Cannabis and exercise : effects of Δ^9-tetrahydrocannabinol on preference and motivation for wheel-running in mice

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ABSTRACT

Recent surveys have revealed close links between cannabis and exercise. Specifically, cannabis usage before and/or after exercise is an increasingly common habit primarily aimed at boosting exercise pleasure, motivation, and performance whilst facilitating post-exercise recovery. However, whether these beliefs reflect the true impact of cannabis on these aspects of exercise is unknown. This study has thus examined the effects of cannabis' main psychoactive ingredient, namely Δ^9-tetrahydrocannabinol (THC), on (i) mouse wheel-running preference and performance and (ii) running motivation and seeking behaviour. Wheel-running preference and performance were investigated using a T-maze with free and locked wheels located at the extremity of either arm. Running motivation and seeking were assessed by a cued-running operant task wherein wheel-running was conditioned by nose poking. Moreover, because THC targets cannabinoid type 1 (CB_1) receptors, i.e. receptors previously documented to control running motivation, this study also assessed the role of these receptors in running preference, performance, and craving-like behaviour. Whilst acute blockade or genetic deletion of CB_1 receptors decreased running preference and performance in the T-maze, THC proved ineffective on either variable. The failure of THC to affect running variables in the T-maze extended to running motivation, as assessed by cued-running under a progressive ratio (PR) reinforcement schedule. This ineffectiveness of THC was not related to the treatment protocol because it successfully increased motivation for palatable food. Although craving-like behaviour, as indexed by a cue-induced reinstatement of running seeking, was found to depend on CB_1 receptors, THC again proved ineffective. Neither running motivation nor running seeking were affected when CB_1 receptors were further stimulated by increasing the levels of the endocannabinoid 2-arachidonoylglycerol. These results, which suggest that the drive for running is insensitive to the acute stimulation of CB_1 receptors, raise the hypothesis that cannabis is devoid of effect on exercise motivation. Future investigation using chronic administration of THC, with and without other

cannabis ingredients (e.g. cannabidiol), is however required before conclusions can be drawn.

1. Introduction

Does cannabis consumption facilitate exercise? If so, does cannabis act on exercise motivation, exercise pleasure, and/or exercise performance? Recent years have seen an expanding number of online press reports from top newspapers (see e.g. Ducharme, 2019; Hesse, 2016; Miller, 2018) and an Outlook in Nature (Nguyen, 2019) that focused on these questions. This media interest is accounted for by a growing number of sportspeople interviews, initially thought to be only anecdotal, highlighting the expanding use of cannabis prior to or after exercise (most often long-distance running). The main reasons for cannabis use are the beliefs that it increases exercise pleasure and performance whilst alleviating after-exercise fatigue symptoms (Nguyen, 2019). Nowadays, the anecdotal reports on the relationship between cannabis and exercise have given way to true scientific interest. Studies based on self-reports in large individual samples confirm that cannabis use is primarily aimed at increasing exercise pleasure (and hence possibly precipitate the so-called "runner's high"), performance, motivation, and after-exercise recovery (Gillman et al., 2015; Huestis et al., 2011; Kennedy, 2017; Ware et al., 2018). However, how these beliefs range compared to each other was unknown until a recent study addressed this issue. The recent legalisation of cannabis use in several states of the United States of America has facilitated the largest survey (i.e. hundreds of aerobic and anaerobic exercise practitioners) on the beliefs underlying cannabis use before/after exercise (YorkWilliams et al., 2019). The results indicate that beliefs linked to exercise pleasure and after-exercise recovery actually surpass the belief that cannabis increases exercise motivation or exercise performance (YorkWilliams et al., 2019). The finding that exercise performance was not the main reason why cannabis was used prior to exercise is in keeping with the observation that cannabis negatively impacts such a performance in certain individuals (Gillman et al., 2015; Huestis et al., 2011; Kennedy, 2017; Ware et al., 2018). Moreover, because cannabis does not have an

ergogenic effect on its own (Ware et al., 2018), it is widely accepted that the positive effects of cannabis on performance, if any, are indirect and are chiefly accounted for by relaxation, well-being, and analgesia (effects that underlie the forbidden use of cannabis use in sport competition by the World Anti-Doping Agency since 2004).

