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Effects of Ethanol, Δ^9 -Tetrahydrocannabinol, or their Combination on Object Recognition Memory and Object Preference in Adolescent and Adult Male Rats

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Abstract

Recent advances have been made in our understanding of the deleterious effects of both ethanol and THC on adolescent behavior and brain development. However, very little is known about the combined effects of EtOH+THC during adolescence, a time in which these drugs are often used together. The purpose of this experiment was to: 1) determine whether EtOH and/or THC induced greater working memory impairment in adolescent than adult male rats using the novel object recognition (NOR) task; and, 2) determine whether the EtOH+THC combination would produce a more potent additive effect in adolescents than adults when compared to these drugs alone. NOR was performed with a 24 hour delay under each of the four drug conditions: vehicle; 1.5g/kg ethanol; 1.0mg/kg THC; and 1.5g/kg EtOH+1.0mg/kg THC, at 72 hour intervals. The results show that there was an age effect on working memory in NOR after the EtOH+THC challenge. Specifically, adolescent animals showed a preference for the familiar object whereas adults showed no preference for the novel or familiar object, the latter being characteristic of a classic working memory deficit. These effects were not dependent on changes in exploration across session, global activity across drug condition, or total object exploration. These novel findings clearly indicate that further understanding of this age-drug interaction is crucial to elucidating the influence that adolescent EtOH+THC use may have on repeated drug use and abuse later in life.

Keywords

Adolescence; Δ^9 -Tetrahydrocannabinol; Ethanol

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1. Introduction

During the past 10 years it has become clear that both ethanol (see [1, 2] for review) and cannabinoids (see [3] for review) affect adolescents and adults differently. For example, both Δ^9 -Tetrahydrocannabinol (THC) and ethanol (EtOH) disrupt spatial learning more potently in adolescent rats, compared to adults [4–6] and ethanol has been shown to disrupt learning more efficaciously in post-adolescent humans than in adults [7]. Conversely, ethanol produces less sedation [8] and motor incoordination [9] in adolescent than adult rats. Thus, the differences in developmental sensitivity to cannabinoids and ethanol between adolescents and adults, varies depending upon the specific behavioral measure assessed. In addition to developmental differences in the behavioral sensitivity of adolescents and adults to ethanol and cannabinoids, differences in the sensitivity of synaptic and neurophysiological functions have also been described [10, 11].

As noted above, cannabinoids also affect learning differently in adolescents, compared to adults. For example, dose-response studies have shown that THC impairs learning in the Morris water maze more potently in adolescent rats than it does in adults [3–5]. These effects suggest that THC may promote inhibition or inhibit excitation more powerfully in the hippocampus of adolescent animals than in adults. It is noteworthy that Moore et al. [3] showed that some of the developmental differences in THC potency may be related to a differential development of tolerance between adolescents and adults.

The fact that both ethanol and THC impair learning and learning-related hippocampal function more potently in adolescents than in adults is obviously of great importance. But, particularly among teens, ethanol and marijuana are often used in combination [12]. Although there have been a number of studies of the combined effects of ethanol and THC, developmental comparisons are conspicuously absent. This is of particular concern given that early misuse of these substances has been linked to an increased likelihood of later substance use and related behavioral problems [13, 14].

Early studies of the combined effects of THC and EtOH in marijuana users and non-users [15] revealed an additive effect of the THC-EtOH combination. The combination produced a larger reduction in divided attention performance in THC non-users than did THC alone or EtOH alone, the latter having no effect by itself. Among THC users, the drug combination was reported to have an ‘antagonistic’ effect, with the combination ameliorating the effect of THC alone at certain dose combinations. The results of subsequent studies have been equivocal with evidence of additive effects when EtOH is combined with low doses of THC [16] but no additive effect when EtOH was combined with higher doses of THC [17, 18]. Inconsistencies also characterize more recent studies of driving performance where the combination of THC and EtOH produced greater impairment than either drug alone [19, 20] on measures of psychomotor performance and simulated driving performance (e.g., speed, lane position, steering deviation) but not on measures of body sway, brake latency, and other driving related performance measures (e.g., speed, steering and headway maintenance [21, 22]). These studies suggest that the interaction between THC and ethanol is complex and depends upon dose, previous drug experience, and the specific performance measure assessed.

