Cannabis smoke and oral Δ9THC enhance working memory in aged but not young adult subjects

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ABSTRACT

With increased legalization of recreational and medical cannabis, use of this drug is growing rapidly among older adults. As cannabis use can impair cognition in young adults, it is critically important to understand how consumption interacts with the cognitive profile of aged individuals, who are already at increased risk of decline. The current study was designed to determine how cannabis influences multiple forms of cognition in young adult and aged rats of both sexes when delivered via two translationally-relevant routes of administration. Acute exposure to cannabis smoke enhanced prefrontal cortex-dependent working memory accuracy in aged males, but impaired accuracy in aged females, while having no effects in young adults of either sex. In contrast, the same cannabis smoke exposure regimen had minimal effects on a hippocampus-dependent trial-unique non-matching to location mnemonic task, irrespective of age or sex. In a second set of experiments, chronic oral consumption of Δ9-tetrahydrocannabinol (Δ9THC) enhanced working memory in aged rats of both sexes, while having no effects in young adults. In contrast, the same oral Δ9THC regimen did not affect spatial learning and memory in either age group. Minimal age differences were observed in Δ9THC pharmacokinetics with either route of administration. Together, these results show that cannabis and Δ9THC can attenuate working memory impairments that emerge in aging. While these enhancing effects do not extend to hippocampus-dependent cognition, cannabis does not appear to exacerbate age-associated impairments in this cognitive domain.

INTRODUCTION

Cannabis use among older adults is growing rapidly, with estimates from the National Survey on Drug Use and Health showing that from 2015 to 2023 the prevalence of past-year use among adults over age 65 rose from 2.4 to 6.9% [1–4]. Many of these individuals report regular use, and among adults ages 50-64 who use cannabis, 34% of women and 39% of men report daily or near-daily use [5,6]. Despite the growing number of older cannabis users, most studies investigating effects of cannabis on cognition have been restricted to young adult subjects.

Older adults exhibit impairments in multiple forms of cognition, including executive functions such as working memory, as well as spatial and episodic/declarative memory [7–12]. These age-associated cognitive impairments can be modeled in rats. Compared to young adults, aged rats show declines in working memory tasks dependent upon prefrontal cortex (PFC) [13–17], as well as in spatial and episodic memory tasks dependent upon the hippocampus and medial temporal lobe [18–22]. Given that cannabis and cannabinoids can impair these same aspects of cognition [23–27], as well as the fact that other drugs with abuse liability (e.g., alcohol and opioids) have greater adverse effects in older individuals [28–31], there is concern that cannabis use in older adults could exacerbate existing cognitive vulnerabilities. In contrast to these concerns, however, a few studies suggest that cannabis may have fewer adverse cognitive effects and even provide some benefit in older compared to younger adults [32,33]. In addition, a few studies show that chronic administration of the primary psychoactive component of cannabis, Δ9-tetrahydrocannabinol (Δ9THC), via osmotic mini-pump enhanced performance of aged male mice across several cognitive tasks, while having no effects or even impairing performance in young adults [34,35]. These results suggest possible benefits of Δ9THC in aged subjects; however, the passive drug delivery in this prior work did not mirror either the temporal dynamics or routes of administration of human cannabis use, which can influence its cognitive consequences via differences in the speed of absorption and/or metabolism [36].

The current study was designed to more fully elucidate the cognitive effects of cannabis delivered through multiple routes of administration and time courses that mimic those frequently used by older adults (acute intake of cannabis smoke and repeated voluntary oral consumption of Δ9THC). Moreover, the study included both male and female subjects (the first preclinical aging study to date examining sex-specific effects of cannabis use on cognition). The cognitive assessments were designed to independently evaluate effects of cannabis and Δ9THC on PFC - and hippocampus-dependent cognition. To assess PFC-dependent working memory, a delayed response task dependent on the medial PFC but not hippocampus was used [37]. In contrast, acute effects of cannabis smoke were assessed on a trial unique non-match to location (TUNL) task sensitive to damage to the hippocampus but not PFC [38,39]. For the repeated-exposure oral Δ9THC consumption study, the water maze was used as a “gold standard” assessment of hippocampal-dependent cognition. Parallel pharmacokinetic studies were performed to aid in the interpretation of age- and sex-dependent effects of cannabis on cognition. The results show that both acute cannabis smoke inhalation and repeated oral Δ9THC consumption can enhance cognition in aged rats, but that these enhancing effects are sex- and cognitive domain-specific.

METHODS

Subjects

Young adult (6-9 months) and aged (24-28 months) Fischer 344 x Brown Norway F1 hybrid rats of both sexes were food restricted to 80-85% of their free-feeding weight during cognitive testing, except as noted below. All procedures were approved by the University of Florida Animal Care and Use Committee.

Experiment 1: Effects of acute cannabis smoke on cognition in young adult and aged rats.

In separate experiments, rats were trained on either a delayed response working memory task (Experiment 1a) or the TUNL task (Experiment 1b). Upon reaching stable baseline performance, the effects of different doses of acute smoke exposure immediately prior to task performance were evaluated.

Smoke Exposure

The smoke exposure regimen was based on [40–42] and chosen to model acute cannabis smoking. Smoke exposure was conducted in an automated cigarette smoking machine [41,43]. During exposure sessions, rats were exposed to smoke from 0, 3, and 5 consecutively-burned cannabis cigarettes (NIDA Drug Supply Program, ~700 mg, 5.6% Δ9THC) using a randomized, within-subjects design such that each rat was tested in separate sessions under each exposure condition, with at least 48 h between exposure sessions.