These findings question the extent to which the belief in the positive effects of cannabis before/after exercise reflect scientifically-proven properties of cannabis. One means of answering this question is through the use of animal models of exercise. However, because cannabis cannot be provided as such to laboratory animals, one prerequisite for the study of cannabis' impact on exercise is to identify the compounds through which cannabis bears its effects. Cannabis is made of hundreds of compounds (Andre et al., 2016) and it is assumed that its effects during/after exercise, including the adverse ones (Kennedy, 2017), are accounted for by the psychoactive properties of Δ^9-tetrahydrocannabinol (THC; Wachtel et al., 2002). Rodent models of exercise chiefly include treadmill-running and wheel-running (swimming is a stress response in laboratory rodents: Porsolt et al., 1978). However, the former relies on a negative reinforcement process because rodents are forced to run to escape electric shocks or air puffs. Hence, wheel-running, by virtue of its volitional use, is the preferred model of exercise (Sherwin, 1998). Accordingly, most investigators place a running wheel in the rodent housing cage, thereby allowing free access to the wheel and on-line measures of running performance. As an illustration, mice housed with running wheels run several kilometres a day (see e.g. Dubreucq et al., 2010), further suggesting that wheel-running is a strong reward in laboratory rodents (see below).

Using home cage wheel-running, we and others have shown that the endocannabinoid system exerts a tonic control on wheel-running performance, as assessed by running distances or durations (Dubreucq et al., 2010; Keeney et al., 2008; Zhou and Shearman, 2004). This tonic control is mediated by CB_1 receptors - the principal cannabinoid receptor in the brain - located in the ventral tegmental area (VTA; Dubreucq et al., 2013), the structure from which project reward-regulating mesocorticolimbic dopaminergic neurones. Because THC's psychoactive effects are accounted for by the stimulation of CB_1 receptors (Huestis et

al., 2001), it is expected that THC augments running performance. Actually, when acutely tested at doses devoid of intrinsic locomotor effects, THC lacked effects on running performance (Dubreucq et al., 2013). However, in keeping with the running paradigm used in this study, i.e. permanent housing with a wheel, thus allowing running with neither any constraint nor any other alternative than resting, this result does not document whether THC impacts (i) preference for running and/or (ii) running motivation. The T-maze test allows preference for a reward to be measured since the reward is located at the extremity of one of the arms of the maze. Therefore, animals have to first make the choice for a distant reward before exerting exploratory efforts to reach that reward. Several T-maze studies have used a running wheel, either provided alone (Hill, 1961) or in concurrence with a second reward placed at the other end of the maze (Correa et al., 2016), but none have explored (i) whether the endocannabinoid system controls running motivation, and if so, (ii) whether the latter is modified by THC administration. Although it has been claimed that the T-maze additionally provides information on reward motivation (Robinson et al., 2005), it is thought that the (exploratory) cost to access the reward in the T-maze is too low to efficiently provide such an information (unless a surmountable barrier is added: Salamone et al., 1994). As opposed to the T-maze, cued-reward instrumental tasks - where e.g. lever pressing is needed for reward access - provide indices ofthe primary reinforcing value of the reward under investigation; indeed, such procedures have confirmed that wheel-running is highly reinforcing (Belke and Garland Jr, 2007; Collier and Hirsch, 1971; Iversen, 1993). Measuring the maximal efforts exerted to reach the reward under progressive ratio (PR) reinforcement schedules provides selective indices of motivation for that reward (Hodos, 1961). Having developed a paradigm wherein wheel-running is conditioned by prior nose poking, we have shown that VTA CB_1 receptors exert a tonic control over running motivation (Muguruza et al., 2019). However, whether acute THC administration affects running motivation in this paradigm remains an open question. Besides measuring motivation for a reward, operant conditioning procedures further permit craving-like behaviour for a reward to be measured by means of a cue-induced reinstatement of reward seeking in animals that have extinguished the cue-reward

association (Shaham et al., 2003; Venniro et al., 2016). Indeed, we have further shown that wheel-running is a reward strong enough to promote seeking after such an extinction period (Muguruza et al., 2019). Again, whether THC affects the intensity of exercise seeking is an issue for which information is still lacking.

