute THC

To replicate and analyze the effects of different THC concentrations in adult mice, the experiments were conducted as outlined in Figure 3. The animals were group-housed, with a maximum of six mice per cage. Animals were tested according to standardized tetrad behavioral protocols (Metna-Laurent et al., 2017). Prior to the start of the behavioral tests, the mice were weighed to ensure accurate dosing of THC. Baseline body temperature was measured using a rectal probe before administering either the vehicle solution or THC at concentrations of 1 mg/kg, 3 mg/kg, 5 mg/kg, or 10 mg/kg

Following the injection, a waiting period of 20 minutes was observed after which locomotor activity was assessed in an open field arena for 30 minutes. Immediately after the test, body temperature was measured again, and the catalepsy test was performed. Finally, the animals were sacrificed, and the brains were dissected and stored at −80 °C for further analysis.

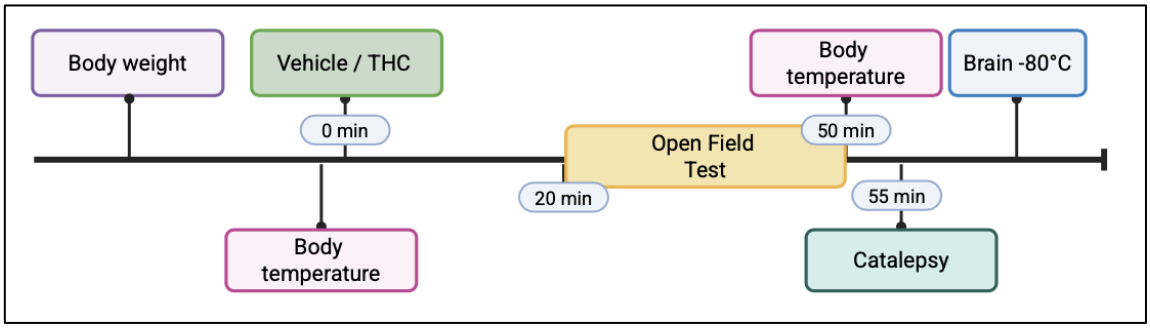

Figure 3. Timeline of Tetrad testing following THC administration (adapted from Metna-Laurent et al., 2017)

Aim 2: acute (*S*)-ketamine

The aim of this experiment was to investigate the acute behavioral effects of (S)-ketamine administration, with a particular focus on changes in locomotor activity. Prior to treatment, the body weight and baseline body temperature of each mouse were measured. The animals were then randomly assigned to receive either a saline solution (serving as the control condition) or an injection of (S)-ketamine at one of three concentrations: 5 mg/kg (Khakpai et al., 2019; McDonnell et al., 2021), 10 mg/kg (Fitzgerald et al., 2019; Ouyang et al., 2021), or 15 mg/kg (Acevedo et al., 2023).

Immediately following the injection, the mice were placed in an open field arena, where their locomotor activity was recorded for 30 minutes to capture acute behavioral responses to the treatment, considered a standardized behavioral readout for ketamine treatments (Irifune et al., 1991). After completion of the open field test, body temperature was measured again to monitor any physiological changes associated with (S)-ketamine administration.

Importantly, the same cohort of mice was subsequently used for the next part of the study, as outlined in Aim 3. This allowed for a continuous and integrated analysis of both acute and follow-up effects within the same subjects.

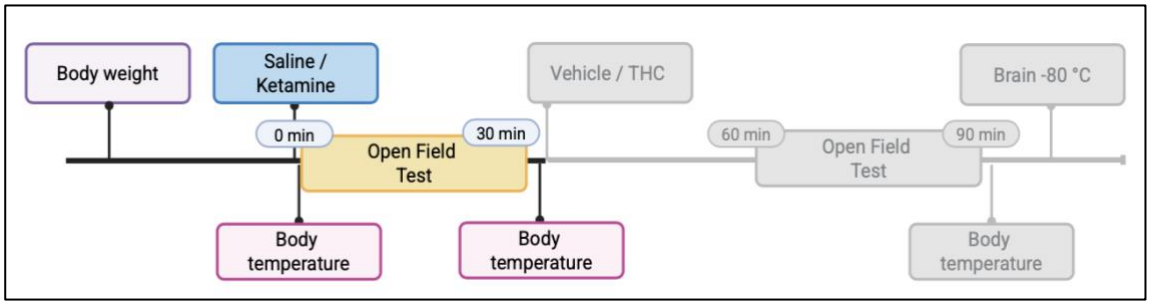

Figure 4. Timeline of behavioral assessments following (S)-ketamine administration

Aim 3: acute ketamine and THC

This experiment aimed to investigate the interaction between (S)-ketamine and a subsequent THC injection, focusing on potential behavioral and physiological effects. Building directly on the procedures described in Aim 2, the same cohort of mice was used to ensure continuity and to reduce variability between experimental groups. After completing the initial (S)-ketamine-related behavioral assessments, the animals received a second treatment: an injection of either the vehicle solution or 5 mg/kg THC. This concentration was chosen because it showed the most pronounced parameters of the tetrad test in the preliminary experiments (Aim 1).

Following the injection, the mice were returned to their home cages for a 30-minute resting period to allow the drug to take effect. Next, they were placed back into the open field arena for an additional 30-minute locomotor activity test, allowing for a direct comparison with their previous behavioral responses.

After the behavioral testing, body temperature was measured once more to assess any physiological changes. Lastly, the mice were sacrificed, and their brains were carefully dissected for subsequent molecular and histological analyses.

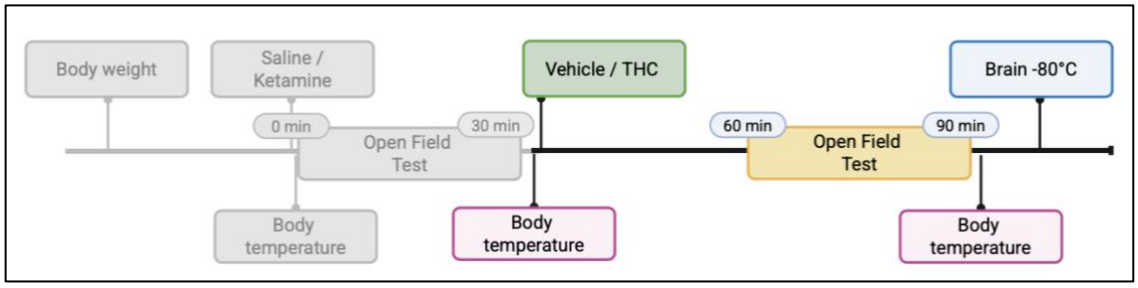

Figure 5. Follow up to Aim 2: THC administation and behavioral evaluation in the same cohort of mice.

Aim 4: THC and (S)-ketamine

In contrast to the previous experiment, the order of drug administration was reversed: mice first received an injection of 5 mg/kg THC, followed by (S)-ketamine at concentrations of 5 mg/kg, 10 mg/kg, or 15 mg/kg. As with earlier experiments, each animal's body weight and baseline body temperature were measured prior to treatment to ensure consistent physiological conditions across groups. The mice were then randomly assigned to receive either the vehicle solution or 5 mg/kg THC via intraperitoneal injection.

Following the THC administration, the animals were returned to their home cages for a 30-minute resting period to allow the drug to take effect (Metna-Laurent et al., 2017). After this interval, body temperature was measured again to monitor any immediate physiological changes. Subsequently, the mice were injected with (S)-ketamine at their respective dosages and immediately placed into the open field arena, where locomotor activity was recorded for 30 minutes.

After completing the behavioral test, the animals were returned to their home cages. Thirty minutes later, they were sacrificed, and their brains were dissected for molecular analyses. This design allowed for the assessment of acute behavioral and physiological effects of THC pre-treatment in combination with varying doses of (S)-ketamine.

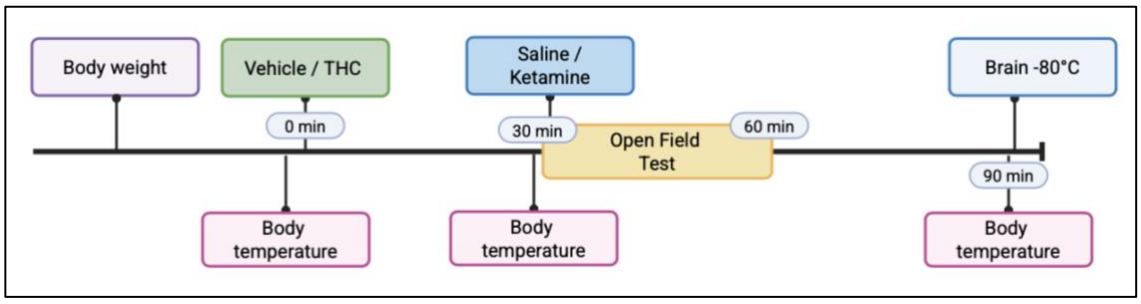

Figure 6. Timeline of sequential THC and (S)-ketamine treatment followed by locomotor activity assessment.