Behavioral Testing

Apparatus. Testing was conducted in touchscreen operant chambers equipped with a touch-sensitive video screen and a food trough. Food pellets (45 mg) reinforced correct responses. During training, a mask divided the touchscreen into a row of 7 squares (Figures 1A, 2A). Prior to the start of testing, rats were shaped to perform the various task components (see [44] and Supplement for details).

Experiment 1a. Working memory task. The delayed response working memory task [13,37] was composed of multiple, self-paced trials, each consisting of a sample, delay, and choice phase. During the sample phase, one panel on the touchscreen (far left or far right, randomized across trials) was illuminated (Figure 1A). Touching the illuminated panel caused the screen to go blank and initiated the delay phase (0, 6, 12, or 24 s), during which the food trough was illuminated and the rat had to nosepoke into the trough. After the delay, both the sample and the panel on the opposite side of the screen were illuminated. A touch on the panel that was not illuminated during the sample phase (i.e., a “non-match”) yielded a food pellet. A touch on the “incorrect” panel caused the screen to go blank and no food was delivered.

Experiment 1b. TUNL Task. The TUNL task [38,39] was similar in structure to the working memory task, but no delays were employed. Each trial was divided into sample and choice phases. During the sample phase, one panel (randomly selected from among the 7 positions) was illuminated (Figure 2A). Touching the illuminated panel caused the screen to go blank and the light in the food trough was illuminated. A nosepoke into the food trough caused two panels on the touchscreen to be illuminated (both the original location and a second location separated by 1, 3, or 5 blank locations). A touch on the panel not illuminated during the sample phase (i.e., a “non-match”) yielded a food pellet. A touch on the “incorrect” panel caused the screen to go blank and no food was delivered.

Data Analyses

The primary performance measure in both tasks was accuracy (% correct trials at each delay or stimulus separation, averaged over the 60-min session). The number of trials completed/session was also assessed. Data were analyzed in SPSS 28.0, and p values less than .05 were considered significant.

Experiment 2: Effects of repeated oral Δ9THC on cognition in young adult and aged rats.

Oral consumption is one of the most prevalent routes of cannabis use in older adults [45]. Experiment 2 was designed to mimic this pattern of consumption to determine its effects on cognition. Rats were trained on a delayed response working memory task until stable performance emerged and then were divided into two groups matched for performance. Rats then consumed either Δ9THC or control gelatin daily, while continuing behavioral testing. After 3 weeks of testing in the working memory task, rats were assessed in the Morris water maze under the same conditions.

Drug administration

Rats were initially trained to consume plain gelatin (to overcome neophobia), followed by daily sessions in which they were given 1 h home cage access to 10.0 g of plain gelatin or gelatin containing Δ9THC (1.0 mg/kg, the highest dose that rats would reliably consume on a daily basis). Gelatin consumption took place in the afternoons and behavioral testing was conducted in the mornings, to eliminate potential acute effects of Δ9THC on performance.

Behavioral Testing

Working memory task. The delayed response task was similar to that used in Experiment 1, but was conducted in standard operant chambers equipped with two retractable levers and a food delivery trough. The task consisted of multiple trials in 40-min sessions. Each trial began with a single lever (the sample, either left or right) extended into the chamber (Figure 3A). A press initiated the delay phase (0-24 s), during which rats nosepoked into the food trough. This was followed by the choice phase, in which both levers extended. A press on the same lever extended in the sample phase ("correct") yielded a food pellet (i.e., rats had to employ a "match" rule). A press on the opposite ("incorrect") lever caused levers to retract and no food delivery. See Supplement and [37,40,46] for details.

Morris water maze task. The maze consisted of a tank filled with opaque water in which an escape platform was hidden beneath the surface in one quadrant (spatial task, Figure 3A) or protruded above the surface and moved locations on each trial (cued task) [18,47,48]. To assess spatial memory, rats received three trials/day over eight days. On each training trial, rats were started at a different location and swam until finding the hidden platform. Every sixth trial was a 30-s probe trial on which the platform was made unavailable for escape. After eight days of spatial training, rats were assessed on the cued task to evaluate visual acuity, sensorimotor abilities, and motivation to escape the water.

Data analyses

In the working memory task, the primary measure was accuracy (% correct trials at each delay, averaged over the 40-min session). In the water maze, the primary measure in the spatial task was search error, calculated either cumulatively across the trial for training trials, or as mean proximity to the platform (mean search error) for probe trials. Swim pathlength was used as the performance measure in the cued task,

Experiment 3: Pharmacokinetics of Δ9THC and its metabolites after different routes of administration

To evaluate Δ9THC pharmacokinetics, rats were exposed to smoke from 5 cannabis cigarettes or consumed gelatin containing 1.0 mg/kg Δ9THC as in Experiments 1 and 2. Blood was collected via tail nicks at different timepoints after consumption and evaluated for Δ9THC and two major metabolites (11-OH-Δ9THC and 11-COOH-Δ9THC) via ultraperformance liquid chromatography-

tandem mass spectrometry (UPLC-MS/MS) as in [43,49]. Plasma concentrations of analytes for which sufficient numbers of samples were above lower limit of quantification (LLOQ, 2.5 ng/mL) were analyzed in SPSS.

RESULTS

Experiment 1: Effects of acute cannabis smoke on cognition in young adult and aged rats.

Experiment 1a. Delayed non-match to sample working memory task. A four-factor repeated measures ANOVA (Age x Sex x Cigarettes x Delay) conducted on task accuracy (Figures 1B, D) indicated an Age x Sex x Cigarettes interaction ($F(2,100)=4.85$, $p=.01$), such that effects of cannabis smoke exposure on accuracy differed as a function of Age and Sex (see Table S1 for full results). In light of this interaction, the effects of Cigarettes were evaluated in each Age and Sex group. Two-factor ANOVAs (Cigarettes x Delay) conducted separately in aged rats of each sex revealed that in aged males, cannabis smoke *enhanced* working memory accuracy (main effect of Cigarettes, $F(2,20)=4.08$, $p=.03$), whereas in aged females, cannabis smoke *impaired* working memory accuracy (main effect of Cigarettes, $F(2,22)=8.46$, $p=.002$). In contrast, there were no main effects of interactions involving Cigarettes in young rats of either sex. To better illustrate these results, Figures 1C and E show individual rat accuracy averaged across the longest two delays, at which cannabis produced the largest group effects. The cigarette dose shown is that which produced the largest effect on accuracy in each group (or at the highest dose in young rats).

