Acute Co-Administration of the Cannabinoid Receptor Agonist WIN 55-212,2 does not Influence 3,4-Methylenedioxymethamphetamine (MDMA)-Induced Effects on Effort-Based Decision Making, Locomotion, Food Intake and Body Temperature

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Abstract

Acute behavioural effects of co-administration of the illegal drug 3,4-methylenedioxymethamphetamine (MDMA, 7.5 mg/kg, s.c.) and the synthetic cannabinoid receptor agonist WIN55212-2 (WIN, 1.2 mg/kg, i.p.) in rats were investigated. MDMA impaired performance on a T-maze effort-based decision making task. MDMA and MDMA+WIN treatment increased body temperature to a similar extent, whereas WIN increased temperature at an earlier time point. Locomotion, exploratory behaviour and food intake was comparable in MDMA and MDMA+WIN-treated animals. MDMA-induced decreased exploration anxiety in the open field was diminished by WIN. In summary, acute co-administration of the cannabinoid receptor agonist did not substantially modulate the MDMA-induced behavioural effects.

Keywords: MDMA; WIN 55212-2; Co-administration; Acute effects; Effort-based decision making; Body temperature; Locomotion; Food intake.

Introduction

Polydrug use has become a challenging issue in research involving human drug users [1]. For example, most 3,4-methylenedioxymethamphetamine (MDMA) users are polydrug users [2]. Cannabis is the most frequently taken illegal co-drug: 98% of a representative sample of MDMA users concomitantly use cannabis [3,4]. Combined effects on physiological, behavioural and cognitive measures are difficult to disentangle and may depend on frequency, duration, and amount of co-consumption. Furthermore, many human studies involve chronic and/or heavy polydrug users, thus causal relationships between single drugs and their effects are hard to discern. Many MDMA users report smoking cannabis concomitantly to enhance positive sensations (for example, euphoria, empathy, prosocial behaviour, energy) or sometime after MDMA consumption to alleviate adverse effects (anxiety, depression, anhedonia and agitation) of the “come-down” [5-7].

Acute MDMA administration leads to a transient, dose-dependent release and reuptake inhibition of the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) and to a lesser extent noradrenaline (NA) and acetylcholine (ACh) [8], particularly in the medial prefrontal cortex (mPFC), striatum and hippocampus [7,9]. Increased 5-HT transmission contributes to the behavioural effects of MDMA in a complex way which additionally seems to depend on the interaction with DA transmission [10]. In accordance with reported effects in human users, acute effects of MDMA-administration in animals include hyperthermia [8,11], hyper-locomotion [12], anxiety (Cote and Sumnall), and hypophagia [13,14]. Few studies have tested the acute effects on more complex behaviour. A decrease in impulsivity in humans as well as a dose-dependent increase of lever pressings for reinforcement in animals by Vollenweider et al [15] was shown after MDMA administration.

In contrast, the main psychoactive compound of cannabis, delta9-tetrahydrocannabinol (delta9-THC) acts as an inhibitory transmitter on presynaptic cannabinoid (CB) type 1 (CB1) and type 2 (CB2) receptors within the endo cannabinoid system. Activation of CB1 seems to modulate neurotransmission [16]. In the CNS, a high abundance of CB1 receptors has been found in basal ganglia, cerebellum, olfactory bulb and hippocampus, moderate density appears in cortical areas [17]. Results from acute studies in animals employing delta9-THC, or one of the more potent synthetic CB1 receptor agonists (e.g., WIN 55-212,2, CP 55,940) or antagonists (e.g., SR 171416) support reports obtained from human studies in that cannabinoids are implicated in the acute regulation of several physiological processes. For example, acute consumption of delta9-THC increases heart rate and postural instability, impulsive responding on certain tasks, decreases alertness and influences time- estimations in humans [18,19]. In animals, activation of CB receptors increases food intake and heart rate, dose-dependently affects locomotor behaviour and decreases body temperature [20-22].

Behavioural and neurobiological effects of sole administration of MDMA or cannabis are well documented (for review see [7,23,24]) and some studies examine the long-term cognitive consequences of co-consumption of these drugs [25-27]. Literature on the consequences of acute co-consumption is less thorough. One human study demonstrated mixed effects: co-administration of cannabis prolonged the onset and duration of MDMA-induced increases in temperature, but had no additive influence on deficits in memory. Delta9-THC-induced psychomotor impairments independent of MDMA [28].

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Positive subjective ratings were increased for the combination of the drugs compared to each drug effects alone [29]. Some evidence from rodent studies shows regulatory effects of co-administration on locomotion, body temperature, and reinforcing effects. For example, prevention of MDMA-induced hyperthermia, anxiety, and a decrease of MDMA-induced hyperactivity due to delta9-THC was observed in rats [12]. Impairment of working memory upon co-administration of low and medium doses of MDMA plus delta9-THC was shown (Young et al., 2005). In terms of drug reinforcing properties, intra-cerebroventricular (i.c.v.) self-administration studies demonstrate a modulation of MDMA- induced reinforcing effects (self-administration or conditioned place preference (CPP)) by cannabinoid agents [30,31]. In mice, exposure to WIN in adolescence later facilitated MDMA-induced CPP [32] or potentiated the rewarding effects of MDMA [33]. In the latter study, the CB1 antagonist SR 171416 did not block the effects of WIN. Furthermore, in CB1-knock-out mice, pre-treatment with delta9-THC still prevented MDMA-induced acute responses [34] pointing towards a CB1-independent mechanism of interaction. The effects of acute co-administration on cognitive tasks, for example choice behaviour, have not been investigated.