Aim 5: THC and (S)-ketamine including rotarod

In this experiment, the procedure closely followed that of Aim 4, with a slight modification in the behavioral testing sequence. After 90 minutes, two additional tests were conducted: the rotarod test, which was used to assess motor coordination and postural control, and the tail suspension test, which in this context was applied to evaluate overall posture and reaction.

Four experimental groups were included: vehicle followed by saline (control group), THC (5 mg/kg) followed by saline, vehicle followed by (S)-ketamine (10 mg/kg) and THC (5 mg/kg) followed by (S)-ketamine (10 mg/kg). For this experiment, we used only one concentration per drug. 5 mg/kg THC had yielded robust results in the preliminary experiments and was therefore used again. We also decided to continue with 10 mg/kg (S)-ketamine, as this dose had a moderate effect on the mice without THC and later in combination with THC, without impairing them too heavily. All treatments were administered intraperitoneally under standardized conditions as described above. Based on our preliminary studies Aim 1 and Aim 2, we were able to determine that these two concentrations were ideal for our further experiments.

At 120 minutes after the initial injection, the animals were sacrificed, and brain tissue was collected for subsequent molecular analyses.

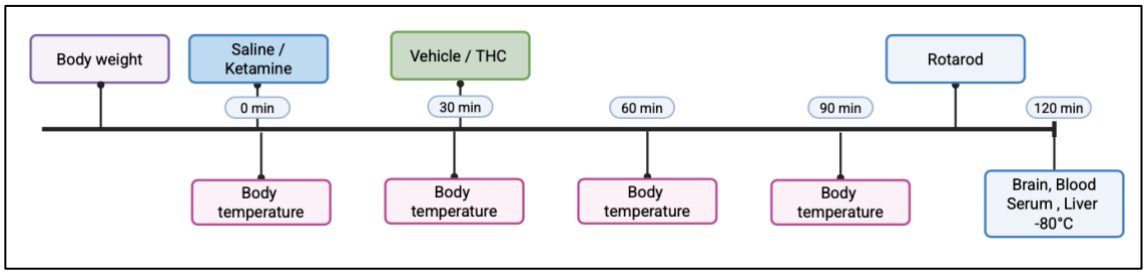

Figure 7. Experimental overview of the final experiment including a THC and (S)-ketamine injection and the motor coordination test on the rotarod.

Tissue

The mice were quickly anesthetized with a lethal dose of isoflurane (Isoflurane Baxter, Baxter Healthcare Corporation, Deerfield, IL, USA) and euthanized in accordance with ethical guidelines by decapitation. All dissections were performed using precision surgical instruments from Fine Science Tools (FST, Heidelberg, Germany), including fine forceps and micro scissors, under sterile conditions. Brains were rapidly removed and sectioned on a metal dissection plate cooled to 4 °C. The left and right hemispheres of the cortex, prefrontal cortex, and hippocampus were carefully dissected and transferred into separate pre-labeled tubes. All samples were immediately snap-frozen and stored at –80 °C for subsequent molecular analysis.

Liver and Blood

For further analysis specific to Aim 5, the livers of the mice were also collected immediately after dissection. Each liver was placed in a sterile 3 cm Petri dish (Greiner Bio-One, Kremsmünster, Austria), which was then sealed with Parafilm® M (Bemis Company, Inc., Neenah, WI, USA) to maintain sterility and prevent drying. In addition to tissue collection, trunk blood was collected directly after decapitation. The blood was allowed to clot at room temperature for 30 minutes and was then centrifuged for 10 minutes at 1200 x g at 4°C. The resulting serum was carefully transferred into clean 1.5 mL reaction tubes (Eppendorf, Hamburg, Germany) and stored at –80 °C for subsequent biochemical analyses.

Data Analysis

Behavioral data from the open field test were analyzed using Noldus EthoVision XT software Version 17.5.1718, which enabled automated tracking of key parameters such as total distance traveled, movement velocity, and time spent in predefined zones. The extracted data were processed and organized using Microsoft Excel for preliminary calculations. Statistical analyses were conducted using GraphPad Prism, where appropriate tests (e.g., one-way ANOVA or Kruskal–Wallis test) were applied depending on data distribution. Graphs were also generated in GraphPad Prism to visually represent

the findings and highlight treatment effects. The schematic illustrations depicting the experimental timeline were created using the online tool BioRender.

Results

Aim 1 Dose Response THC

To evaluate the acute effects of Δ^9-tetrahydrocannabinol (THC) on physiological and behavioral parameters, mice were administered increasing doses of THC (1, 3, 5, and 10 mg/kg, i.p.), followed by open field testing 30 minutes post-injection (Figure 3; Metna-Laurent et al., 2017).

We first found a clear, dose-dependent reduction in body temperature in mice treated with higher THC concentrations (5 mg/kg and 10 mg/kg), showing a significant hypothermic effect compared to the control group (Figure 8a). In contrast, mice that received the vehicle solution showed no notable change in body temperature between pre- and post-test measurements. Similarly, the 3 mg/kg THC group did not exhibit a significant temperature difference. Interestingly, the 1 mg/kg THC group appeared to show a slight increase in body temperature, suggesting a potential hyperthermic response at low doses.

In the open field test, we found a clear dose-dependent reduction in locomotor activity (hypolocomotion) across all THC-treated groups (Figure 8b). All concentrations of THC resulted in a statistically significant decreased in the distance traveled compared to the control group. This effect was particularly pronounced in the 5 mg/kg and 10 mg/kg groups, which showed a highly significant suppression of locomotion.

A more detailed view of locomotor activity through illustrating the average distance moved in 5-minute intervals further reveals the dose-dependent hypolocomotor effect of THC, with higher doses consistently associated with reduced movement across all time bins (Figure $8b_1$).

Representative movement tracks and heatmaps further visualized these behavioral impairments (Figure 8c). While vehicle- and 1 mg/kg-treated mice showed widespread arena exploration, higher THC doses resulted in highly restricted movement, with mice spending extended time in the corners or along the edges

In addition to general locomotor activity, specific behavioral parameters were analyzed to further assess the acute effects of THC. While the number of rotations per meter (Figure 8d) and grooming behavior (Figure $8e_1$), indicators of impaired motor control, showed a slight increase at higher THC concentrations, these changes were not

statistically significant. In contrast, supported rearing (i.e., rearing with contact to the wall) was clearly reduced starting at 3 mg/kg, with a marked decrease observed at 5 and 10 mg/kg, indicating a suppression of vertical exploratory behavior (Figure $8e_1$). A similar trend was observed for unsupported rearing, which decreased with increasing doses of THC, especially at 10 mg/kg (Figure $8e_2$). Interestingly, mice treated with 1 mg/kg THC displayed a notable increase in unsupported rearing compared to controls. This suggests a biphasic effect of THC on exploratory activity, enhancing behavior at low doses while suppressing it at higher doses.

The catalepsy test was performed to assess THC-induced motor rigidity. In the control group, all mice were able to grasp the horizontal bar without difficulty and kept their entire body on the bar during the experiment.

In contrast, mice treated with 10 mg/kg THC could not be evaluated using this test. All individuals exhibited a pronounced and immediate startle-like reaction, commonly referred to as the "popcorn effect" (Metna-Laurent et al., 2017), even in calm conditions. This reaction made it impossible to position the animals on the bar without triggering uncontrolled movement.

In the 5 mg/kg THC group, two out of three mice displayed clear cataleptic behavior, while the other mice showed similar popcorn effects making measurements impossible. One mouse remained immobile for an average of 80 seconds across three trials, while another maintained the imposed posture for over 5 minutes in a single trial.

These results indicate a dose-dependent trend in THC-induced catalepsy, although consistent quantification at higher doses was limited by variability in behavioral responses, particularly the strong startle effects.