Experiment 1b. TUNL task. A four-factor repeated measures ANOVA (Age x Sex x Cigarettes x Separation) revealed an Age x Sex x Cigarettes interaction ($F(2,110)=3.77$, $p=.03$), such that effects of cannabis smoke exposure on task accuracy differed as a function of both Age and Sex (Figures 2B, D; see Table S3 for full statistical results). To better elucidate this interaction, two-factor ANOVAs (Cigarettes x Separation) were conducted separately in each Age and Sex group. Unlike in the delayed response task, however, no main effects or interactions involving Cigarettes emerged. To better illustrate these results, Figures 2C and E show individual rat accuracy at the largest separation, at which cannabis produced the greatest group effects. The cigarette dose shown is that which appeared to have the numerically largest effect on accuracy.

Experiment 2: Effects of repeated oral Δ9THC on cognition in young adult and aged rats.

Delayed match-to-sample working memory task. Prior to Δ9THC or control gelatin consumption, comparison of working memory accuracy (four-factor ANOVA, Age x Drug x Sex x Delay) revealed no main effects or interactions involving Drug ($Fs<1.65$, $ps>.13$), indicating comparable performance between drug groups prior to Δ9THC consumption (Figure S2A and Table S6). Rats regularly consumed all the gelatin during consumption sessions, with no differences across groups (Figure S2B and Table S7). To evaluate effects of chronic Δ9THC on working memory, data were averaged across the last five sessions of testing in Week 3 and assessed using a four-factor ANOVA (Age x Drug x Sex x Delay). There was a main effect of Age ($F(1,180)=10.38$, $p=.003$) and a Delay x Age interaction ($F(6,180)=2.88$, $p=.01$), such that aged rats performed worse than young, particularly at longer delays (Figure 3B). Most importantly, there was a Delay x Age x Drug interaction ($F(6,180)=2.45$, $p=.03$), indicating that, across delays, Δ9THC had distinct effects in young and aged rats.

To further explore this interaction, three-factor ANOVAs (Delay x Sex x Drug) were conducted separately in each age group. Among aged rats, there was a significant Delay x Drug interaction

(F(6,84)=2.76, p=.02), indicating enhanced accuracy in the Δ9THC compared to the control group, particularly at longer delays (Figure 3B). In contrast, the same analysis in young rats revealed no main effect or interaction involving Drug (see Table S5 for full results). To better assess Δ9THC effects in aged rats, Fig. 3C shows averaged accuracy across the longest two delays at baseline prior to drug consumption and at the 3-week timepoint. Whereas aged control rats exhibited no change in performance across weeks (t(16)=0.12, p=.90), aged rats consuming Δ9THC showed a significant improvement across this period (t(16)=3.31), p=.004), supporting the interpretation that chronic oral Δ9THC alleviates age-associated working memory impairment.

Morris water maze task. A four-factor repeated measures ANOVA (Age x Sex x Drug x Trial Block) conducted on performance across training trials (Figure 3D) revealed a main effect of Trial Block, such that rats improved their performance (reduced search error) across training (F(3,78)=18.05 p<.001), as well as a trend toward a main effect of Age (F(1,29)=4.00 p=.056) such that aged rats had less accurate searches than young, but no main effects or interactions involving Drug. A similar pattern of results was evident on probe trials (Figure 3E), as well as on pathlength data on the cued task (see Table S9 and Figure S3 for full results), indicating that chronic Δ9THC consumption did not affect either spatial or cued task performance.

Experiment 3: Pharmacokinetics of Δ9THC and its metabolites after different routes of administration

Following cannabis smoke exposure, peak plasma Δ9THC concentrations in both young adult and aged rats were achieved 10 min after smoke exposure (Figure 4A). Although aged rats tended to have higher C_{max} values than young, these differences were not reliable (two-factor ANOVA, main effect of Age, F(1,33)=3.34, p=.08; Age x Sex interaction, F(1,33)=0.120, p=.73). Comparison of plasma Δ9THC at 10-60 min (the timepoints at which there were sufficient data to analyze; Figure 4A) using a three-factor ANOVA (Age x Sex x Timepoint) also revealed no main effect of Age (F(1,33)=0.95, p=.34), or Age x Timepoint interaction (F(3,99)=2.40, p=.07; see Table S10 for full results). There were too few data points to evaluate the short-lived 11-OH-Δ9THC metabolite (Table S11); however, analysis of the secondary metabolite 11-COOH-Δ9THC revealed no group differences (Figure 4B).

Following consumption of gelatin containing 1.0 mg/kg Δ9THC, plasma Δ9THC concentrations were below the LLOQ at all timepoints in all but a few rats (Table S11). Levels of 11-OH-Δ9THC were similarly low, but 11-COOH-Δ9THC was more detectable, particularly at the 30 and 60 min timepoints (Figure 4C). A three-factor ANOVA conducted on data at these two timepoints (Age x Sex x Timepoint) showed no main effect of Age (F(1,32)= 3.19, p=.08) but there was a Timepoint x Age interaction (F(1,32)= 13.38, p<.001), such that plasma 11-COOH-Δ9THC levels were higher in young adult than aged rats at the later timepoint.