This study aimed to further elucidate the interactive effects seen after acute co-administration of MDMA and Cannabis. The synthetic specific CB receptor agonist WIN 55212-2-2 (WIN) was used to investigate interactions of MDMA and CB receptor stimulation, aiming to narrow the range of effects of delta9-THC in the central nervous system. For the first time, the effect of acute MDMA administration as well as the combined administration of MDMA and a cannabinoid agonist on effort-based choice behaviour is investigated, in addition to locomotion, food intake and body temperature measures.

Material and Methods

Animals

In total, 39 adult naive male Wistar rats (Harlan, Borchen, Germany) weighing 230-300 grams were used in these experiments. Upon arrival, animals were allowed to habituate for 4-5 days in a vivarium under standardized conditions (4-6 animals per Makrolon type IV cage; tap water ad libitum; 12 hour light/dark cycle, lights on at 7am; temperature 22+/− 2°C) and were handled regularly. During the habituation and handling period, standard lab chow was available ad libitum. Controlled feeding (12g/animal/day) started two days before the first training session. All animal experiments were conducted in accordance with the principles of animal care and the international laws on animal experiments (Directive 2010/63/EU) and were approved by the local authorities.

Drugs

All drugs were prepared freshly before administration and were injected in a volume of 1ml/kg. WIN 55212-2-2 (SIGMA-Aldrich, Steinheim, Germany) was dissolved in 2% Tween®80 (Serva, Heidelberg, Germany) and 98% NaCl solution (0.9% NaCl, Fresenius Kabi GmbH, Bad Homburg, Germany) and injected at a dose of 1.2mg/kg, intraperitoneally (i.p.). MDMA hydrochloride (synthesized in the Institute of Inorganic Chemistry, Prof. Nagel, University of Tübingen. Identity and chemical purity was verified) was dissolved in phosphate buffered saline (PBS), stabilizing a well-tolerated pH-value of the solution, and injected at a dose of 7.5mg/kg, subcutaneously (s.c.). PBS injection served as vehicle control. Single doses which have been shown to be behaviourally relevant (Drews et al., 2005; Young et al., 2005) were chosen in order to minimize the number of animals used in this study.

Behavioural tests

Behavioural testing was conducted in a between subjects design. Each treatment group consisted of n=10 rats, except the combination (MDMA+WIN) group (n=9). Each animal underwent the same training and testing procedures. However, the sequence of tests was altered in order to minimize the influence of repeated substance administration and previous behavioural tasks. All animals started with training and subsequent testing in the effort-based decision making task. Half of each treatment group were tested in an open field one week later, followed by a food preference test another week later. The second half of the group underwent the food preference task first, followed by the open field tests. After each tests, a wash-out period of seven days was allowed for all animals. Contrary to other reports [35], there were no observable adverse effects upon first, or subsequent, exposure to the cannabinoid agonist. MDMA was administered 30mins and WIN 10mins prior to testing to allow examination of behaviour during the peak time of effect. The combined treatment was timed in such a way that the peak time of the effects accumulated at the time of testing. Half (n=5) of the control (vehicle) group was injected 10mins prior to testing, the other half (n=5) 30mins before.

Effort-based decision making

The effects of MDMA, WIN, and the combination of both drugs on effort-based decision making were tested in a T-maze paradigm. This task allows monitoring of cost-benefit choice behaviour, i.e., how much effort the animal is willing to exert to obtain a (larger) reward. At the end of the reward arms of the T-maze (measurements of each arm: 60 cm x 15 cm x 30 cm (L x W x H)) either two or four pellets (BioServ®, UK Dustless Precision Pellets®, 45mg) were placed in a metal food well. While the arm containing two pellets (low reward arm, LR) was freely accessible for the rats, a 30 cm barrier made of wire mesh was placed in the arm containing four pellets (high reward arm, HR). The HR was the right side T-maze arm for half of the animals, and left side for the other half. All animals were habituated to the apparatus as well as increasing heights of the barrier and pre-trained until as a group they reached baseline level of ≥ 80% choice of HR for three consecutive days. Intertemporal interval (ITI) was 1 min. Habituation and pre-training sessions took place once a day for an average of 16 days (for further detail on apparatus and training method see [36], adapted from the original study by Salamone (1994)). For the test the animals received drugs or vehicle as described above and performed two forced choice runs (one to each arm, in pseudo-randomized order), prior to ten free choice runs. The percentage of choices for the HR arm was calculated for each treatment group.

Body temperature

Temperature was measured with an in-ear thermometer (Thermoscan, IRT3020 CO, Braun, Switzerland) at three different time points: A baseline measurement one day before the first testing in the effort-based task was done in order to rule out effects of the injection procedure. A second measurement was done before the animals were tested (i.e., at the respective peak times of effects of the substances, T1), and a third time 1 hour after the test (T2). At each time point, temperature was measured three consecutive times, and the mean of these three measures was considered the temperature value for that time point.