We decided to continue with a concentration of 5 mg/kg THC in further experiments based on the following parameters. After administration of THC, a hypothermic effect was observed at concentrations of 5 and 10 mg/kg. However, analysis of locomotion showed that the mice that had received 10 mg/kg hardly moved and that their movement was strongly influenced by the THC. In addition, as described above, it was only possible to observe cataleptic behavior in the mice when 5 mg/kg THC was administered.

In summary, only when 5 mg/kg THC was administered, the TETRAD parameters, hypothermia, hypolocomotion, and catalepsy were fulfilled. Based on this robust foundation, this concentration will be used for future experiments.

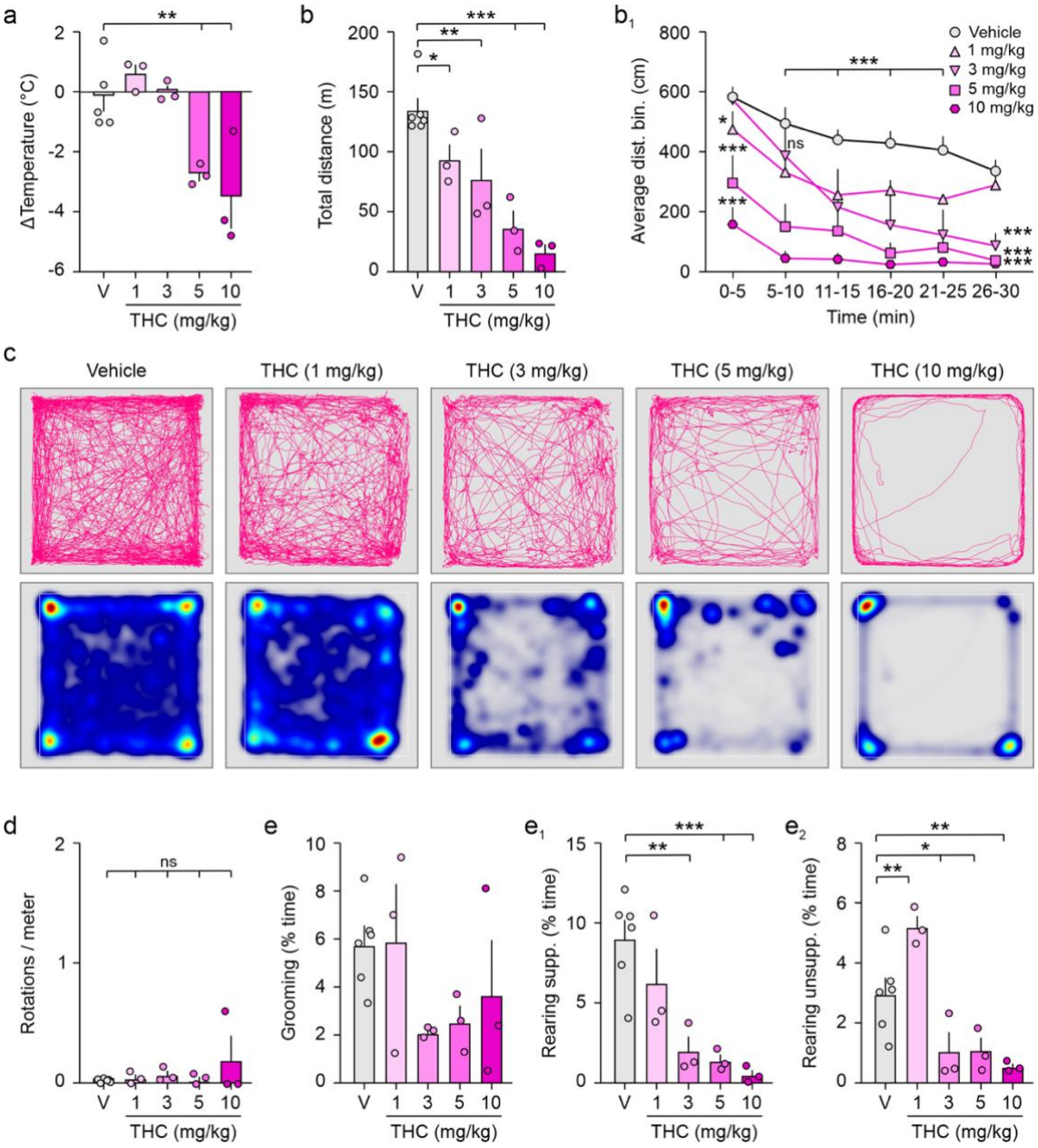

Figure 8. ***Dose-response stimulation with THC.*** *(**a**) Differences in core body temperature post THC exposure (n = 3-5 per condition). (**b,b₁**) Distance travelled in the open field arena. (**c**) Example traces and heat maps during the open field trial. (**d**) Number of rotations around their central axis. (**e-e₂**) Grooming and rearing times during the open field trial. *P<0.05, **P<0.01, ***P<0.001.*

Aim 2 Dose Response acute Ketamine

To evaluate the acute effects of (S)-ketamine on physiological and behavioral parameters, mice were administered increasing doses of (S)-ketamine 5 mg/kg (Khakpai et al., 2019; McDonnell et al., 2021), 10 mg/kg (Fitzgerald et al., 2019; Ouyang et al., 2021) and 15 mg/kg (Acevedo et al., 2023), i.p.), followed by open field testing 30 minutes immediately post-injection (Figure 4).

Following the injection of 5 mg/kg (S)-ketamine, a slight increase in body temperature was observed both before and after the open field test, comparable to the response seen with the control solution. In contrast, administration of 10 mg/kg (S)-ketamine resulted in a significant reduction in body temperature. At a higher dose of 15 mg/kg (S)-ketamine, a slight decrease in body temperature was observed in most animals, which also showed a significant difference compared to the control group (Figure 9a).

To further investigate this effect, we next examined the total distance moved (in meters) across the different concentrations of (S)-ketamine. No significant differences were observed in the overall distance moved over the 30-minute testing period (Figure 9b). However, a more detailed analysis of locomotor activity in 5-minute intervals, revealed a significant increase in locomotion (hyperlocomotion) following administration of 5 mg/kg (S)-ketamine in the first 10 minutes. In contrast, a significant decrease in locomotor activity (hypolocomotion) was observed at the 15 mg/kg dose in the first 10 minutes (Figure $9b_1$).

The tracking plots and heatmaps illustrate the movement patterns and spatial distribution of the mice during the open field test. The figures show that the movement of the mice did not differ significantly within the 30-minute open field test, despite different (S)-ketamine concentrations (Figure 9c). The rotations around the body axis revealed that the number of rotations per meter increased with higher doses of (S)-ketamine (Figure 9d). No significant changes were observed in grooming behavior (Figure 9e) across the different treatment groups. In contrast, both supported (Figure $9e_1$) and unsupported rearing (Figure $9e_2$) were significantly reduced following (S)-

ketamine administration, indicating a dose-dependent suppression of vertical exploratory activity.