Though there are few detailed studies, the animal literature is more consistent. For example, additive effects were observed in a pharmacological study of operant learning [23] and a study of working memory using the object recognition task [24]. Although the literature on the combined effects of ethanol and THC indicates that there are some significant interactions, there are also studies indicating no additive or synergistic effects. Moreover, there have been very few animal behavioral studies that address underlying mechanisms

related to these interactions, and there have been no developmental comparisons of the efficacy or potency of the ethanol-THC combination. Since memory is impaired more potently in adolescents by each of these drugs, we hypothesized that ethanol, THC, and their combination would more potently impair novel object recognition memory in adolescents than in adults and that this relative impairment would be greatest in the combined drug condition.

2. Methods

2.1 Animals

All procedures were reviewed and approved by Duke University's Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River, Raleigh, NC) were housed on a reverse 12:12-hour light-dark cycle and were provided ad libitum access to food and water. Adolescent animals (n=7) were postnatal day 30 and adult animals (n=6) were postnatal day 70 at the beginning of the experiment. In order to habituate the animals to the testing chambers, each animal was placed into the chamber for 15 minutes on each of two successive days and then completed a full training procedure including the 24-hour delay prior to the initiation of recorded testing.

2.2 Drug Preparation and Dosing

THC was obtained from RTI (Durham, NC). It was prepared as a 0.105mg/ml solution in vehicle (1:1:18, 95% EtOH, normal saline, Tween80). Ethanol (95%) was prepared as a 0.1578g/ml solution in an identical vehicle. The combination solution was prepared similarly. Drug solutions were administered i.p. at 9.5ml/kg. Due to the number of drug by age treatment conditions, a within-subjects (repeated measures) design was used in which each animal received novel object recognition testing under each of the four drug conditions (vehicle, 1.5g/kg ethanol, 1.0mg/kg THC, and 1.5g/kg EtOH+1.0g/kg THC) at 72 hour intervals. Doses were selected based on prior work from our lab as well as the extant literature in an effort to avoid floor or ceiling effects. Prior work from our group has demonstrated that EtOH doses of 1g/kg and 2g/kg impairs spatial learning in adolescents but not adults [6]. The 1.0mg/kg was chosen based on our previous work [4, 5, 25] as well as the recognition of reports from the human literature suggesting that co-administration of EtOH and THC may result in increased plasma THC levels thereby increasing the effective dose of THC [26]. The order of drug treatment conditions was counterbalanced across test sessions.

2.3 Novel Object Recognition

Testing within each treatment condition occurred across two trials on successive days. During the first trial (AA Trial) each animal was placed into the testing chamber for five minutes with two identical objects placed in the far corners of the chamber. This task was specifically chosen as a one learning trial, one test trial procedure because it allows for the assessment of a pharmacological treatment on a single learning experience. Most other learning and memory tasks require repeated training trials across days, making the study of pharmacological effects more akin to sub-chronic dosing rather than true acute dosing.

The animal's behavior was recorded using digital video software and subsequently analyzed by trained observers (AnyMaze, Stoelting, Chicago, IL). Two independent raters reviewed and scored each video to calculate the object preference score. Individuals collecting the data and each rater were blind to the animal's age and dose condition. Both raters were trained to criterion by the study author (MLR). Subsequent analysis of inter-rater agreement on the data reported below was excellent ($r=0.96$). The amount of time spent exploring each object was recorded (in seconds). Exploration was operationally defined as the time during which the animal's head was oriented toward the object, and was within two centimeters of

the object. Immediately upon completion of the AA Trial, each animal was removed from the chamber and received an i.p. injection of the designated vehicle/drug solution. Twenty-four hours later the second trial (AB Trial) was conducted to assess memory for the objects. During the AB Trial each animal was placed into the chamber for five minutes in the presence of one of the objects from the AA Trial and a novel object, which the animal had never before encountered. Behavior was recorded and quantified as described above. Novel object preference was quantified using inspection time as follows: $(\text{novel} - \text{familiar})/(\text{novel} + \text{familiar})$. Using this metric, scores approaching zero reflects no preference while positive values reflect preference for the novel object and negative numbers reflect preference for the familiar. This metric was chosen because it controls for individual differences in the total object exploration time, not compensating for this can increase variability and distort the results [27, 28].