Locomotor activity

Animals were placed in infrared beam-controlled acrylic glass chambers (ActiMot-system; TSE, Bad Homburg, size: 44.7 cm x
Food preference test

Animals were placed in a standard Makrolon type II cage with two glass food wells each containing pellets (Bio-Serv®, UK Dustless Precision Pellets®, 45mg) or breeding chow (Altromin, Lage, Germany). Breeding chow and pellets only differed in palatability, not in protein (22.5%, 18.7%, respectively) or fat (5%, 5.6%, respectively) content. Animals were allowed to free-feed for 10mins. Animals had not eaten for 20+/-2 hours when testing was conducted. Mass of eaten food was weighed for each animal.

Data analysis

For statistical analysis, analyses of variance (ANOVAs) were conducted with SigmaStat2.03 for Windows (SPSS Inc., Chicago, IL, USA), followed by post hoc Tukey tests for pairwise multiple comparisons. For all measurements, p<0.05 was considered a significant difference.

Results

Effort-based decision making

One animal from the MDMA+WIN group was unable to complete the task and therefore was excluded, thus yielding n=10 for MDMA, WIN and vehicle groups, and n=8 for the MDMA+WIN group for the statistical analysis. MDMA- and MDMA+WIN-treated animals chose the high reward (HR) arm less often compared to the WIN and vehicle groups (51% and 57% vs. 82% and 95%, respectively, Figure 1). A one-way ANOVA yielded a significant difference of HR choices between treatment groups [F(3,34)= 3.50, p=0.026]. The post-hoc test revealed a significant difference between the vehicle and MDMA group (mean HR choice= 95%, 51%, respectively, p = 0.034). While the combined treatment (MDMA+WIN) reduced choices for the HR, this effect failed to reach significance (Mean: 57%, p=0.117). (Figure 1)

Body temperature

One hour after behavioural testing (T2), body temperature was increased in rats treated with MDMA (mean: 37.4°C) and MDMA+WIN (mean: 37.5°C) compared to baseline (BL) and to measurements 30 minutes after administration (T1). WIN-treated animals showed an increase in temperature 10 minutes after administration (37.3°C, BL: 36.6°C; (Figure 2). A two-way ANOVA yielded a significant effect of time point of measurement [F(2,44)=15.41, p<0.001] as well as a significant interaction of time point x substance (F(6,44)= 3.40, p=0.008). Post-hoc test revealed significant differences comparing treatment groups MDMA+WIN vs. WIN measured before the first run (T1) (p<0.007). Furthermore, significant differences were found within MDMA and MDMA+WIN groups between measurements taken one hour after behavioural testing (T2) and BL (p=0.001 and p<0.001) as well as between T1 and T2 (p=0.017 and p<0.001). Within the WIN group, the difference between T1 and BL was significant (p<0.001).

Locomotor activity and exploratory behaviour

MDMA and MDMA+WIN-treated animals showed an increase in activity (mean: 63% and 61%, respectively) as well as in number of rearings (mean: 53 and 43, respectively), compared to the WIN (20% and 12) or vehicle (20% and 10) group (Figure 3). In addition, MDMA-treated rats spent more time in the centre of the open field than the remaining three treatment groups (mean: 15% vs. 8% (MDMA+WIN), 4% (WIN) and 6% (vehicle)). For locomotor activity, a two-way ANOVA yielded significant effects of treatment group [F(3,244)= 310.74, p<0.001], time interval [F(6,244)= 27.46, p<0.001], and interaction of substance x time interval [F(18,244)= 2.18, p=0.004]. Post-hoc test revealed significant differences between MDMA and MDMA+WIN groups compared to WIN- and vehicle-treated groups (p<0.001) over all 35 minutes, as well as within each 5-minute time interval (p<0.001). WIN- and vehicle-treated animals significantly reduced their activity between the first and all subsequent intervals (p<0.001), as well as between the second and the second last (WIN, p=0.05) or the third last (vehicle, p=0.011) intervals.

A two-way ANOVA comparing the number of rearings yielded significant effects for substance [F(3,244)= 73.56, p<0.001] and time interval [F(6,244)= 3.24, p=0.004]. Post-hoc test revealed significantly increased number of rearings for the MDMA group compared to the other three treatment groups (p<0.001 (WIN and vehicle), p=0.021 (MDMA+WIN)), as well as for the MDMA+WIN group compared to the WIN and vehicle groups (p<0.001).

In terms of differences within time intervals, MDMA-treated animals reared significantly more often than WIN- and vehicle-treated groups in all but the very first time interval (p<0.001 to p=0.006). MDMA+WIN-treated animals reared significantly more often than both WIN- and vehicle-treated animals in the last three intervals (p<0.001) and the third interval (p=0.029; p=0.044, respectively), as well as compared to WIN during the second and fourth interval (p=0.003 and p=0.042, respectively). Vehicle-treated animals showed a reduction in the number of measured rearings between the first and third to seventh time interval (p<0.001 to p=0.025) (For significant differences (p<0.001) within the time intervals, see Figure 3). A two-way ANOVA analysing the time spent in the centre of the open field yielded a significant effect for substance [F(3,244)=17.88, p<0.001]. Post-hoc test confirmed that MDMA-treated animals spent significantly more time in the centre compared to all three remaining treatment groups (p<0.001).
distribution of CB1 receptors may cause an indirect inhibition on and dopamine transporter (DAT) [40]. The abundant and overlapping dendrites, and may interfere with the serotonin transporter (SERT) on 5-HT and DA neurons not only pre-synaptically, but also on whereas delta9-THC decreases 5-HT neurotransmission in the and dopaminergic neurotransmission, especially in the mPFC [37], synthetic CB1 receptor antagonist SR141617A increased serotonergic on transmitter release in a range of brain regions. For example, the this difference was not statistically significant.