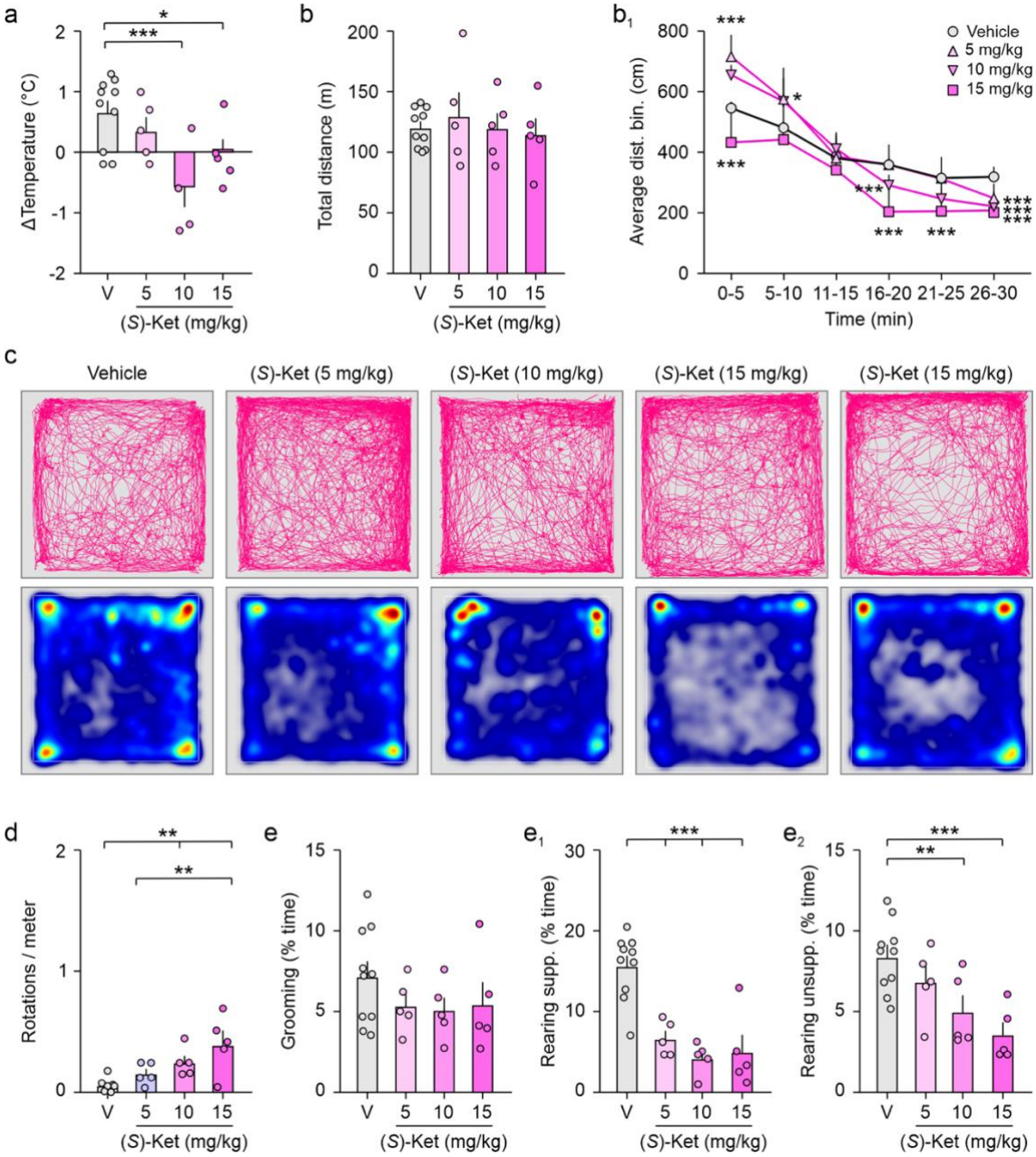

*Figure 9. **Treatment with (S)-ketamine.** (a) Differences in core body temperature post (S)-ketamine injection (n = 4-10 per condition). (b,b1) Distance travelled in the open field arena. (c) Example traces and heat maps during the open field trial. (d) Number of rotations around their central axis. (e-e2) Grooming and rearing times during the open field trial. *P<0.05, **P<0.01, ***P<0.001.*

Aim 3 Acute (S)-ketamine and THC

In this experiment, we continued with the same cohort of mice from Aim 2 to assess the effects of 5 mg/kg THC alone and on top of various doses of (S)-ketamine (5, 10, or 15 mg/kg) (Figure 5.)

Core body temperature measurements revealed a robust hypothermic response in all THC-treated groups compared to vehicle-injected controls. Administration of THC alone led to a significant temperature reduction of slightly more than 2 °C. A similar level of hypothermia was observed in mice pre-treated with 10 mg/kg (*S*)-ketamine, followed one hour later by 5 mg/kg THC. Interestingly, mice pre-treated with either 5 mg/kg or 15 mg/kg (S)-ketamine prior to THC exhibited a more pronounced temperature drop of approximately 3 °C (Figure 10a).

In terms of locomotor activity, a significant reduction in total distance moved was observed in all groups receiving THC, regardless of the (S)-ketamine dose administered beforehand. This suppression in locomotion was particularly evident when analyzing the distance traveled in 5-minute bins. Specifically, a marked reduction in activity was observed between minutes 5 - 10 and again from minutes 16 - 25 in the THC-only group, a pattern that was mirrored across all ketamine-THC combination groups (Figure 10b, b1). These behavioral changes were further supported by the tracing plots and heatmaps, which demonstrated a restricted movement pattern in the (S)-ketamine and THC-treated mice (Figure 10c).

Additional behavioral parameters assessed in the open field included grooming, rearing supported, and rearing unsupported. Across all THC-treated groups, there was a significant reduction in these exploratory behaviors compared to controls, particularly in vertical activity (Figure 10e, e_1, e_2). Importantly, the parameter "rotations per meter," which was found to increase with higher doses of (S)-ketamine in the previous experiment, was not elevated in any of the THC-treated groups, suggesting that THC may suppress the rotational or stereotypic behaviors otherwise induced by ketamine (Figure 10d).

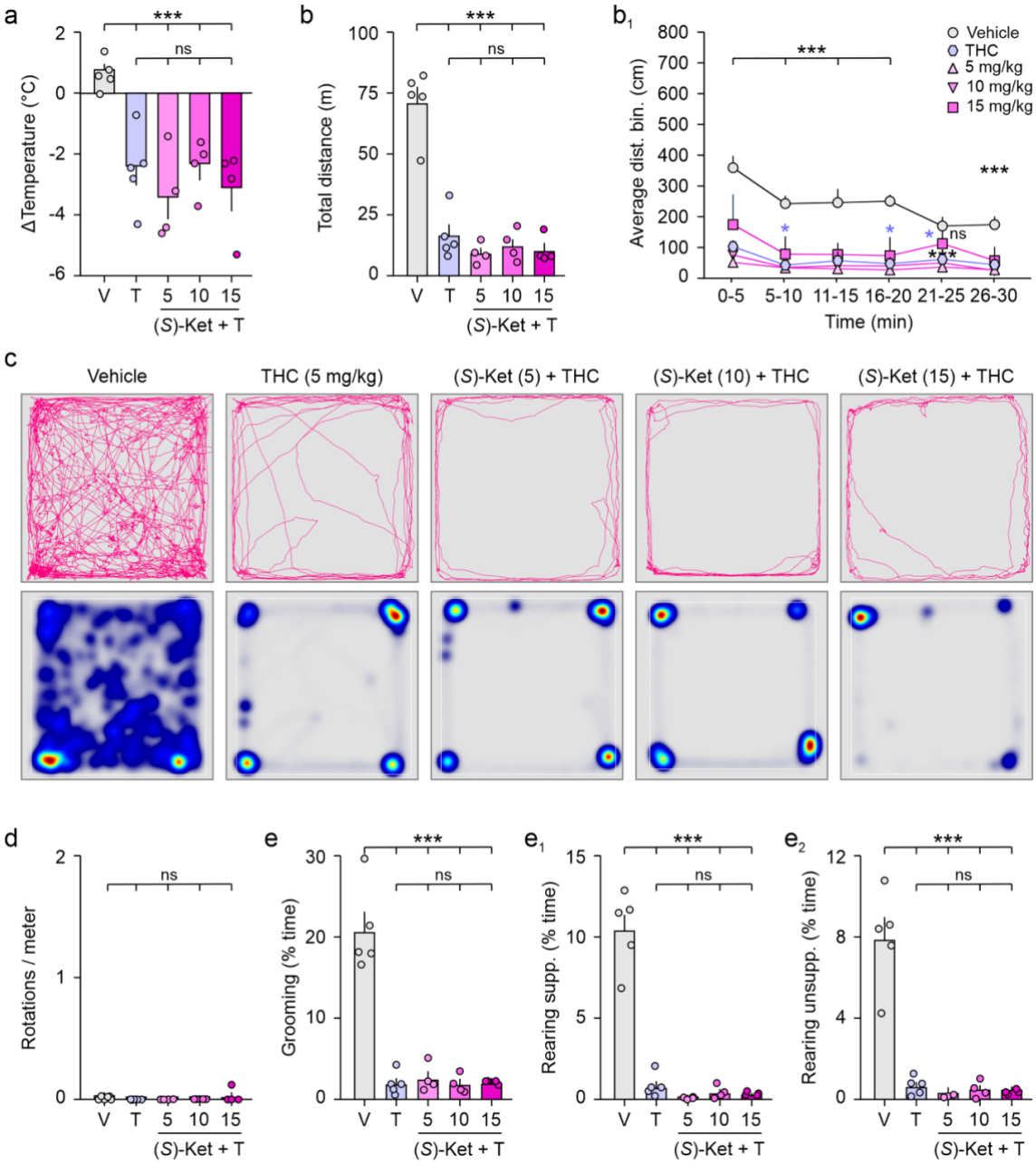

Figure 10. ***Combined Effects of (S)-Ketamine and THC.*** *(a) Differences in core body temperature post (S)-ketamine and THC exposure (n = 4-5 per condition). (b,b1) Distance travelled in the open field arena. (c) Example traces and heat maps during the open field trial. (d) Number of rotations around their central axis. (ee2) Grooming and rearing times during the open field trial. *P<0.05, **P<0.01, ***P<0.001.*

Aim 4 THC and (S)-ketamine

In Aim 4, animals were first administered 5 mg/kg THC, followed by an injection of (S)-ketamine at varying concentrations (5, 10, or 15 mg/kg) 30 minutes later (Figure 6).