2.4 Apparatus

Behavioral testing was performed in two identical enclosures constructed of gray PVC plastic (9.5mm) measuring 70cm (L) $\times$ 41cm (W) $\times$ 33cm (H). Objects were made of plastic, glass or ceramic and varied in height (6cm – 15cm) and width (6cm – 8cm). Objects were weighted down (when necessary) to reduce the likelihood that the animals could maneuver the objects around the box. There were 5 sets of novel and familiar objects used and consisted of porcelain figurines or household objects (e.g., pepper grinder, hand soap dispenser). Objects were paired based on similarity of interest as established in a previous pilot study. One set was used during the habituation phase and the remaining sets were used in the subsequent vehicle/drug conditions. The order in which object sets were used was randomized across animals and no animal was exposed to the same set in more than one drug condition. The position of the novel object for each trial was counterbalanced. Objects and apparatus were wiped down with a dilute (10%) vinegar solution and then water between animals.

2.5 Statistical Analyses

Repeated measures analysis of variance was used to assess differences between drug conditions. Age was used as an independent variable to assess the presence of neurodevelopmental differences in the effects of these drugs/combinations. The primary dependent measure was the preference score described above. Additional control measures included total activity in the AA and AB trials, as well as total object inspection time in the AB trial. One sample t-tests were used to determine if preference scores were reliably different from zero and planned paired t-tests were used to assess differences between vehicle and drug/composition conditions. Alpha was established at $p = 0.05$.

3. Results

The purpose of this experiment was to assess the neurodevelopmental effect of ethanol + THC relative to each drug alone using the novel object recognition task as an index of learning and memory..

3.1 Novel object preference

Results reveal a significant main effect of age, $F(1, 11) = 11.59$, $p = 0.006$, and a main effect of drug treatment, $F(3, 33) = 13.27$, $p < 0.001$. These results indicate that adolescent and adult animals differed significantly in object recognition learning, and that the drug treatments significantly altered object recognition learning. However, the age by treatment interaction was not significant, $F(3, 33) = 0.07$, $p = 0.976$, (Figure 1a). The absence of a statistically significant interaction is consistent with traditional ANOVA procedures in the presence of an ordinal interaction [29]. Visual inspection of Figure 1 reveals a quantitatively

different effect in the EtOH+THC condition and a qualitatively different response pattern in adolescents and adults in this treatment condition.

As the figure indicates, among adolescent animals, the EtOH+THC combination appeared to result in a preference for the familiar object during the AB Trial as indicated by the negative object preference score. Among adults, the drug combination merely drove the score to near zero, indicating a lack of memory for the familiar object but not a preference for it. To test these hypotheses, we used one-sample t-tests to assess whether the object preference scores differed significantly from zero in the EtOH+THC treatment condition. We found that the negative object preference score for adolescent animals in the EtOH-THC treatment condition differed significantly from 0, $t(6) = -3.22$, $p = 0.018$, while scores for adult animals did not, $t(5) = 0.71$, $p = 0.518$. This indicates that the drug combination resulted in a preference for the familiar object in adolescents, whereas in the adults it impaired novel object recognition performance but did not produce a preference for the familiar object. These findings were further confirmed by a sequence of planned post-hoc tests revealing that EtOH+THC treatment altered object preference scores in both adolescent, $t(6) = 4.23$, $p = 0.006$, and adult, $t(5) = 4.28$, $p = 0.008$, animals relative to vehicle treatment. These findings confirm that the EtOH+THC combination significantly altered object recognition in adolescents and adults relative to vehicle treatment. In contrast, neither drug alone altered object recognition in adolescents (ethanol: $t(6) = 1.11$, $p = 0.31$; THC: $t(6) = 0.23$, $p = 0.83$) or adults (ethanol: $t(5) = 1.25$, $p = 0.27$; THC: $t(5) = 0.18$, $p = 0.87$). It is also important to note that these effects are not explained by changes in object exploration across sessions (AA trials), changes across drug condition (AB trials), or by changes in global activity across drug condition (AB trials) as demonstrated by the following analyses.