Discussion

Virtually all published research in young adult subjects shows that cannabinoids impair multiple forms of cognition [23–25,27]. Cannabinoid effects on cognition in older adults have been less well studied, despite this population representing the fastest-growing group of cannabis users. The growing prevalence of cannabis use among older adults in combination with their increased vulnerability to cognitive decline makes it critical to determine how cannabinoids affect cognition in this age group. Surprisingly, the few studies that have addressed this topic find no differences between older adults who use cannabis and age-matched controls on measures of attention, episodic memory, executive functions, and processing speed [32,33,50], suggesting distinct actions of cannabis on cognition across the lifespan. The present results in rats show that acute

exposure to cannabis smoke enhanced working memory in aged males but impaired working memory in aged females, while having minimal effects in either sex on an episodic/spatial memory task. Chronic oral consumption of Δ9THC enhanced working memory in both sexes, while also having no effect on spatial memory. Neither drug regimen affected performance in young adults of either sex. These findings show that, at least under some conditions, cannabinoids have the potential to remediate age-related cognitive impairments.

The results of the present studies are consistent with some previous findings in aged rodents. Chronic administration of the CB_1 receptor agonist WIN-55,212-2 via osmotic minipump enhanced water maze performance in aged rats [51]. More recently, both chronic Δ9THC (via osmotic minipump) and an acute Δ9THC injection remediated cognitive deficits across a range of tasks in aged mice [52,53]. The current findings advance this prior work in several ways. First, the routes of administration employed here mimic those used by the majority of cannabis users (including older adults [5]). As route of administration can dramatically alter Δ9THC pharmacokinetics [36], it is important to account for this variable in translating approaches and results across species. Second, unlike in prior work, subjects of both sexes were tested under the same conditions. The effects of aging on some aspects of cognition can differ in males and females (e.g., [54,55]) as can the effects of exogenous cannabinoids on cognition and behavior [56]. Thus, it is important to evaluate both sexes in determining how cannabinoids affect age-related cognitive impairments.

A notable difference between the current and previous work on cannabinoid effects in aged rodents is the absence of enhancing effects on hippocampus-mediated cognition. Whereas prior work found that Δ9THC (and the CB_1 agonist WIN-55,232-2) improved performance on the Morris water maze in aged rodents [51–53], no such effects were observed here with chronic oral Δ9THC, or with acute cannabis smoke in the TUNL task (despite cannabis smoke being as behaviorally active in the TUNL as in the working memory task, as indexed by the reduction in number of trials completed; Figure S1). The reason for this difference is unclear (see the next paragraph for more on this point). Another notable aspect of the current findings is the fact that acute cannabis smoke impaired working memory in females, while enhancing performance in males. Δ9THC pharmacokinetics were similar in aged males and females following smoke exposure, and although there is other evidence for sex differences in cannabinoid signaling, it is unclear how they interact with advanced age [57–61]. Another possibility, however, stems from the robust sex differences in baseline performance in aged rats in Experiment 1a, such that aged females performed comparably to young adults whereas aged males were considerably impaired. Previous work in young adult rats showed that acute cannabis smoke enhances working memory only in rats (of both sexes) that performed poorly at baseline [40], suggesting that baseline performance rather than sex accounts for the sex difference in acute cannabis smoke effects in aged rats in this experiment. This is supported by Experiment 2, in which aged control rats of both sexes were impaired relative to young, and both benefitted from chronic Δ9THC.

In considering the translational implications of these findings, it is important to consider cannabis use patterns amongst older adults. Many older adults report using cannabis regularly to treat chronic medical conditions, with edibles (49%) and smoking (29%) being the most common routes of intake [45,62]. In addition, the doses of Δ9THC ingested through both smoke and oral routes in this study were relatively low, and although behaviorally active ([41,42] and current data), they likely produced limited psychoactive effects (e.g., rats did not show a taste aversion to oral Δ9THC). These low doses could account for the absence of effects on hippocampus-dependent cognition compared to previous work [51–53,63], although the different drugs, routes of administration, and species employed render such comparisons challenging. Importantly, however, the levels of Δ9THC exposure in the present study are well below those shown to produce cognitive impairments in young rats, including in both working memory and water maze tasks [40,64–66] (see also [67] for related findings). Finally, note that exposure to smoke alone (aside from its Δ9THC content) was unlikely to have contributed to its cognitive effects, as

exposure to smoke from placebo cigarettes (from which cannabinoids are removed) does not mimic effects of cannabis smoke on cognition [40,68].

Δ9THC exposure in aged mice causes a host of changes in the hippocampus, including increases in dendritic spine density and synaptic protein expression that are linked to enhanced hippocampus-dependent cognition [35,69]. In contrast, the mechanisms by which cannabis/ Δ9THC might enhance working memory in aging are less clear. Performance on delayed response tasks is thought to depend upon sustained PFC pyramidal neuron activity, which is regulated by input from GABAergic interneurons [70]. Age-related working memory impairments are linked to alterations in PFC pyramidal neuron excitability, due in part to increased GABAergic tone [71–73]. CB_1 receptors are highly expressed on PFC GABAergic interneurons [60,74–76], and acute activation of these receptors could suppress GABA release, thus disinhibiting pyramidal neuron activity [77]. Such pyramidal neuron disinhibition could provide a mechanism by which cannabis acutely improves PFC-dependent working memory. In contrast to PFC, age-related impairments in hippocampus-mediated cognition in both rats and humans are due in part to hyperexcitability of pyramidal neurons in the CA3 subregion [78,79]. Given that CB_1Rs are also highly expressed by a subset of hippocampal GABAergic interneurons [80], Δ9THC-induced suppression of GABAergic activity might exacerbate hippocampal excitability, and provide more limited cognitive benefits in aging.