As observed in the previous aim, THC alone induced a robust hypothermic response, lowering core body temperature by approximately 2 °C compared to controls. When (S)-ketamine was administered after THC, an even more noticeable temperature reduction was recorded. In all (S)-ketamine groups, body temperature dropped by more than 5 °C, a significant reduction both in comparison to the THC-only group and to vehicle injected controls. This indicated a strong synergistic effect of (S)-ketamine on THC-induced hypothermia (Figure 11a).

Behavioral assessment was conducted using a 30-minute open field test following both injections. Total distance traveled was significantly decreased in all drug-treated groups, including THC alone and all THC and (S)-ketamine combinations, compared to vehicle-treated controls (Figure 11b). When locomotor activity was analyzed in 5-minute intervals, a persistent and significant suppression of movement across all drug-treated groups was observed, consistent throughout the testing period. The results of the preliminary experiment Aim 2 showed that (S)-ketamine alone causes brief hyperactivity in mice. However, the results of this experiment Aim 4 showed that this hyperactivity was blunted by the prior administration of THC (Figure 11b_1).

Tracing plots revealed significant qualitative differences in movement patterns. While THC-only mice still showed exploratory trajectories, the addition of increasing doses of (S)-ketamine resulted in a progressive shift toward stereotypic behavior characterized by tight, repetitive rotations rather than linear movement (Figure 11c). This behavioral alteration was quantified by the parameter “rotations per meter” which showed a clear dose-dependent increase with higher (S)-ketamine concentrations (Figure 11d).

Vertical exploration was also markedly affected. The number of unsupported rearing events showed a significant reduction in the THC-only group as well as in the 5 and 10 mg/kg (S)-ketamine groups (Figure 11e_2). Furthermore, both grooming and supported rearing were significantly reduced in all THC and (S)-ketamine groups compared to controls, indicating a suppression of self-care behavior, motor control and total movement (Figure 11e, e_1).

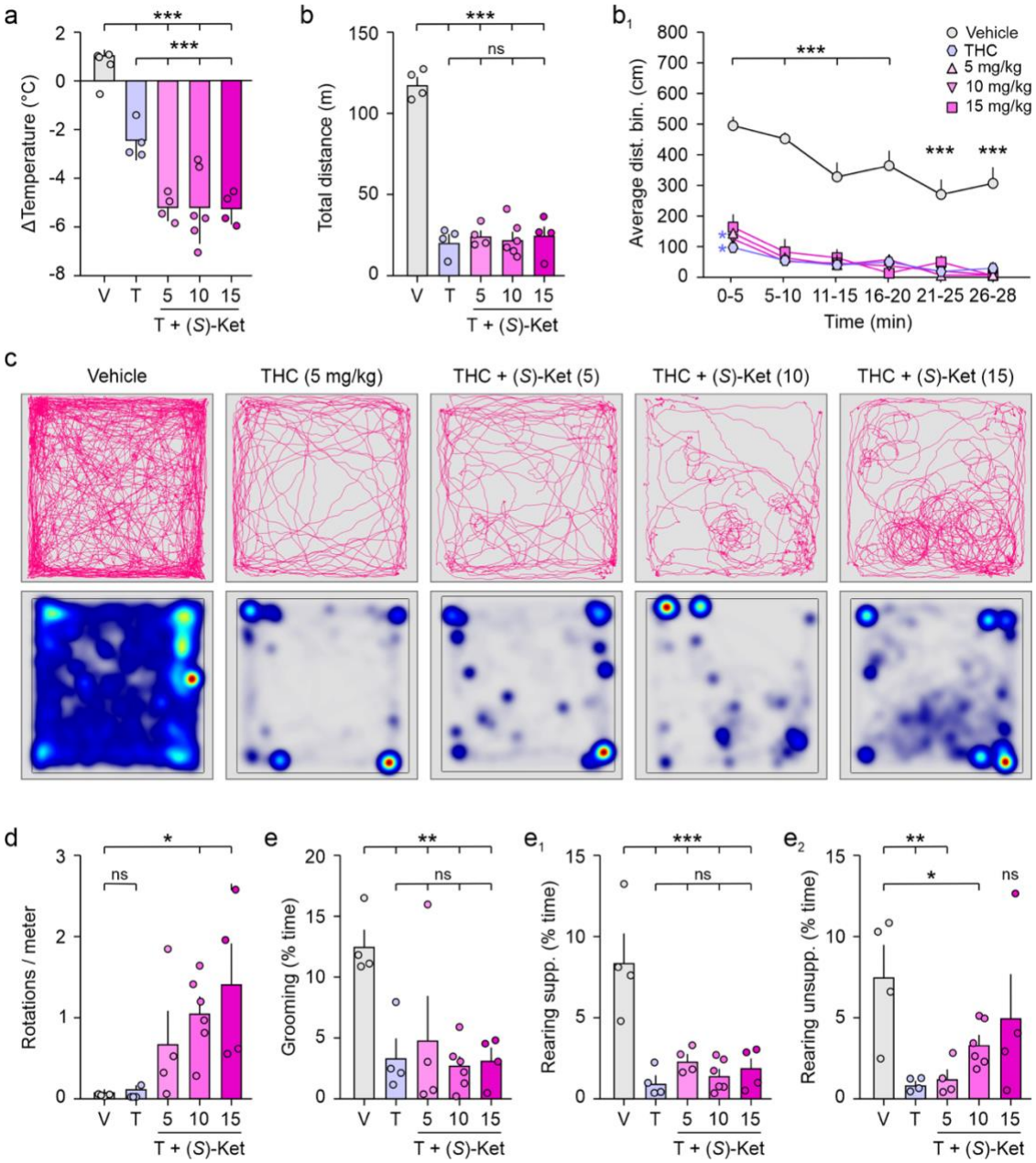

*Figure 11. Interaction of THC and (S)-ketamine. (a) Differences in core body temperature post (S)-ketamine and THC exposure (n = 4-6 per condition). (b,b1) Distance travelled in the open field arena. (c) Example traces and heat maps during the open field trial. (d) Number of rotations around their central axis. (e-e2) Grooming and rearing times during the open field trial. *P<0.05, **P<0.01, ***P<0.001.*

Aim 5 THC and (S)-ketamine including Rotarod

In this experiment, we analyzed the interaction between THC and (S)-ketamine in more detail and carried out additional behavioral tests. Based on our preliminary experiments, in which we tested different concentrations, we were able to see that 5 mg/kg THC is a solid basis for our experiments. Furthermore, under our laboratory conditions, a concentration of 10 mg/kg (S)-ketamine proved to be ideal. In Aim 2, we can see that hypothermic effect was induced by acute (S)-ketamine, as well as hyperactivity in the first few minutes of the open field test. In combination (Aim 4), when THC was injected first and then (S)-ketamine, it was evident that the concentration of 10 mg/kg provided robust data without causing excessive impairment in the mouse, as was the case with the higher concentration of 15 mg/kg.

In order to best monitor interaction of THC and (S)-ketamine, body temperature was measured at four timepoints. Before the injection of 5 mg/kg THC at time 0 and 30 minutes later, a significant drop in core body temperature of about 2 °C was observed by minute 30. In the control group, a slight temperature increase occurred over the same period. At timepoint 30, mice received either 10 mg/kg (S)-ketamine or a saline injection. This ketamine administration led to a further significant drop in body temperature, both in mice that had received THC beforehand and in those that had not. By timepoint 90, mice treated only with (S)-ketamine had largely returned to baseline temperature, and the THC-only group showed a partial recovery. In contrast, the combination group (THC + ketamine) showed a continued decrease in temperature. At this timepoint, the core body temperature of this group was significantly lower than in both single-treatment groups, indicating an additive hypothermic effect and a prolonged effect of ketamine in the presence of THC (Figure 12a).