Food preference test

All animals consumed more pellets than breeding chow. When comparing the amount of pellets consumed, MDMA- and MDMA+WIN-treated animals are less than the vehicle and WIN-treated groups (mean: 4.5 and 2.8 grams vs. 6.0 and 6.8 grams, respectively) (Figure 4). A two-way ANOVA yielded significant effects for type of food $[F(1,70)=167.66, p<0.001]$, substance $[F(3,70)=6.14], p<0.001]$, and the interaction substance x type of food $[F(3,70)= 4.88, p=0.004]$. Post-hoc tests revealed that all animals preferred pellets over breeding chow ($p<0.001$). Rats from the vehicle group consumed significantly more pellets than the MDMA ($p=0.017$) and MDMA+WIN group ($p<0.001$). WIN-treated animals consumed significantly more pellets than the MDMA+WIN group ($p<0.001$). When comparing total amount of food intake, vehicle and WIN-treated groups are significantly more than MDMA+WIN-treated animals ($p=0.004$ and $p=0.002$, respectively). MDMA+WIN-treated animals consumed less food than MDMA-treated animals (2.8grams vs. 4.7grams), however this difference was not statistically significant.

Discussion

Various studies suggest an interaction of MDMA influence on different neurotransmitters and the inhibitory effects of CB receptors on transmitter release in a range of brain regions. For example, the synthetic CB1 receptor antagonist SR141617A increased serotonergic and dopaminergic neurotransmission, especially in the mPFC [37], whereas delta9-THC decreases 5-HT neurotransmission in the nucleus accumbens [38,39]. Furthermore, CB1 receptors are expressed on 5-HT and DA neurons not only pre-synaptically, but also on dendrites, and may interfere with the serotonin transporter (SERT) and dopamine transporter (DAT) [40]. The abundant and overlapping distribution of CB1 receptors may cause an indirect inhibition on dopaminergic and serotonergic neurons by influencing GABAergic inhibition of DA neurons [41]. An effect of WIN on glutamatergic [42] and cholinergic [43] synapses has been shown as well, offering further sites for interaction. Therefore, the DA and 5-HT release and reuptake inhibition properties of MDMA would hereby interact with the inhibitory effects of WIN via the CB1 receptor and result in opposing, additive or regulative effects in motivation and effort, as well as temperature regulation and locomotor behaviour.

Effort-based decision making

MDMA impaired choice behaviour based on effort irrespective of co-administration of WIN. As far as to current knowledge, no previous study has investigated the effect of acute MDMA administration on effort-based choice behaviour. Exact mechanisms on how MDMA-induced alterations in 5-HT and DA release could influence the fronto-striatal circuitry [44] or DA release in the nucleus accumbens [45] responsible for regulating effort-based choice remain speculative. The impact of central 5-HT release on general aspects of behaviour, e.g. motivation, may be important here. Furthermore, acute MDMA administration did not impair locomotion, but decreased food intake (see below). Therefore, increased 5-HT release might have decreased appetite and thus, motivation for climbing the barrier to obtain the high reward. Whatever the underlying causes, our data indicates that acute MDMA effects do not only include physiological responses like hyperthermia or increased activity, but may also immediately impair cognitive functions like decision making.

Although only MDMA-alone significantly differed from the control group, responses of the combined treatment group closely paralleled those of MDMA-treated animals. The lack of a significant effect is probably due to side effects observed in the MDMA+WIN-treated animals such as increased head waving, defecation, salivation and overall slow responses and movements. One MDMA+WIN-treated animal was excluded from the analysis due to inability to move further than the decision point of the T-maze. These impairments may have been adverse effects upon the first acute simultaneous administration of MDMA and WIN, as this behavioural pattern was not, or to a lesser extent, observed in subsequent experiments. Young et al. (2005) reported similar behavioural impairments upon combined administration of delta9-THC (1mg/kg) and MDMA (5mg/kg) in a within subjects design. As in our study, no major adverse effects of administration of either MDMA or the cannabinoid agonist alone were evident, thus observed impairments seem to be due to the combination, not the single doses, of the substances. Overall, and especially taking into account the observed side effects of co-administration, it is not possible to draw firm conclusions about the influence of the combined consumption on effort-based decision making based on these results. However, MDMA-administration seems to have an effect on effort-based choice behaviour with or without WIN co-administration.

Our results show no effect of WIN (1.2mg/kg) on effort-based decision making in rats. This contrasts with an earlier study employing operant chambers showing that acute treatment with 1.2 mg/kg or 1.8 mg/kg WIN significantly reduced the number of lever presses for pellets (“break point”) in a progressive-ratio task compared to a lower dose of WIN (0.6mg/kg) or vehicle treatment. Moreover, a significant decrease in the total number of lever presses was detected, indicating a complex role of cannabinoids in the control of reward-related behaviour [46]. However, although both paradigms aim at investigating the influence of CB1 activation on effort-based reward obtainment, there are of course differences between T-maze- and instrumental tasks, which preclude a direct comparison. However, our data indicate no influence of CB1 receptor activation on this behavioural paradigm.
Body temperature

Our results support previous studies demonstrating an increase in body temperature upon consumption of MDMA in humans [47,48] and rats [7]. The peak temperature measured 1hr after behavioural testing contrasts with previous findings showing significant increases compared to baseline levels at 20-30 minutes after administration of MDMA (12.5mg/kg, i.p.) [9]. However, depending on the route of administration (s.c. versus i.p. injection) hyperthermic effects can vary in time due to different absorption and metabolizing rates. Furthermore, according to Green at al. (2003) peak temperatures are observed 40-60 minutes after i.p. administration.