It is less likely that acute Δ9THC actions on CB_1Rs account for the enhancing effects on working memory in Experiment 2. Although circulating levels of the secondary Δ9THC metabolite 11-COOH-Δ9THC were higher in young adult than aged rats (and particularly in females, consistent with [43,81,82]), these levels would have been extremely low at the time of cognitive testing 20 h after oral administration (Figure 4 and Table S11). An alternative mechanism concerns Δ9THC actions on inflammatory signaling. Cognitive impairments accompanying aging are associated with dysregulated inflammatory signaling and increased circulating levels of pro-inflammatory cytokines [83–91]. Within the brain, pro-inflammatory factors can act on both microglia and neurons via specific receptors, and can ultimately modulate neuronal excitability [87,92–97]. Δ9THC has anti-inflammatory effects both *in vitro* and *in vivo*, including attenuation of microglial activation and suppression of pro-inflammatory cytokine expression [98–100], suggesting that chronic Δ9THC could attenuate age-related increases in inflammatory signaling and ultimately remediate cognitive deficits [51,63].

The results of this study demonstrate that cannabis does not impair, and can provide benefit to, cognition in older subjects, but that these benefits vary as a function of sex, duration of exposure, and/or the form of cognition being measured. It is also important to highlight that the route of administration between acute and chronic studies differed, and that their distinct pharmacokinetic profiles could impact the effects of cannabinoids on cognition as a function of sex or age, rendering direct comparisons between the two experiments challenging. In addition, we did not assess potential age differences in the pharmacokinetics of Δ9THC in brain. A further factor to consider is that the experiments evaluated effects of Δ9THC alone, and did not investigate contributions of other cannabis constituents such as cannabidiol, which can interact with the effects of Δ9THC on cognition and other aspects of behavior [34,101,102]. Future work will assess effects of cannabidiol on cognition in the context of aging. Moving forward, it will be important to begin identifying mechanisms driving the pro-cognitive effects of cannabis in aging.

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AUTHOR CONTRIBUTIONS

SZ, AS, CRM, BS, & JLB designed the research; SZ, EAG, AAG, ECB, & ASS conducted experiments; SZ, ECB, ASS, AS, & BS performed data analyses; MF, TH, & LRM provided critical reagents; SZ, AS, BS, & JLB wrote the initial draft of the manuscript; all authors contributed to data interpretation, manuscript editing, and final approval of the submitted manuscript.

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COMPETING INTERESTS

ECB founded Planted in Science Consulting, LLC after her work on this study. No other authors declare competing interests.