The rotarod test was performed following the final temperature measurement. When we analyze the results of the rotarod test on mice that received either THC or (S)-ketamine, we can see that they took less time to fail than the control group, but this difference was not significant regardless of the drug. However, mice in the THC and (S)-ketamine group showed a significantly shorter latency to fall compared to controls, suggesting impaired motor coordination. Now it can be seen once again that the combination of both drugs clearly led to more pronounced and significant results,

whereas the individual drug only had a mild effect on the mice (Figure 12a$_1$). Each animal was tested three times, and the mean value is shown. Trials were aborted if the mouse began to rotate passively without active walking.

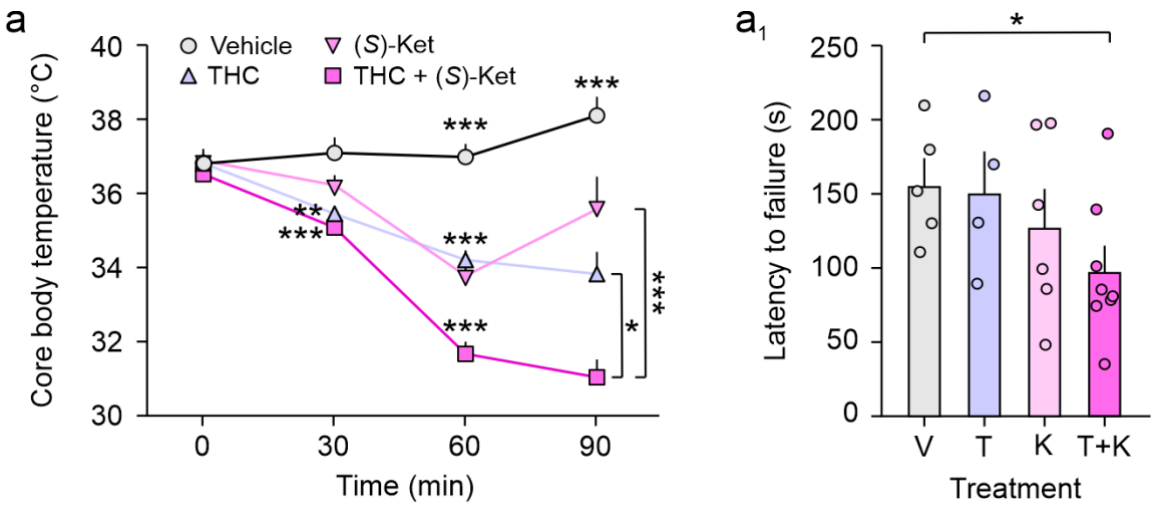

Figure 12. Co-administration of THC and (S)-Ketamine (a) Core body temperature over time post THC and (S)-ketamine Treatment (a$_1$) Motor coordination assessed by the rotarod.

Conclusion on findings

In summary of the results, we can first say that in Aim 1 we were able to demonstrate a strong dose-dependent effect of THC on core body temperature, locomotion, and catalepsy which replicated the literature. Furthermore, Aim 2 showed, (S)-ketamine acutely induces hyperactivity in the first few minutes when measuring locomotion, as described in the literature.

When (S)-ketamine is administered first and then THC is injected, there appears to be no cumulative interaction between the two drugs, instead, the body temperature and movement of the mice are similar to that of mice that received only THC. However, if we change the order of the substances, and THC is administered first followed by (S)-ketamine, there is a significant reduction in temperature and locomotion compared to the mice that received only THC. Similarly, in the following experiment, we were able to replicate our results and again observe the same effects.

From this, we can conclude that the order in which the drugs are administered makes a significant difference, and further experiments must be conducted to investigate the reason for this finding.

Discussion

To date, there is no published work that has systematically investigated the combined administration of THC and (S)-ketamine or the possible interactions between these two substances. In this study, our first aim was to determine the baseline effects of each substance in our laboratory under standardized conditions in our in-house C57BL/6 mouse strain. This allowed us to identify behaviorally and physiologically relevant concentrations that could then be used in combination experiments to investigate potential additive or synergistic effects. By first characterizing the isolated effects of THC and (S)-ketamine, we established a solid foundation for interpreting the results of co-administration of these compounds.

Aim 1 acute THC

The aim of the first experiment was to assess the classical cannabinoid "Tetrad" effects of Δ^9-tetrahydrocannabinol (THC) in mice, while adapting the protocol to reflect concentrations relevant to human exposure. The Tetrad test is a well-established behavioral model used to characterize the effects of cannabinoids and typically includes four parameters: hypolocomotion, catalepsy, hypothermia, and analgesia (Metna-Laurent et al., 2017). These effects are considered hallmark indicators of CB_1 receptor activation.

In our study, it was crucial to characterize the specific behavioral and physiological effects of THC with our laboratory conditions and mouse strain, as the effects of THC strongly differ between mouse lines, sex, and age (Lulek et al., 2023). The experimental procedures were adapted from an established Tetrad protocol (Metna-Laurent et al., 2017), although the analgesia component was excluded to reduce animal burden and to focus on parameters most relevant to our subsequent behavioral studies. Furthermore, in contrast to the original publication, we chose not to include the highest tested dose of 15 mg/kg THC, as we already observed that 10 mg/kg induced a near-complete lack of mobility.

The central question of this study was to identify the THC concentrations at which the characteristic Tetrad effects, excluding analgesia, consistently emerge, without inducing complete immobilization or anesthesia-like states. Establishing this dose-response

relationship was essential for validating our in-house model and for informing later experiments on THC interactions with other drugs, such as (*S*)-ketamine.

We observed a dose-dependent effect on body temperature, with higher doses leading to a greater decrease, with a significant reduction in body temperature at 5 and 10 mg/kg THC.

The THC induced hypothermia is already well described in the literature (Elmes et al., 2019; Lulek et al., 2023; Varvel et al., 2005) and one mechanism could be the CB_1 receptor activation in the hypothalamus, which plays a central role in thermoregulation (Wenger & Moldrich, 2002). In addition to its effects on body temperature, THC administration also significantly impairs locomotor activity in a dose-dependent manner. A progressive hypolocomotion was observed with increasing THC doses. Notably, a significant reduction in movement was already detectable at a dose of 1 mg/kg, with even more pronounced and highly significant decreases in locomotor activity observed at 5 mg/kg and 10 mg/kg. These findings support the well-documented suppressive effects of cannabinoids on motor behavior and suggest that even low doses of THC are sufficient to induce measurable behavioral alterations in mice.

The results regarding body temperature and locomotor activity are consistent with the findings reported in the reference study (Metna-Laurent et al., 2017) we used as a template and could be reliably replicated in our experiment.

As already emphasized in the section on the results, the catalepsy test was difficult to perform. Our results indicate a dose-dependent effect of THC on cataleptic behavior, with clear immobility observed at 5 mg/kg. However, at 10 mg/kg, the assessment was not possible due to the “popcorn effect,” a strong startle-like response that disrupted the test. This suggests that while moderate doses induce catalepsy, higher doses may trigger competing hyperreactive behaviors, preventing reliable measurement. These findings highlight the complex and dose-sensitive effects of THC on motor function, consistent with previous reports on cannabinoid-induced motor changes. An ideal THC dose would reliably produce the characteristic cannabinoid effects measured in the tetrad test, namely hypothermia, hypolocomotion and catalepsy, without inducing excessive sedation or behavioral artifacts that could interfere with further analyses. Based on our results, we selected a dose of 5 mg/kg THC as the most suitable, as it consistently produced measurable effects across all tested parameters while still

allowing for sufficient behavioral assessment. In addition, this dose is commonly used in the literature, further supporting its relevance and comparability for future studies

Aim 2 acute (S)-ketamine

The objective of this research was to investigate the acute effects of (S)-ketamine under our specific laboratory conditions using our in-house mouse strain, with the goal of identifying an appropriate concentration for future experiments. Given the known variability in ketamine's effects depending on dose, strain, age, and environment (W.-C. Chen et al., 2023), it was essential to first establish a baseline behavioral and physiological profile in our setup. To this end, we assessed body temperature changes and performed a detailed analysis of behavioral patterns in the open field test following administration of different doses of (S)-ketamine.

In terms of body temperature, we observed a highly significant reduction following administration of 10 mg/kg (S)-ketamine compared to mice receiving the control solution. This reduction appears to be dose-dependent and is consistent with earlier findings, such as those described in a 1978 study (Lin et al., 1978), which reported a similar thermoregulatory response in rats to ketamine. A more recent study by Vučković et al. from 2014 (Vučković et al., 2014) further confirmed this effect in rats, demonstrating significant temperature reductions at ketamine doses of 10 mg/kg and above. The authors attributed the hypothermia to NMDA receptor blockade in hypothalamic thermoregulatory centers, potentially involving serotonergic pathways (Fahim et al., 1973). Our findings at 10 mg/kg (S)-ketamine are in line with these observations, supporting the role of NMDA antagonism in centrally mediated temperature regulation.