3.2 Total object exploration (AA trial)

Due to the repeated measures design, we sought to rule out any decline in exploratory behavior across sessions (independent of drug condition). Using a statistical strategy similar to that described above, total object exploration time changed as a function of age, $F(1, 11) = 16.26$, $p = 0.002$, but not as a function of session number, $F(3,33)=0.87$, $p=0.47$, or of the interaction between age and session number, $F(3,33)=0.64$, $p=0.98$. This indicates that adolescent animals engaged in more object exploration during the AA Trials than adult animals, but that exploration did not differ across testing sessions, nor was the age difference influenced by session number. Total object exploration was not analyzed as a function of age and drug solution because animals did not receive the designated vehicle/drug solution until after the AA trial.

3.3 Total object exploration (AB trial)

This analysis was performed to determine if total object inspection time in the AB trials changed as a function of age or drug condition. As before, inspection time was higher among adolescents than adults in the AB trial, $F(1,11)=13.38$, $p=0.004$, with no differences among the drug conditions, $F(3,33)=1.67$, $p=0.19$. Unlike the analysis of exploration during the AA trial, there was a significant interaction between age and drug, $F(3,33)=3.7$, $p=0.02$ (figure 1). In addition, there were significant differences between drug conditions among the adolescents, $F(3,18)=4.3$, $p=0.019$, but no differences between drug conditions among the adults, $F(3,15)=0.35$, $p=0.79$. Although these results mimic the overall effect observed on the object preference variable (an inherent interaction between age and drug condition), the actual pattern of effects are not parallel to those reported above. Among adolescents, AB trial object exploration declines from a maximum in the vehicle condition in a relatively linear trend across the EtOH and THC conditions to a low in the EtOH+THC condition (left side, figure 1b). Among adults, there were no differences between drug conditions (right side, figure 1b).

3.4 Global activity (distance traveled in the AB trial)

Global activity during the AB trial was higher among adolescent animals than adults, $F(1,11)=14.94$, $p=0.003$. The effect of drug treatment on global activity approached significance, $F(3,33)=2.73$, $p=0.06$, and the interaction between age and drug treatment was not significant, $F(3,33)=1.41$, $p=0.26$. Similarly, differences in global activity provide no explanation for the preference for the familiar object among adolescent animals after treatment with EtOH+THC.

4. Discussion

The principal findings in this experiment are that 1) adolescent and adult rats perform differently on the object recognition memory task; 2) there was a main effect of drug treatment that was driven by the effect of the combined EtOH-THC treatment condition; and 3) the EtOH-THC combination produced qualitatively different effects in adolescent and adult animals. In adolescents, the EtOH+THC combination induced a preference for familiar objects as demonstrated by a statistically significant negative preference score. In adults, the drug combination appears to have induced a memory deficit demonstrated by a lack of preference for the novel object. These findings were not due to differences in object inspection time or global activity during the AB trials, and there was no evidence that animals spent progressively less time exploring the objects across sessions. It bears repeating that this age-dependent effect represents an important qualitative difference in how the EtOH-THC combination affects behavior in adolescent and adult animals. These findings are of particular interest because the induction of a preference for the familiar object in a novel object recognition task runs counter to the animals' natural behavioral tendency, which is a preference for novelty (see [28] for review). This suggests that the combination of ethanol and THC may be sufficiently reinforcing in adolescent animals to induce a preference for recently encountered stimuli, but not sufficiently reinforcing to do so in adults. It is important to note that these data were collected using male rats only. There is literature documenting important differences between male and female adolescent animals for both EtOH and THC (e.g., [4, 31]). A thorough understanding of the neurodevelopmental effects of the EtOH+THC combination will require studies that employ both males and females at different stages of development.