Co-administration of WIN did not influence MDMA-induced hyperthermia. Significantly higher temperature compared to both baseline and pre-test was measured 1hr after behavioural testing for the MDMA+WIN group. In fact, temperature changes were akin to those seen in the MDMA group. These results are in contrast with a finding by Morley (2004), showing that delta9-THC and CP 55,940 prevent MDMA-induced hyperthermia in rats. However, these cannabinoid agents were administered according to a different injection scheme (4x 2.5 mg/kg). A study in humans demonstrated that delta9-THC co-administration does not prevent MDMA-induced temperature increase [28]. Our results do not support the modulatory role of acutely administered CB1/2 receptor ligands on MDMA-induced hyperthermia. Rather, a modulation of the WIN-induced rise in temperature by MDMA appears.

WIN (1.2mg/kg) led to a significant increase in body temperature 10 minutes after administration compared to baseline. Earlier studies on cannabinoid effects on body temperature found that low doses of delta9-THC (0.05 and 0.1 mg/kg) caused hyperthermia, while doses of 1.0, 2.0 and 5.0 mg/kg induced hypothermia [49]. In contrast to our study, cannabinoid receptor agonists WIN55,212-2 and CP55,940 led to hypothermia, which was reversed by the selective CB1 receptor antagonist SR 141716 [35]. On the other hand, it was recently shown that the endogenous cannabinoid anandamide increases temperature when administered intracerebroventricularly, an effect which is reduced by co-administration of a CB1 receptor antagonist [50]. Furthermore, low

Figure 3: Effects of MDMA, WIN, MDMA+WIN and vehicle (n=10;10;9;10, respectively) on locomotion (a), exploratory (b) and anxiety-like (c) behaviour. (a) and (c) are depicted as % time of 5-minute intervals for a total of 35 minutes, (b) as absolute number of rearings per interval. Significant differences of p<0.001 are depicted only. Open triangles and open circles indicate differences between the first and the denoted time interval within vehicle and WIN groups, respectively. Closed triangles and squares denote differences between MDMA vs. WIN and vehicle groups, respectively. Closed circles and rhombuses indicate differences between MDMA+WIN and WIN and vehicle groups, respectively.

Figure 4: Effects of acute administration of MDMA, WIN, MDMA+WIN and vehicle (n=10;10;9;10, respectively) on food preference. Closed square shows significant difference (p<0.005) in total food intake compared to vehicle and WIN groups; hash symbol denotes difference (p<0.001) in amount of pellets consumed compared to vehicle and WIN groups; asterisk shows difference (p<0.05) in amount of pellets consumed compared to vehicle group.
In the current experiment, WIN-only treatment did not affect locomotor activity and exploratory behaviour.

MDMA as well as MDMA+WIN treatment significantly increased locomotor activity compared to the WIN- as well as vehicle- group (Figure 3a). These effects were stable over the 35 minutes test duration and support other studies demonstrating hyperactivity upon acute MDMA-administration [7,51]. Rats treated with 1.2 mg/kg WIN showed activity levels akin to the vehicle group. Co-administration of WIN therefore does not have an attenuating effect on MDMA-induced increases in locomotor activity.

Compared to MDMA-treated animals, MDMA+WIN administration led to a significant overall reduction in exploratory behaviour (number of rearings) over the 35 minute measurement (Figure 3b). No habituation was observed over time. However, there was no difference when comparing any of the individual time intervals. Therefore, co-administration of WIN seems to have a small, if any, modulating effect on MDMA-induced exploratory behaviour. In contrast to these results, rodent studies administering delta9-THC and MDMA found that the cannabinoid had an attenuating effect on MDMA-induced hyperactivity in rats [12] and mice [34]. The discrepancy between the previously described and our results may be due to the different test paradigms, dose-dependent biphasic effects of cannabinoids [35], and a more unspecific effect of delta9-THC compared to WIN.

In terms of the time spent in the centre of the open field, a measure for exploration anxiety, MDMA+WIN-, WIN- and vehicle-treated animals (see Figure 3c). MDMA seems to have an anxiolytic effect. In contrast, increased anxiety levels were found in MDMA-only treated rats [52] and mice [53] on various anxiety-related measures. However, this result is congruent with Morley et al. (2005) demonstrating a decreasing effect of delta9-THC on MDMA-induced anxiety measured in an emergence test. The attenuating effect of WIN on MDMA-induced decreased exploration anxiety is not due to differences in locomotion as the MDMA+WIN group displayed equal levels of hyperactivity as the MDMA group whilst not differing from vehicle group in the anxiety measure. Future studies seeking to elucidate the influence of CB1 agonism on (MDMA-induced) anxiety should employ a more direct measure as well as various CB agents.

Rats treated with 1.2 mg/kg WIN showed activity, vertical exploratory behaviour as well as exploration anxiety levels akin to the vehicle group. Furthermore, there were no differences in habituation, i.e. a reduction of activity over the measured intervals within the 35 minutes occurred in WIN- and vehicle-treated animals equally. As noted previously, CB1 agonists may have biphasic effects according to dose, inducing hyperactivity at low doses and severe motor deficits at larger doses [35]. In line with this, locomotor activity in the open field has been reported to be increased by 0.6 mg/kg, but not by higher doses of WIN [46]. A dose of 1 mg/kg does not affect either ambulation or the frequency of rearings, while higher doses (3 or 5.6mg/kg) reduce both measures [54]. Various reports point to involvement of CB1 antagonism (for example, by SR 141716), but not agonism, in anxiogenic effects [55-58]. In the current experiment, WIN-only treatment did not affect any of the measures.