Interestingly, an increase in body temperature after 30 minutes in the open field arena can also be found in the literature following administration of a control solution. This suggests that environmental factors, such as prolonged exposure to a novel testing arena, may contribute to a mild stress-induced hyperthermia in control animals, whereas ketamine at higher doses counteracts this physiological response through its sedative or dissociative properties.

As described in the results section, no significant differences were observed in the total distance moved over 30 minutes compared to the control group. However, a more

detailed temporal analysis of locomotor activity revealed a time-dependent pattern. Mice that received 5 mg/kg (S)-ketamine exhibited pronounced hyperlocomotion within the first 5 minutes post-injection, a finding that aligns well with previous reports in the literature describing ketamine's stimulant-like effects at low doses. In contrast, administration of 15 mg/kg (*S*)-ketamine led to a marked attenuation of movement, highlighting the characteristic biphasic effect of ketamine: stimulation at low doses and suppression at higher doses. Similar dose-dependent effects were reported by Chen et al. 2023 (W.-C. Chen et al., 2023) who, despite using higher doses (25 and 50 mg/kg), observed hyperlocomotion at the lower dose and reduced or delayed activity at the higher dose in C57BL/6J mice. This supports our findings and highlights the biphasic behavioral response to ketamine.

We observed that with increasing doses of (S)-ketamine, mice displayed a higher number of rotations around their body axis. This type of stereotyped behavior has been previously described and is often linked to alterations in the dopaminergic system. While earlier studies associated rotation behavior with dopaminergic agents like amphetamine or quinpirole (Kokkinidis & Anisman, 1979; Shannon et al., 2000) more recent findings also point to ketamine's ability to induce similar patterns. In line with our results, the group of Galvanho et al., (2020) demonstrated that ketamine induces stereotyped behaviors in mice, and highlighted a modulatory role for dopaminergic and serotonergic receptors, suggesting a complex interaction between multiple neurotransmitter systems. Both supported and unsupported rearing were reduced in a dose-dependent manner with increasing ketamine concentrations. This decline in vertical exploration likely reflects a general suppression of exploratory drive and motor activity, possibly due to ketamine's dissociative and sedative effects at higher concentrations, which is described in the literature (Galvanho et al., 2020).

Overall, our results indicate a dose-dependent effect of (*S*)-ketamine on thermoregulation and behavior, including locomotion, rotational activity, and rearing. These findings support previously described biphasic effects and suggest that intermediate doses (e.g., 10 mg/kg) may be suitable for further studies targeting behavioral activation.

Aim 3 Acute (S)- ketamine and THC

When the same mice were subsequently administered 5 mg/kg THC, we observed distinct effects on both thermoregulation and behavior.

What we observed was that the administration of THC led to a significant drop in core body temperature in all groups that had previously received (S)-ketamine. Consistent with our results from previous experiments, THC alone induced a robust hypothermic response of approximately 2 °C. Notably, this effect appeared stronger in mice pre-treated with 5 mg/kg or 15 mg/kg (S)-ketamine, as these groups showed a greater reduction in core temperature than the THC-only group. However, statistical analysis showed that the decrease in the THC control group was not significantly different from the ketamine pre-treated groups. In particular, the group that received 10 mg/kg (S)-ketamine followed by THC showed a temperature drop closely to the THC-only group, indicating no additive effect at this dose combination.

These findings highlight the complexity of how these two psychoactive substances influence thermoregulation, potentially involving NMDA receptor blockade (Autry et al., 2011; Zhang et al., 2021) and cannabinoid receptor activation (Pertwee, 2006; Thorsen et al., 2025).

When examining the 30-minute recordings from the open field test, we observed that, similar to Aim 1, the locomotor activity of the mice was reduced following THC administration, regardless of whether they had previously received different concentrations of (S)-ketamine. This confirms that THC has a strong suppressive effect on spontaneous movement.

Interestingly, even the control group, which did not receive THC or ketamine, showed a reduced total distance traveled during this session. This can likely be linked to the reduced novelty of the environment, as the mice were exposed to the open field arena for the second time and therefore exhibited less exploratory behavior. As previously described in a study from 2023 (W. Chen et al., 2023) environmental familiarity reduces exploratory drive and novelty-induced locomotion, which likely contributes to the observed decrease in activity.

Similar to the reduction in total distance moved, which is additionally demonstrated in the tracking plots and heatmaps, all other behavioral parameters, such as grooming,

supported rearing, and unsupported rearing are also markedly suppressed following THC administration.

Nonetheless, our data suggest that the reduction in core temperature and the attenuated behavioral effects is primarily driven by the THC administration itself.

Aim 4 THC and (S)-ketamine

However, when we reversed the order of substance administration, injecting 5 mg/kg THC first, followed by varying concentrations of (S)-ketamine, we observed a different outcome. Specifically, body temperature drops significantly compared to the group receiving only THC. Regardless of the (S)-ketamine dose administered, the temperature difference between the pre-THC baseline (time point 0) and the post-ketamine measurement after the open field test (time point 60) is approximately 5°C. In contrast, the group treated with THC alone showed a smaller temperature reduction of about 2°C, consistent with previous experiments.

These contrary results compared to Aim 3 are evident not only in body temperature but also in the locomotor activity of the mice. Despite (S)-ketamine being administered 30 minutes after THC, followed by immediate open field testing, the mice's movement remains markedly reduced and dampened. However, in the 5-minute time bins, mice receiving 15 mg/kg (S)-ketamine exhibit a short phase of significant hyperlocomotion in the first 5 minutes compared to the THC-only group. This suggests that high-dose (S)-ketamine can still briefly override the suppressive motor effects of THC.

As previously observed in Aim 2 with acute (S)-ketamine administration, a characteristic rotational behavior emerges following ketamine treatment. When THC is administered prior to (S)-ketamine, this rotational behavior becomes significantly more pronounced, reminiscent of higher concentrations of ketamine use (Galvanho et al., 2020). Tracking data (Figure 11c) clearly showed that the mice exhibit little exploratory movement beyond spinning around their own axis. This effect is dose-dependent, becoming more severe with increasing (S)-ketamine concentrations on top of the THC pre-treatment.

Further analysis of locomotor confirmed, that the prior administration of THC intensifies the rotational phenotype. When examining rotations per meter traveled, a clear increase is seen in the groups receiving THC before (S)-ketamine, indicating that rotation becomes

the dominant form of locomotion. Tracking maps and heatmaps support this observation: instead of exploring the arena, the mice primarily rotate in place.

Figure 13 Rotational behavior in mice.

Interestingly, when analyzing the percentage of rearing, an apparent but inconsistent dose-dependence emerged with escalating (S)-ketamine concentrations. However, upon manual video review, it became evident that the software had misclassified certain behaviors: what was detected as "rearing" was in fact the mouse falling onto its side, likely due to loss of motor coordination caused by excessive rotation.

This behavior, falling over during rotational movement, was exclusively observed in the THC followed by (S)-ketamine condition and was absent in THC-only or ketamine-only groups. The motor instability suggests an impaired coordination of limb movements, potentially pointing to dysfunction in motor circuitry, such as the basal ganglia or cerebellum (Klaus et al., 2019; Manto et al., 2012).

Such a phenotype has been described in the literature (Galvanho et al., 2020), where ketamine-induced rotation was linked to disrupted dopaminergic signaling and NMDA receptor antagonism. The combination with THC may exacerbate this through additional modulation of CB_1 receptor pathways and cross-talk with dopaminergic systems.

Particularly outstanding, in addition to the intense hypothermia and hypolocomotion, was the persistent behavioral impairment in mice that received THC and then (S)-ketamine. Even 90 minutes after THC injection (namely, 60 minutes after (S)-ketamine administration), these animals were still visibly impaired, showing motor deficits and reduced exploration.

In contrast, as shown in Aim 2, the mice that received only (S)-ketamine had largely recovered after 60 min and exhibited behavior that was indistinguishable from that of the control animals. This discrepancy emphasizes that the combination of THC and (S)-ketamine may prolong or potentiate the pharmacologic effects of ketamine.