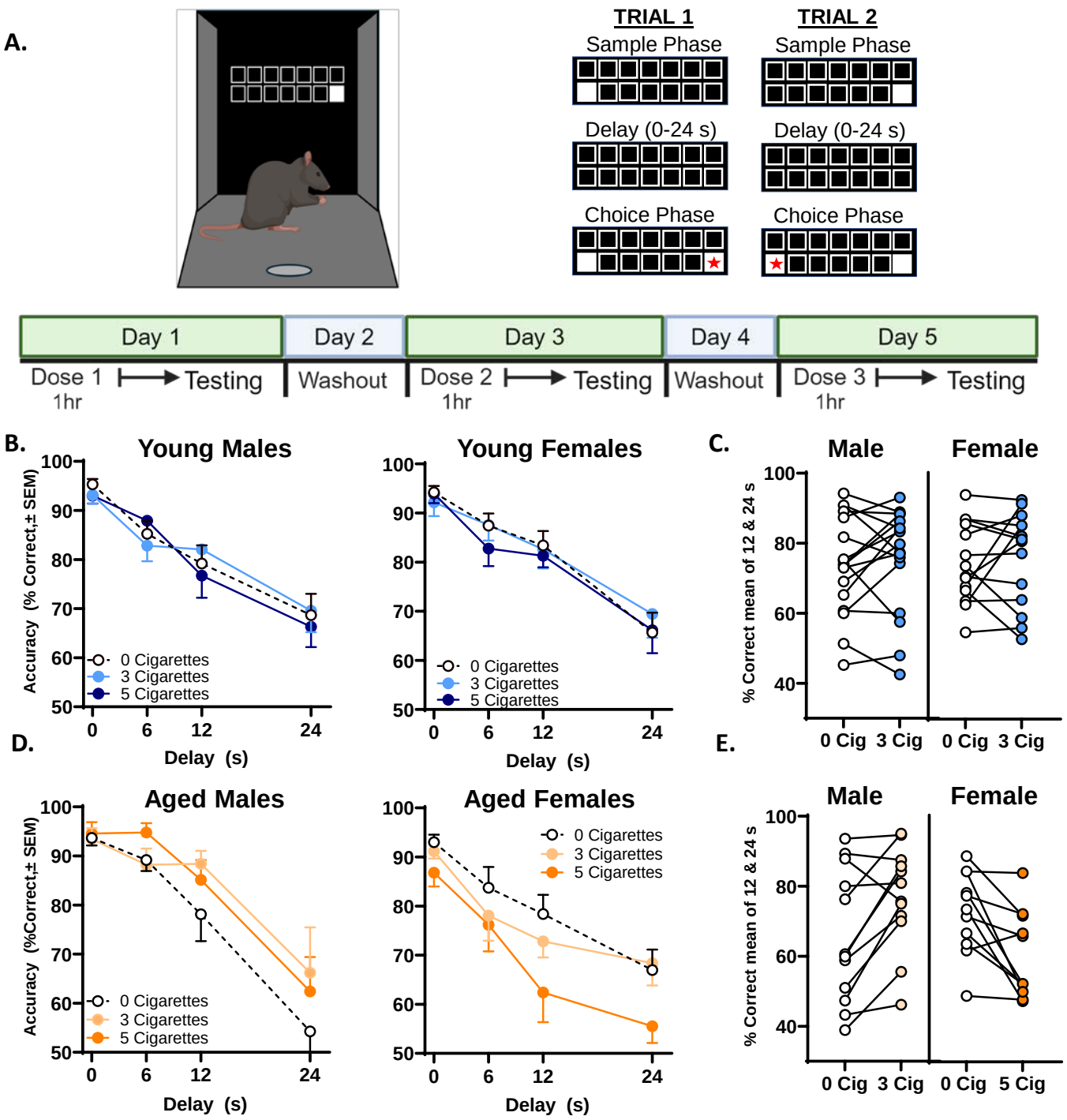
FIGURE LEGENDS

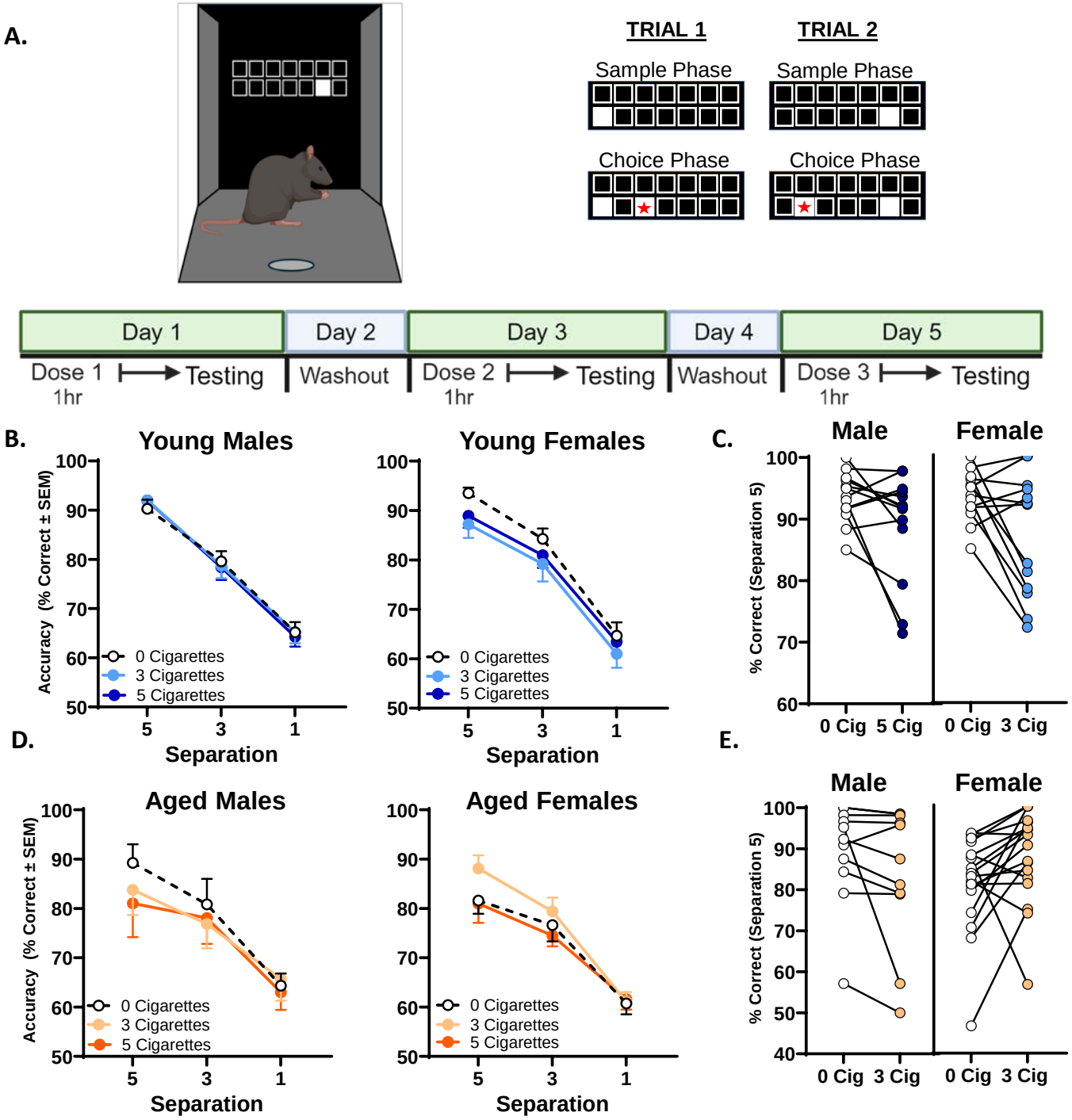
Figure 1. Effects of acute exposure to cannabis smoke on working memory task performance. Young adult (n=17 M, n=14 F) and aged (n=11 M, n=12 F) rats were tested in a non-match-to-sample delayed response task following acute exposure to cannabis smoke, using a within-subjects design. **A.** Top left illustrates the touchscreen apparatus, and top right illustrates two examples of trials in the task (red star indicates correct choice on each trial). Bottom shows timeline of testing with acute smoke exposure. **B.** There was no effect of smoke exposure on working memory accuracy in young adult male or female rats. **C.** There was no significant change in individual performance at 12-24 s delays (averaged) from the 0 to 3 cigarette condition in either young males or females. **D.** Among aged rats, acute cannabis smoke enhanced working memory accuracy in males but impaired accuracy in females. **E.** At the 12-24 s delays (averaged), aged males showed improved accuracy in the 3 cigarette condition, whereas aged females showed impaired accuracy in the 5 cigarette condition. See Table S1 for details of statistical analyses.