### Food preference

MDMA reduced intake of pellets, which is congruent with previous studies demonstrating that MDMA consumption reduces food intake and appetite in humans [59,60] and animals [13,61]. Co-administration of WIN does not seem to have an effect on MDMA-induced hypophagia, as MDMA+WIN treated animals consumed even less than the MDMA group (means of total food intake: 2.79 vs 4.55 grams), but this difference was not statistically significant. Although i.c.v. administration of WIN has been shown to decrease extracellular 5-HT and 5-HIAA in hypothalamic brain areas [62], this effect seems to be overruled by the strong MDMA-induced increase of 5-HT release and the associated reduction in food intake [63]. Food intake is a process mediated by stimulation of 5-HT receptors [62,63].

WIN-treated animals did not differ from the vehicle-treated group in terms of food consumption. This result was somewhat unexpected since previous studies demonstrated an increase in food intake after delta9-THC or WIN administration compared to vehicle groups [64]. For example, i.p. administration of WIN at doses of 0.5, 1 and 2 mg/kg caused a significant increase in food intake from 1h-6h after injection [62]. However, Merroun et al. (2009) did not find significant differences when comparing the non-cumulative amounts of food intake between vehicle- and WIN- (1 or 2 mg/kg) treated animals. As with activity levels and body temperature, activation of CB1/2 receptors tends to evoke dose-dependent biphasic responses. WIN at doses of 1 and 2 mg/kg promoted hyperphagia, whereas administration of a higher dose (5 mg/kg) significantly inhibited food intake in partially satiated rats [62]. Drews et al. (2005) even found a significant reduction in the amount of pellets consumed by animals treated with 1.8 mg/kg WIN. If orogenic effects of WIN appear subsequent to maximal blood concentration levels, hyperphagia may have been evoked only partially in the current study as testing took place 10 minutes after administration.

### Conclusion

MDMA led to decreased choices of the HR option in an effort-based decision making task. Furthermore, previously well-documented increases in activity and body temperature as well as decreased food intake were replicated. Overall, our behavioural tests do not support a modulatory role of WIN regarding MDMA-induced acute effects. Apart from an augmenting effect on body temperature, WIN administration alone did not yield effects distinct from vehicle treatment. Although there was a wash-out period of seven days between each test, addictive or habituation effects cannot be completely ruled out. Future studies could vary the administration schedule and doses. In the current study, we used doses which in other animal studies have been shown to be behaviourally relevant (e.g., [44,65]). However, since the dosage used in these experiments was relatively high, a lower dose of MDMA could reveal a putative potentiating effect of WIN. Many MDMA users consume cannabis concomitantly to enhance positive sensations or “come-down” [66,67]. The neurobiological mechanisms underlying behavioural effects of MDMA, as well as co-consumption of cannabis, remain somewhat unclear as a complex interplay between 5-HT and DA release as well as activation of different 5-HT subtypes must be considered. Administration of specific CB1 agonists and manipulation of certain 5-HT receptors, and/or verification of DA and 5-HT-transmitter levels in brain areas known to be involved in behavioural responses, could further elucidate underlying pharmacological mechanisms. From these experimental tests in rats, we conclude that acute co-administration of a CB agonist does not substantially attenuate the MDMA-induced behavioural effects.
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References