The present study has thus examined the acute impact of THC administration on (i) preference for wheel-running and running performance in a T-maze wherein animals had the choice between two arms containing at their extremities either a free wheel or a locked wheel, and (ii) wheel-running motivation and craving-like behaviour, as assessed through a PR session and a cue-induced reinstatement of running seeking session respectively, using operant conditioning procedures. In the final series of experiments, we wondered whether the effects of THC on running motivation and seeking mimicked those elicited by an endogenous overstimulation of CB_1 receptors. To this end, mice were pretreated with the monoacylglycerol lipase (MAGL) inhibitor JZL184 (Long et al., 2009), which increases the levels of the endocannabinoid 2-arachidonoylglycerol (2-AG), before being tested either under a PR reinforcement schedule or in a cue-induced reinstatement of running seeking session.

2. Materials and methods

2.1. Animals

T-Maze experiments involved male C57BL/6N mice (Elevage Janvier, Le Genest-Saint-Isle, France) aged 8-12 weeks, and 8-14 week-old male constitutive CB_1 receptor mutant (CB_1 KO) mice and their wild-type (CB_1 WT) littermates (Bellocchio et al., 2010; Dubreucq et al., 2013; Muguruza et al., 2019). Operant conditioning procedures used 8-12 week-old males from a C57BL/6N-derived mouse line bred in our animal facilities, namely the *Cnr1*$^{flox/flox}$ (CB_1-floxed) line, and conditional mutants lacking floxed CB_1 receptors in cortical glutamatergic neurons - due to the expression of the *Nex*-Cre recombinase (Glu-CB_1 KO) - and their wild-type littermates (Glu-CB_1 WT; Bellocchio et al., 2010; Dubreucq et al., 2013; Muguruza et al., 2019). Mutant and wild-type mice, all bred in our animal facilities, were in a mixed genetic background with a predominant C57Bl/6N contribution. Note that CB_1-floxed mice behave similarly to C57Bl/6N with regard to the reinforcing value of wheel-running and its control by CB_1 receptors (Muguruza et al., 2019). All mice were genotyped (at 2-3 weeks-old) and regenotyped (at the end of experiments), as described previously (Bellocchio et al., 2010; Dubreucq et al., 2013; Muguruza et al., 2019).

2.2. Housing

At least one week before the beginning of the experiments, all mice were individually housed (to avoid inter-individual aggression) with (T-maze experiments) or without (operant conditioning experiments) a running wheel similar to that used in the T-maze (see below). Mice were located in a thermoregulated room (21-22°C) placed under a partly inverted 12-h light/12-h dark cycle, with the lights turning off at 2.00 PM (T-maze experiments) or at 9.00 AM (operant conditioning experiments). Except for one series of experiments which required

a restriction feeding regimen (see below), mice were all provided with food and water *ad libitum*.

2.3. T-maze experiments

The maze was made of three grey Perspex arms (8-cm large x 14-cm high). One arm, harbouring the start box (11-cm), was 35-cm long. The two other arms, opposing each other, were 45-cm long, including a compartment (16-cm long x 20-cm wide) placed at their respective ends. Each compartment housed a free or a locked 12-cm diameter running wheel (Intellibio, France). The right/left arm locations of the free/locked wheel were inverted between two successive mice. Except for one series of experiments conducted under light exposure (see below), all experiments were run with a red lamp placed above the T-maze to deliver a 0.2-lux illumination to the start box.

The first day of test, mice were placed in the start box and then freed through a sliding door to allow them to explore the T-maze for 5 min without either running wheel in the wheel compartments. One to two hours later, each mouse was placed back in the starting chamber before being freed to explore the T-maze for 5 min with the free and locked wheels. The next day, mice were put back in the starting chamber before being allowed to explore the starting arm and one of the two wheel-containing arms for 150 sec, the second arm being blocked by a sliding door. At the end of this period, mice were put back in the starting chamber before repeating the previous test, except that the blocked arm was now free whilst the free arm was now blocked. The two tests achieved the second day were repeated in the opposite order the third day. On test days 4, 5, and 6, only one daily session was conducted wherein mice were left free to explore the T-maze for 5 min. The initial latency to enter the free wheel, the respective numbers of entries into the free wheel and the locked wheel, the duration of running in the free wheel, the time spent in the locked wheel, and the total number of entries in the arms were all video-recorded by means of a sensitive camera placed above the apparatus connected to a computer in an adjacent room. All behavioural variables were scored by means of a customised EVENTLOG program. Preference ratios were calculated