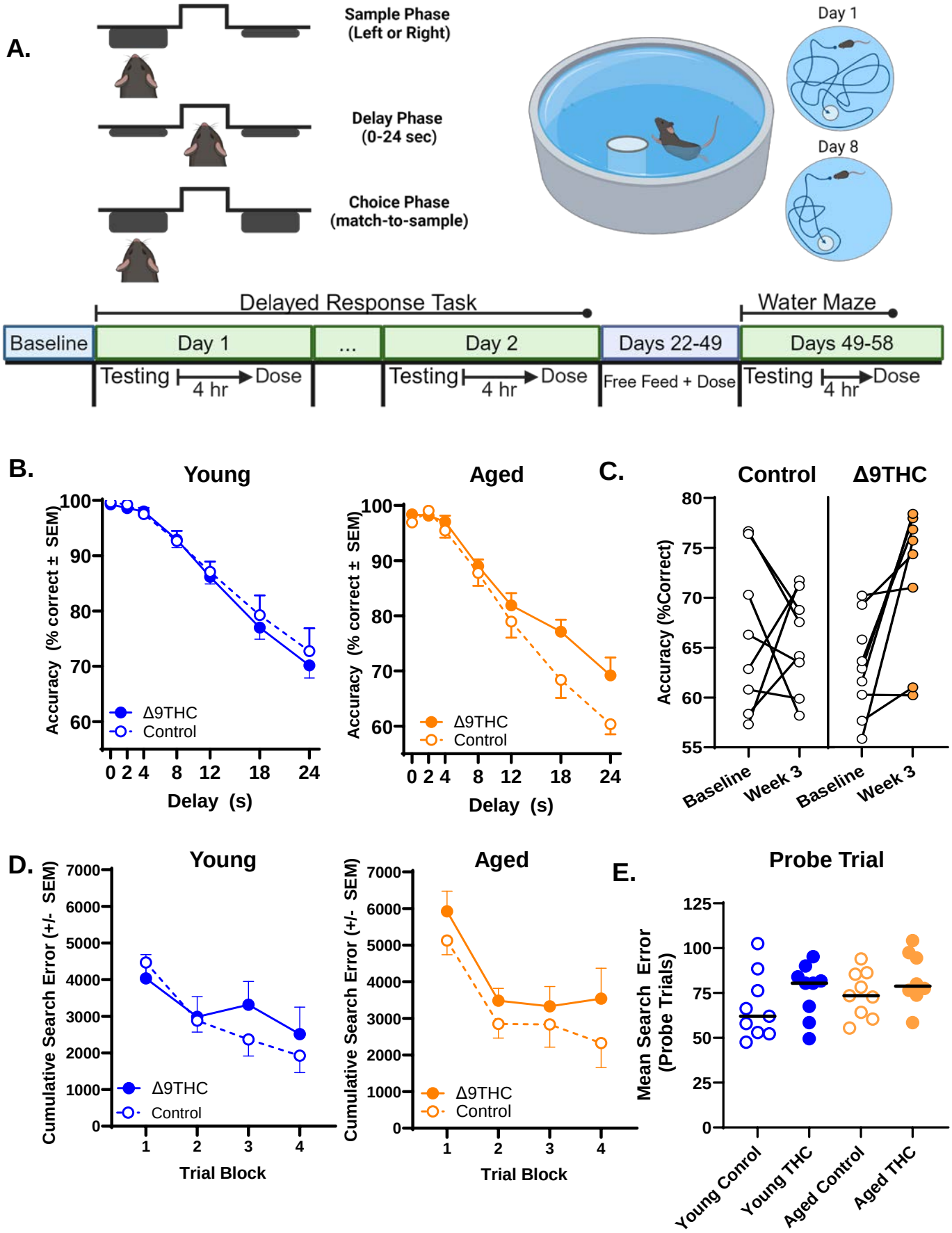
Figure 2. Effects of acute exposure to cannabis smoke on trial-unique non-match-to-location (TUNL) task performance. Young adult (n=17 M, n=13 F) and aged (n=11 M, n=18 F) rats were tested in a trial-unique non-match-to-location task following acute exposure to cannabis smoke, using a within-subjects design. **A.** Top left illustrates the touchscreen apparatus, and top right illustrates two examples of trials in the task (red star indicates correct choice on each trial). Bottom shows timeline of testing with acute smoke exposure. **B-C.** TUNL task accuracy in young adult male and female rats. **D-E.** TUNL task accuracy in aged male and female rats. Although the omnibus ANOVA indicated distinct effects of cannabis smoke in young adult and aged rats of each sex, follow-up analyses revealed no significant effects in any individual age or sex group. See Table S3 for details of statistical analyses.