One possible explanation for this prolonged impairment is THC-induced inhibition of liver enzymes, particularly cytochrome P450 isoforms such as CYP2C9 and CYP2B6, which are involved in THC and ketamine metabolism. In vitro studies have shown that THC and its metabolites act as competitive inhibitors of several cytochrome P450 (CYP450) enzymes, particularly CYP2B6 and CYP2C9 (Nasrin et al., 2021). Among these enzymes, CYP2C9 is particularly important as it is responsible for about 70 % of THC metabolism in humans. The enzyme CYP2C9 mainly converts the active metabolite 11-OH-THC and into the inactive 11-COOH-THC (Yabut et al., 2024).

This interaction is particularly important in the context of drug-drug interactions: (S)-ketamine is also metabolized by CYP2B6 and CYP2C9 (Yanagihara et al., 2001), which catalyze its biotransformation into norketamine, a less potent metabolite (Dinis-Oliveira, 2017; Nguyen et al., 2022). When THC is administered before (S)-ketamine, it may inhibit these common metabolic pathways, leading to a reduction in the metabolic clearance of ketamine. This could lead to higher plasma and brain concentrations of unmetabolized (S)-ketamine, prolonging and enhancing its pharmacodynamic effects.

Another possible explanation for the prolonged and intensified behavioral suppression observed in the group that received THC prior to (S)-ketamine lies in a potential disruption of adrenaline signaling. Ketamine is known to activate the sympathetic nervous system (Kovacova et al., 2024), which results in the release of catecholamines such as adrenaline and noradrenaline (Chu et al., 2025), contributing to its characteristic arousing effects, increased locomotor activity, change body temperature, and heightened alertness. In our study, these effects were clearly seen in mice treated with ketamine alone (Aim 2), where the animals recovered within 60 minutes post-injection. However, when THC was administered beforehand, this recovery did not occur. This could be due to THC's ability to interfere with peripheral and central sympathetic pathways (Chayasirisobhon, 2020; Zou & Kumar, 2018). An in vitro study using isolated rabbit adrenal glands showed that cannabinoids suppress adrenaline secretion by activating presynaptic CB_1 receptors. Resulting in a release of acetylcholine from preganglionic sympathetic neurons. According to the authors, this mechanism likely accounts for the reduced plasma adrenaline levels observed following cannabinoid administration in pithed rabbits (Niederhoffer et al., 2001).

Translating this to our mouse model, pre-treatment with THC may dampen the adrenal medulla's response to sympathetic input, therefore blunting the adrenaline rush normally triggered by ketamine. So, ketamine's typical sympathomimetic effects may be reduced or absent, contributing to the intensified hypolocomotion, exaggerated hypothermia, and rotational behavior seen in the combined treatment group. These findings suggest a synergistic effect, where THC inhibits the capacity of ketamine to engage its usual compensatory arousal mechanisms, particularly via interference with adrenaline-mediated pathways.

Taken together, these findings highlight the critical importance of the administration sequence when combining THC and (S)-ketamine. The more pronounced effects observed, ranging from extreme hypothermia and restricted mobility to exaggerated turning behavior and motor instability, suggest that pretreatment with THC significantly alters the animal's physiological response to subsequent exposure to (S)-ketamine. However, to date, no studies have systematically investigated the combination of THC and (S)-ketamine in this specific order of administration. As a result, the exact mechanisms underlying the apparent amplification or potentiation of both physiological and behavioral effects remain unclear.

Although our results indicate a strong interaction between the two active substances, further research is needed to investigate the molecular mechanisms involved.

Aim 5 THC and (S)-ketamine including Rotarod

To further investigate these results, in our final experiment, we combined the previously determined concentrations of 5 mg/kg THC and 10 mg/kg (S)-ketamine and investigated both the temperature and the behavior. As can be seen from the temperature curve, we were able to successfully repeat the results of previous experiments. By measuring the body temperature at four time points, we were able to follow the course of the temperature precisely. Remarkably, mice treated with (S)-ketamine alone returned to near baseline temperature at minute 90 - 60 minutes after injection. In contrast, mice that received THC at time 0 followed by ketamine showed a continuous drop in body temperature with a difference of about 6 °C.

Since impaired motor coordination was previously observed in the THC and (S)-ketamine group, we included the rotarod test in this experiment. As expected, the mice in the

combined treatment group remained on the rotating rod for a significantly shorter time, indicating a pronounced motor impairment.

In the rotarod test, the control and THC-only groups showed comparable performance, suggesting that at the administered dose (5 mg/kg), THC alone did not significantly impair motor coordination. The ketamine-only group (10 mg/kg) exhibited a slight reduction in rotarod time, however since the test was taken 60 minutes after the (S)-ketamine injection and the animals were back not normal, this result is not surprising. However, the most striking result emerged in the combined THC and (S)-ketamine group, which demonstrated a significant decline in performance. This suggests a synergistic or interaction effect, where the co-administration of both substances leads to greater motor impairment than either drug alone. This points toward a possible interaction effect when the two drugs are combined, rather than a simple additive effect of their individual actions.

Although the analysis of temperature and motor coordination confirmed our previous findings, the underlying cause of these effects remains unclear. To gain further insight, we collected additional tissue samples, specifically blood, liver, and brain, from the animals in this final experiment. The serum may be used in future molecular analyses, such as ELISAs, to quantify circulating levels of ketamine, adrenaline, or other relevant biomarkers in the respective treatment groups.

Additionally, the liver samples allow us to examine enzyme activity, which could provide valuable information about metabolic processes and bring us closer to understanding the mechanisms underlying the observed effects.

Despite the valuable findings from our experiments, several limitations should be noted. First, the sample size per group was relatively small, which may limit the statistical power and generalizability of the results. Second, although the behavioral tests showed clear differences, they do not provide mechanistic insights on their own. Without direct molecular or pharmacokinetic measurements, such as brain or serum concentrations of THC, ketamine or downstream signaling molecules. We can only speculate about the signaling pathways involved.

In addition, the timing of drug administration and testing was based on established protocols, but it is possible that different intervals could lead to different results, especially given the pharmacodynamic profiles of THC and ketamine. While the rotarod

test is commonly used to assess motor coordination, it does not distinguish between motor deficits, sedation, or motivational changes, all of which could be affected by drug treatment.

Although we have collected brain, liver and blood samples, these tissues have not yet been analyzed, which means that we currently have no molecular confirmation of the proposed mechanisms. Future studies should include detailed biochemical testing and possibly brain region specific analyses to better link the behavioral results to the underlying biological changes.

Conclusion

In this study, the acute physiological and behavioral effects of (S)-ketamine, THC and their combination were investigated in healthy mice, with a focus on thermoregulation and motor coordination. Both substances individually caused only moderate changes in body temperature and locomotion. However, when we administered both drugs, it led to a synergistic decrease in body temperature and a limitation of motor function. These results were consistent across all our experiments and suggest a possible potentiation or interaction between the two substances when administered together.

One possible explanation for our observed results could be due to overlapping mechanisms of the substances (Figure 1). Both (S)-ketamine and THC act on neuronal circuits involved in movement, motivation and the regulation of body temperature like the hypothalamus, cerebellum and basal ganglia. Furthermore, their influence on dopaminergic and glutamatergic systems may underlie the observed behavioral deficits, especially when both drugs are administered at the same time. In addition, both THC and (S)-ketamine are known to affect peripheral organ function. This would include the liver and adrenal glands, affecting both drug clearance and epinephrine availability, adding another layer of complexity to the possible molecular mechanism underlying their synergistic effects.

For this reason, brain, liver and blood samples were taken during the last experiment, which we can analyze in future experiments. The serum samples from these mice could be used to quantify the concentrations of stress hormones or (S)-ketamine metabolites by ELISA or mass spectrometry. Additionally, the sampled liver tissue can provide information on the activity of liver enzymes.

Overall, our results of this work highlight the importance of studying drug interactions in preclinical models. This is essential to highlight that the use of (S)-ketamine for clinical purposes is becoming increasingly important. At the same time, the legalization of cannabis is increasing and therefore its consumption is also on the rise. As a result, it is mandatory for us to understand how these substances interact. This knowledge is critical not only for optimizing therapeutic approaches but also for predicting potential risks associated with multi-drug use.

Future work should focus on detailed molecular analyses, as well as the long-term effects of repeated exposure, to fully uncover the neurobiological consequences of co-use of THC and ketamine.

Disclaimer

I hereby declare that Artificial Intelligence (AI) tools were used exclusively for supportive tasks such as enhancing grammar, refining language clarity, and assisting with formatting. The intellectual content, research design, data analysis, interpretation, and all scientific conclusions presented in this thesis are entirely the result of my own independent work.

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