Still, these findings may shed light on why the combined use of EtOH and THC is more prevalent in adolescents than in adults. If the drug combination is more reinforcing in younger individuals, they would be more motivated to use it on a regular basis. The present data also suggest that combined use of THC and EtOH by adolescents could elevate their risk for the development of abuse of either drug alone. We know that the liability for EtOH addiction increases with early onset adolescent drinking [30], and the present results indicate that it will be important to assess the extent to which that finding may be influenced by the combined use of THC. It is also notable that EtOH-induced dopamine (DA) release in the nucleus accumbens (NAc) is mediated, at least in part, by CB1 receptors [31]. Since adolescent rats have been shown to be more responsive to some of the neurobehavioral effects of THC [4, 5], it may be that they are also more responsive to the potentiating effects of THC on EtOH-induced DA release. Moreover, CB1 receptors in adolescent rats desensitize to repeated THC administration less rapidly than do those in adults [3]. This suggests that they may remain more capable of promoting EtOH-induced enhancement of DA release for a longer period of time, thus enhancing the reinforcing effects of co-administered EtOH. Taken together, these implications of the present data may lead to both new clinical and mechanistic conceptualizations regarding the combined use of EtOH and THC by adolescents. Although the developmental focus of the present data is new, this is not the first instance in which THC or an EtOH-THC combination has been shown to evoke a preference for the familiar. Ciccocioppo et al. [24] showed that high dose THC (10mg/kg)

or high dose THC + EtOH produced a preference for the familiar object in the NOR task following a 1-minute delay in alcohol preferring rats. Moreover, high dose THC or moderate dose THC (5mg/kg) + EtOH produced a preference for the familiar object in the NOR task following a longer, 15-minute delay. Interestingly, those findings were interpreted as impairments in working memory although the animals in those dose conditions had no difficulty distinguishing the novel from the familiar object. They simply showed a preference for the familiar rather than the novel. The present data add the developmental comparison, and indicate a distinct preference for the familiar in adolescent animals treated with THC + EtOH.

Additional possible explanations for our observed preference development include EtOH withdrawal effects and THC induced preference or aversion. With respect to withdrawal, there is evidence that THC may decrease maximum EtOH bioavailability in humans by 30% while doubling the latency to peak BAC [32]. Thus, our adolescent animals exposed to the EtOH-THC combination may have experienced a withdrawal-induced anxiogenesis 24 hours after dosing, at the time of the AB trial. This anxiogenesis could explain their preference for the familiar. Though plausible, this mechanism seems unlikely since it would not explain previously observed preferences for the familiar with shorter, 1 and 15 minute delays or the preferences observed after THC alone [24]. Finally, although the latency to peak BAC may have been delayed by the co-administration of THC, it would also presumably have lowered EtOH bioavailability thus making EtOH withdrawal less likely.

Another possibility is that EtOH increased the behavioral potency or plasma levels of the co-administered THC. Co-administration of EtOH and THC in humans increases euphoria, and THC plasma levels [26]. If EtOH potentiates the reinforcing effects of THC that could explain the development of preferences for recently familiarized stimuli. This would be consistent with the observation that that EtOH + THC produced preference for familiar objects in adults [24], similar to what we have observed in adolescent animals. Although this interpretation should be made cautiously because studies on the reinforcing effects of THC are equivocal (see [33, 34]), the present data do suggest that the reinforcing properties of THC may be more readily potentiated by ethanol during adolescence.

Regardless of the precise underlying mechanism, the present findings represent yet another distinctive difference in drug sensitivity between adolescent and adult animals. Given the frequency with which EtOH and THC are used together by adolescents, it is imperative that we gain a better understanding of the underlying pharmacology of concomitant use of these substances. Furthermore, we believe such an understanding may have direct translational relevance to the impact of early drug exposure on later dependence and abuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Adolescent animals show lower object recognition than adults.
- EtOH+THC lowers object recognition more than EtOH or THC, relative to vehicle.
- EtOH+THC produces a clear preference for familiar objects in adolescent animals.
- EtOH+THC produces a clear working memory deficit in adult animals.

Figure 1.

Effect of age (adolescent, $n=7$; adult, $n=6$) and drug condition ($\square$ vehicle; $\blacksquare$ Ethanol; $\blacksquare$ THC; $\square$ Ethanol + THC) on novel object recognition performance were significant, $p = 0.006$, and $p = 0.001$, respectively. (Panel a) Recognition index: $(\text{Novel} - \text{Familiar})/(\text{Novel} + \text{Familiar}) \pm \text{SEM}$. (Panel b) Total object inspection time: $(\text{Novel} + \text{Familiar}) \pm \text{SEM}$. ¹ $p < 0.05$ compared to zero; ² $p < 0.05$ compared to vehicle.