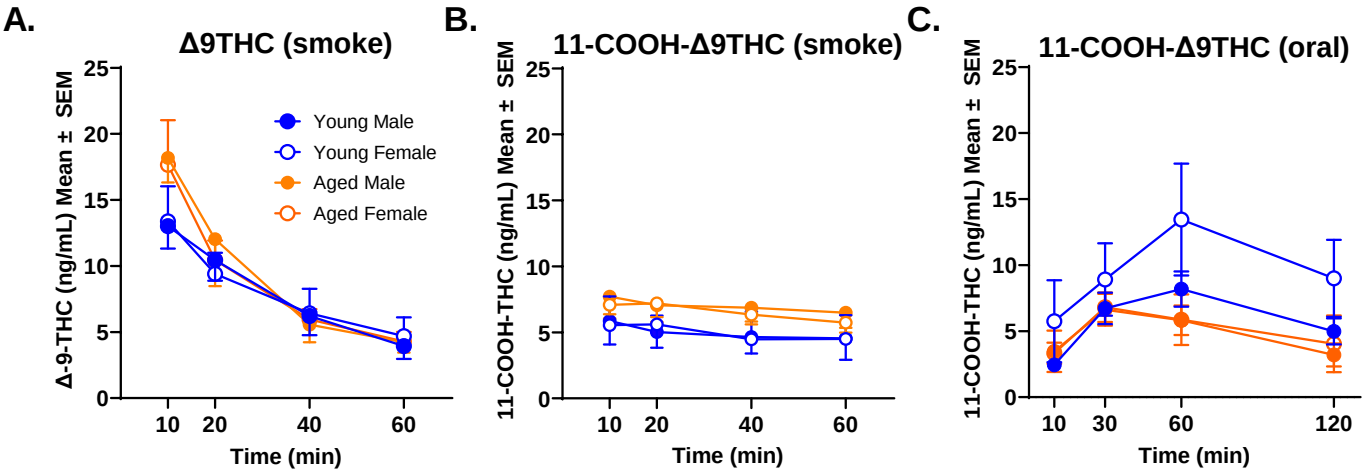
Figure 3. Effects of chronic oral consumption of Δ9THC on working and spatial memory. Young adult (n=10 control (5M, 5F), n=10 Δ9THC (5M, 5F)) and aged (n=9 control (5M, 4F), n=9 Δ9THC (5M, 4F)) rats were tested in a match-to-sample delayed response task and the Morris water maze task, while undergoing daily consumption of Δ9THC or plain (control) gelatin. **A.** Top left illustrates a single trial in the working memory task, and top right illustrates the water maze apparatus and swim paths on two example trials. Bottom shows timeline of testing with chronic Δ9THC consumption. **B.** At 3 weeks of gelatin consumption, there was no effect of Δ9THC on working memory accuracy in young adult rats. In contrast, Δ9THC enhanced working memory accuracy in aged rats, particularly at long delays. **C.** Among aged rats, those in the control group showed no change in accuracy from baseline (pre-gelatin) to Week 3, whereas those in the Δ9THC group showed a significant improvement in accuracy across the same time period. **D.** In the water maze task, there were no significant effects of Δ9THC on search accuracy (cumulative search error) in either age group during training trials. **E.** Similarly, there were no significant effects of Δ9THC on memory for the platform location during the interpolated probe trials (mean search error, averaged across all four trials). Note that as there were no main effects or interactions involving sex, data are collapsed across sex for all measures. See Tables S5, S9 for details of statistical analyses.

Figure 4. Plasma concentration-time profiles of Δ9THC and 11-COOH-Δ9THC after cannabis smoke exposure and oral administration of Δ9THC in young adult and aged rats. **A.** Peak levels of Δ9THC were evident 10 min following acute cannabis smoke exposure, but age and sex differences did not reach statistical significance (young adult: n=9 M, n=8 F; aged: n=8 M, n=12 F). **B.** Following acute cannabis smoke exposure, there were no age or sex differences in 11-COOH-Δ9THC levels. **C.** Levels of 11-COOH-Δ9THC peaked at 30-60 min following acute oral Δ9THC consumption, with levels being higher in young than aged rats, particularly at the 60-min timepoint (young adult: n=12 M, n=6 F; aged: n=10 M, n=8 F). See Table S10 for details of statistical analyses.


A.
TRIAL 1
Sample Phase
Delay (0-24 s)
Choice Phase
TRIAL 2
Sample Phase
Delay (0-24 s)
Choice Phase
Day 1
Day 2
Day 3
Day 4
Day 5
Dose 1
1hr
Testing
Washout
Dose 2
1hr
Testing
Washout
Dose 3
1hr
Testing
B.
Young Males
Young Females
Accuracy (% Correct,± SEM)
0 Cigarettes
3 Cigarettes
5 Cigarettes
Delay (s)
C.
Male
Female
% Correct mean of 12 & 24 s
0 Cig
3 Cig
D.
Aged Males
Aged Females
Accuracy (%Correct,± SEM)
E.
5 Cig


A.
TRIAL 1
TRIAL 2
Sample Phase
Sample Phase
Choice Phase
Choice Phase
Day 1
Day 2
Day 3
Day 4
Day 5
Dose 1
1hr
Testing
Washout
Dose 2
1hr
Testing
Washout
Dose 3
1hr
Testing
B.
Young Males
Young Females
Accuracy (% Correct ± SEM)
0 Cigarettes
3 Cigarettes
5 Cigarettes
Separation
C.
Male
Female
% Correct (Separation 5)
0 Cig
5 Cig
0 Cig
3 Cig
D.
Aged Males
Aged Females
Accuracy (% Correct ± SEM)
0 Cigarettes
3 Cigarettes
5 Cigarettes
Separation
E.
Male
Female
% Correct (Separation 5)
0 Cig
3 Cig
0 Cig
3 Cig


A.
Sample Phase
(Left or Right)
Delay Phase
(0-24 sec)
Choice Phase
(match-to-sample)
Day 1
Day 8
Delayed Response Task
Water Maze
Baseline
Day 1
...
Day 2
Days 22-49
Days 49-58
Testing
4 hr
Dose
Free Feed + Dose
B.
Young
Aged
Accuracy (% correct ± SEM)
Δ9THC
Control
Delay (s)
C.
Control
Δ9THC
Accuracy (%Correct)
Baseline
Week 3
D.
Cumulative Search Error (+/- SEM)
Trial Block
E.
Probe Trial
Mean Search Error
(Probe Trials)
Young Conrol
Young THC
Aged Control
Aged THC


A.
Δ9THC (smoke)
Δ-9-THC (ng/mL) Mean ± SEM
Time (min)
Young Male
Young Female
Aged Male
Aged Female
B.
11-COOH-Δ9THC (smoke)
11-COOH-THC (ng/mL) Mean ± SEM
Time (min)
C.
11-COOH-Δ9THC (oral)
11-COOH-THC (ng/mL) Mean ± SEM
Time (min)