1. Editorial team (2010) The EMCDDA annual report 2010: the state of the drugs problem in Europe. Euro Surveill 15.
2. Daniellsen AK, Wennberg P, Hibell B, Romeljáj A (2012) Alcohol use, heavy episodic drinking and subsequent problems among adolescents in 23 European countries: does the prevention paradox apply? Addiction 107: 71-80.
3. Wu LT, Parrott AC, Ringwall CL, Yang C, Blazer DG (2009) The variety of ecstasy/MDMA users: results from the National Epidemiologic Survey on alcohol and related conditions. Am J Addict 18: 452-461.
4. Parrott AC, Milani RM, Gouzouliis-Mayfrank E, Daumann J (2007) Cannabis and Ecstasy/MDMA (3,4-methylenedioxyamphetamine): an analysis of their neurophychobiological interactions in recreational users. J Neural Transm 114: 959-968.
5. Parrott AC, Gouzouliis-Mayfrank E, Rodgers J, Solowij N (2004) Ecstasy/MDMA and cannabis: the complexities of their interactive neurophychobiological effects. J Psychopharmacol 18: 572-575.
6. Boys A, Marsden J, Strang J (2001) Understanding reasons for drug use amongst young people: a functional perspective. Health Educ Res 16: 457-469.
7. Green AR, Methan AO, Elliott JM, O'Shea E, Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxyamphetamine (MDMA, "ecstasy"), Pharmacol Rev 55: 463-508.
8. Cole JC, Summlll HR (2003) The pre-clinical behavioural pharmacology of 3,4-methylenedioxyamphetamine (MDMA). Neurosci Biobehav Rev 27: 199-217.
9. Methan AO, Esteban B, O'Shea E, Elliott JM, Colado MI, et al. (2002) The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxyamphetamine (MDMA, "ecstasy") to rats. Br J Pharmacol 135: 170-180.
10. Bankson MG, Cunningham KA (2001) 3,4-Methylenedioxyamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. J Pharmacol Exp Ther 297: 846-852.
11. Docherty JR, Green AR (2010) The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxyamphetamine (MDMA, ecstasy) and its derivatives. Br J Pharmacol 160: 1029-1044.
12. Morley KC, Li KM, Hunt GE, Mallet PE, McGregor IS (2004) Cannabinoids prevent the acute hyperthermia and partially protect against the 5-HT depleting effects of MDMA ("Ecstasy") in rats. Neuropharmacology 46: 954-965.
13. Frith CH, Chang LW, Lattin DL, Walls RA, Hamm J, et al. (1987) Toxicity of methylenedioxyamphetamine (MDMA) in the dog and the rat. Fundam Appl Toxicol 9: 110-119.
14. De Souza I, Kelly JP, Harkin AJ, Leonard BE (1997) An appraisal of the pharmacological and toxicological effects of a single oral administration of 3,4-methylenedioxyamphetamine (MDMA) in the rat. Pharmacol Toxicol 80: 207-210.
15. Byrne T, Baker LE, Poling A (2000) MDMA and learning: effects of acute and neurotoxic exposure in the rat. Pharmacol Biochem Behav 66: 501-508.
16. Wilson RI, Nicolli RA (2002) Endocannabinoid signaling in the brain. Science 296: 678-682.
17. Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, et al. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11: 563-583.
18. McDonald J, Schleifer L, Richards JB, de Wit H (2003) Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology 28: 1356-1365.
19. Zuurman L, Ippel AE, Moin E, van Gerven JM (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol 67: 5-21.
20. Ameri A (1999) The effects of cannabinoids on the brain. Prog Neurobiol 58: 315-348.
21. Elphick MR, Egertová M (2001) The neurobiology and evolution of cannabinoid signaling. Philos Trans R Soc Lond B Biol Sci 356: 381-408.
22. Iversen L (2003) Cannabis and the brain. Brain 126: 1252-1270.
23. Morton J (2005) Ecstasy: pharmacology and neurotoxicity. Curr Opin Pharmacol 5: 79-86.
24. Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, et al. (2004) Cannabinoid physiology and pharmacology: 30 years of progress. Neuropsychopharmacology 47 Suppl 1: 345-358.
25. Daumann J, Hensen G, Thimm B, Rezk M, Tili B, et al. (2004) Self-reported psychopathological symptoms in recreational ecstasy (MDMA) users are mainly associated with regular cannabis use: further evidence from a combined cross-sectional/longitudinal investigation. Psychopharmacology (Berl) 173: 398-404.
26. Croft RJ, Mackay AJ, Mills AT, Gruzelier JG (2001) The relative contributions of ecstasy and cannabis to cognitive impairment. Psychopharmacology (Berlin) 153: 373-379.
27. Rodgers J, Buchanan T, Scholay AB, Hefferman TM, Ling J, et al. (2001) Differential effects of Ecstasy and cannabis on self-reports of memory ability: a web-based study. Hum Psychopharmacol 16: 619-625.
28. Dumont GJ, Kramer C, Sweep FC, Touw DJ, van Hasselt JG, et al. (2009) Cannabis coadministration potentiates the effects of "ecstasy" on heart rate and temperature in humans. Clin Pharmacol Ther 85: 160-166.
29. Dumont GJ, van Hasselt JG, de Kam M, van Gerven JM, Touw DJ, et al. (2011) Acute psychomotor, memory and subjective effects of MDMA and THC co-administration over time in healthy volunteers. J Psychopharmacology 25: 478-489.
30. Sala M, Braida D (2005) Endocannabinoids and 3,4-methylenedioxymethamphetamine (MDMA) interaction. Pharmacol Biochem Behav 81: 407-416.
31. Braida D, Sala M (2002) Role of the endocannabinoid system in MDMA intracerebral self-administration in rats. Br J Pharmacol 136: 1089-1092.
32. Rodríguez-Arias MA, Manzanedo C, Roger-Sánchez C, Do Couto BR, Aguilar MA, et al. (2010) Effect of adolescent exposure to WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference. Prog Neuropsychopharmacol Biol Psychiatry 34: 166-171.
33. Manzanedo C, Rodríguez-Arias M, Daza-Losada M, Maldonado C, Aguilar MA, et al. (2010) Effect of the CB1 cannabinoid agonist WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference in mice. Behav Brain Funct 6: 19.
34. Tourniol C, Ledent C, Maldonado R, Valverde O (2008) CB1 cannabinoid receptor modulates 3,4-methylenedioxyamphetamine acute responses and reinforcement. Biol Psychiatry 63: 1030-1038.
35. Chaperson F, Thébault MH (1998) Behavioral effects of cannabinoid agents in animals. Crit Rev Neurobiol 13: 243-281.
36. Walton ME, Bannerman DM, Rushworth MF (2002) The role of rat medial frontal cortex in effort-based decision making. J Neurosci 22: 10996-11003.
37. Darmani NA, Jancayi JK, Kumar N, Cim J (2003) Behaviorally active doses of the CB1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover. Pharmacol Biochem Behav 75: 777-787.
38. Sano K, Mishima K, Koushi E, Orito K, Egashira S, Nakaoka K, et al. (2003) Effects of delta 9-tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. Neuroscience 151: 320-329.
39. López-Moreno JA, González-Cuevas G, Moreno G, Navarro M (2008) The
pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. Addict Biol 13: 160-187.