as the time spent running in the free wheel over the total time spent in both free and locked wheels. Data from CB_1 KO mutants and their CB_1 WT littermates are reported as the mean ± SEM of the performances recorded during days 4-6. For tests with pharmacological intervention (C57Bl/6N mice), the performances were recorded 30 min after acute drug (SR141716, THC) or vehicle administration on day 6. As indicated above, mice were tested during the dark phase of the light/dark cycle in keeping with their nocturnal activity. However, in one series of experiments aimed at examining the impact of THC when mice are naturally inactive, mice were trained (days 1-3) as described above (i.e. under the dark phase) but exposed to T-maze tests (and THC treatments) during the light phase (days 4-6) under a 56-lux illumination.

2.4. Conditioned running procedures

2.4.1. Experimental set-up

The set-up included operant chambers (28 cm x 26 cm x 38 cm; Imetronic, France) located in a room adjacent to the housing room. These chambers, placed inside wooden casings (60 cm x 62 cm x 49 cm), were ventilated to guarantee air circulation and to provide background noise. The rear wall had a hollow for mounting a 20-cm-wheel that was locked or unlocked (by means of a brake-pad) according to predefined experimental conditions. The central wheel was flanked by 2 small holes set into the rear wall, allowing the animal to 'poke' its nose through, with cue-lights located above nose poke ports. An additional light was placed above the wheel, which illuminated the wheel while it was unlocked. Nose pokes could be either "active" (simultaneously leading to cue-light illumination above the active port, wheel unlocking, and illumination of the wheel) or "inactive" (having no consequence). The left/right allocation of active/inactive ports was switched between animals. A grilled floor was placed above a drawer to allow for easy removal of solid/liquid waste material. All devices in the operant chambers were linked to a computer (Polywheel software, version 5.2.2; Imetronic, France). The number of active/inactive nose pokes, the number of running

sequences, and the running duration of each rewarded sequence were detected and transmitted online (Hurel et al., 2019; Muguruza et al., 2019).

2.4.2. Wheel-running under fixed ratio (FR) and PR reinforcement schedules

The operant protocol consisted of daily 1-h sessions, as previously described (Hurel et al., 2019; Muguruza et al., 2019). The first day, mice were placed in the chambers, with the light above the unlocked running wheel remaining illuminated during the whole session. The nose poke ports were covered up by metal pieces and the cue-light above the active port remained off. This phase – which was performed on 2 consecutive days – was aimed at habituating the mice to the operant chambers, the wheel, and the wheel-light indicating availability of the reward. When learning sessions began on the third day (session 1), the wheel locking/unlocking mechanism and the nose poke ports became fully operational. The wheel was unlocked for 1 min (wheel brake released) following nose pokes the mouse performed in its allocated active port. The other port, although accessible, remained inactive. Learning sessions began with FR1 sessions during which a single active nose poke was sufficient to simultaneously illuminate the cue-light above the port for 10 sec and unlock the running wheel for 1 min under light. When this time period elapsed, the wheel-light extinguished and the brake was applied, so that the mouse had to step down from the wheel and execute a further nose poke in order to unlock it again. Nose pokes made in the active port while the wheel was already unlocked were without effect. After completing the FR1 schedule of reinforcement (6 daily sessions), mice were moved on to the FR3 condition where a 1-min wheel-running period was contingent on 3 consecutive active nose pokes. This experimental condition was repeated over 6 sessions. The day after the last FR3 session, four mouse groups were formed on the basis of similar mean nose pokes scores during this last FR3 session (to avoid a priori biases). Mice were then injected with THC or JZL184 (or their corresponding vehicle) 30 min or 120 min, respectively, before being tested under a linear PR schedule of reinforcement. Under this schedule, the number of active nose

pokes required to free the running wheel was incremented by 3 between each rewarded step with a time limit of 15 min between two successive steps.