40. Lau T, Schloss P (2008) The cannabinoid CB1 receptor is expressed on serotonergic and dopaminergic neurons. Eur J Pharmacol 578: 137-141.

41. Pistis M, Porcu G, Melis M, Diana M, Gessa GL (2001) Effects of cannabinoids on prefrontal neuronal responses to ventral tegmental area stimulation. Eur J Neurosci 14: 96-102.

42. Shen M, Piser TM, Seybold VS, Thayer SA (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 16: 4322-4334.

43. Gessa GL, Casu MA, Carta G, Mascia MS (1998) Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. Eur J Pharmacol 355: 119-124.

44. Floresco SB, St Onge JR, Ghoda-Shariff S, Winstanley CA (2008) Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. Cogn Affect Behav Neurosci 8: 375-389.

45. Assadi SM, Yücel M, Pantelis C (2009) Dopamine modulates neural networks involved in effort-based decision-making. Neurosci Biobehav Rev 33: 383-393.

46. Drews E, Schneider M, Koch M (2005) Effects of the cannabinoid receptor agonist WIN 55,212-2 on operant behavior and locomotor activity in rats. Pharmacol Biochem Behav 80: 145-150.

47. Freedman RR, Johanson CE, Tancer ME (2005) Thermoregulatory effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology (Berl) 183: 249-256.

48. Mohamed WM, Ben Hamida S, Cassel JC, de Vasconcelos AP, Jones BC (2011) MDMA: interactions with other psychoactive drugs. Pharmacol Biochem Behav 99: 759-774.

49. Taylor DA, Fennessy MR (1977) Biphasic nature of the effects of delta9-tetrahydrocannabinol on body temperature and brain amines of the rat. Eur J Pharmacol 46: 93-99.

50. Fraga D, Zanoni CI, Rae GA, Parada CA, Souza GE (2009) Endogenous cannabinoids induce fever through the activation of CB1 receptors. Br J Pharmacol 157: 1494-1501.

51. Spanos LJ, Yamamoto BK (1989) Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)-MDMA] on locomotion and serotonin syndrome behavior in the rat. Pharmacol Biochem Behav 32: 835-840.

52. Morley KC, McGregor IS (2000) (+/-)-3,4-methylenedioxymethamphetamine (MDMA, `Ecstasy`) increases social interaction in rats. Eur J Pharmacol 408: 41-49.

53. Ferraz-de-Paula V, Stankevicius D, Ribeiro A, Pinheiro ML, Rodrigues-Costa EC, et al. (2011) Differential behavioral outcomes of 3,4-methylenedioxymethamphetamine (MDMA-ecstasy) in anxiety-like responses in mice. Braz J Med Biol Res 44: 429-437.

54. Järbe TU, Ross T, DiPatrizio NV, Pandarinath L, Makriyannis A (2006) Effects of the CB1R agonist WIN-55,212-2 and the CB1R antagonists SR-141716 and AM-1387: open-field examination in rats. Pharmacol Biochem Behav 85: 243-252.

55. Moreira FA, Grieb M, Lutz B (2009) Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab 23: 133-144.

56. Rodgers RJ, Evans PM, Murphy A (2005) Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naive and plus-maze-experienced mice. Behav Pharmacol 16: 405-413.

57. Patel S, Cravatt BF, Hillard CJ (2005) Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. Neuropsychopharmacology 30: 497-507.

58. Haller J, Varga B, Ledent C, Freund TF (2004) CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behav Pharmacol 15: 299-304.

59. Vollenweider FX, Gamma A, Liechti M, Huber T (1998) Psychological and cardiovascular effects and short-term sequelae of MDMA (`ecstasy`) in MDMA-naive healthy volunteers. Neuropsychopharmacology 19: 241-251.

60. Kirkpatrick MG, Gunderson EW, Perez AY, Haney M, Foltin RW, et al. (2012) A direct comparison of the behavioral and physiological effects of methylamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology (Berl) 219: 109-122.

61. Jean A, Conductor G, Mannique C, Bouras C, Berta P, et al. (2007) Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. Proc Natl Acad Sci U S A 104: 16335-16340.

62. Merroun I, Errami M, Hoddah H, Urbano G, Porres JM, et al. (2009) Influence of intracerebroventricular or intraperitoneal administration of cannabinoid receptor agonist (WIN 55,212-2) and inverse agonist (AM 251) on the regulation of food intake and hypothalamic serotonin levels. Br J Nutr 101: 1569-1578.

63. Lam DD, Garfield AS, Marston OJ, Shaw J, Heisler LK (2010) Brain serotonin system in the coordination of food intake and body weight. Pharmacol Biochem Behav 97: 84-91.

64. Kirkham TC (2005) Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol 16: 297-313.

65. Young JM, McGregor IS, Mallet PE (2005) Co-administration of THC and MDMA (`ecstasy`) synergistically disrupts memory in rats. Neuropsychopharmacology 30: 1475-1482.

66. Winstock AR, Griffiths P, Stewart D (2001) Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. Drug Alcohol Depend 64: 9-17.

67. Schulz S (2011) MDMA & cannabis: a mini-review of cognitive, behavioral, and neurobiological effects of co-consumption. Curr Drug Abuse Rev 4: 81-86.