2.4.3. Cue-induced reinstatement of running seeking

In another series of experiments, mice were placed under 12 FR (6 FR1 followed by 6 FR3) sessions of conditioned wheel-running as described above. Twenty four hours after the last FR3 session (session 12), mice underwent daily 1-h extinction sessions for 7 consecutive days. Throughout the extinction procedure, neither active nose poke ports nor cue lights were active and the running wheel remaining locked, as previously described (Muguruza et al., 2019). The day after the last extinction session (session 19), mouse groups - with identical scores during that session - were pretreated with either (i) THC or its vehicle, or (ii) JZL184 or its vehicle. Thirty minutes (THC experiments) or two hours (JZL184 experiments) later, a cue-induced reinstatement session was performed (session 20). Two minutes after this session began, a single 10-sec lighting of the cue above the active nose poke port appeared. Then, when the animal performed one active nose poke (as for the FR1 schedule) the cue-light was lit again for 5 sec (the wheel remaining locked, including for the rest of the session). Next, three active nose pokes were required (as for the FR3 schedule) to switch on the light; this procedure was then kept constant throughout the session. Whatever the number of active nose pokes required to light the active port, the running wheel remained locked whilst the cue light above the wheel remained inactive (Muguruza et al., 2019).

2.5. Conditioned feeding procedures

Owing to the stimulatory effect of THC on palatable feeding, we verified that the highest dose of THC used in this study (1 mg/kg) was pharmacologically efficient by measuring its acute impact on motivation for palatable feeding in food-restricted mice. The operant chambers described above were configured so as to host on their left panel a recessed pellet

tray surrounded by two nose poke ports (Hurel et al., 2019; Muguruza et al., 2019). Cue-lights were placed above both the nose poke ports and the feeder to indicate effectiveness of the nose pokes and pellet distribution, respectively. The rear side (where the running wheel and its corresponding nose poke ports and cue-lights are located; see above) was covered by grey Perspex. Note that the operant protocol consisted of 30-min daily sessions to avoid premature satiety.

The daily food consumption and the body weight of each mouse were recorded every day for a week before mice were given a limited quantity of food so as to maintain their body weight to 90 % levels of their free-feeding weight. Prior to the onset of the operant conditioning procedure, animals were first habituated to the 20-mg chocolate pellets used in the operant chambers (Dustless precision pellets F05301; Plexx, The Netherlands for BioServ) by being provided with 5 pellets/day for 3 days in their home cages. Thereafter, mice were placed in the chambers with the cue light above the pellet tray remaining illuminated while the two NP ports were covered-up by metal objects. Immediately after placement of the mouse in the operant chamber, 17 food pellets were successively distributed to the tray. This first conditioning session was aimed at habituating the mice to both the operant chamber, the feeder, and the cues indicating pellet distribution. When learning sessions began, the feeder was empty whilst the NP ports were fully operational. During FR1 sessions, a single active NP was sufficient to simultaneously illuminate the cue-lights above the active nose poke port and the feeder and to dispense one pellet. NP in the inactive port were counted but had no effect. The pellet distribution was followed by a 15-s time-out period during which NP activity was ineffectual To compare with operant running experiments, the number of FR1 sessions was fixed to 6, a number sufficient to reach performance stability. After completing the FR1 schedule of reinforcement, mice moved on to the FR3 condition, i.e. mice had to NP 3 consecutive times in the active port to get one food pellet. As above, this experimental condition was repeated over 6 sessions. The day after the last FR3 session, two mouse groups were formed on the basis of similar mean nose poke scores during this last FR3 session. Mouse groups were then injected with 1 mg/kg THC or

its vehicle 30 min before being tested under a linear PR schedule of reinforcement similar to the one described above, except that there were no time limits between steps in keeping with the short (i.e. 30-min) duration of the PR session (Hurel et al., 2019; Muguruza et al., 2019).

2.6. Drugs

SR141716 and JZL184 were from Interchim (Montluçon, France, for Caiman Chemical) whilst THC was from THC-Pharm GmbH (Frankfurt, Germany). SR141716 (3 mg/kg) or its vehicle (DMSO, final concentration: 1.25%) were diluted in one droplet of Tween 80 and then in 0.9% NaCl. THC (0.1-1 mg/kg) or its vehicle (a mixture of ethanol and Cremophor-EL at final concentrations of 5%) were dissolved in 0.9 % NaCl (final concentration of ethanol: 0.395 g/kg). JZL184 (8 mg/kg) or its vehicle (DMSO, final concentration: 10%) were diluted in one droplet of Tween 80 and then in 0.9% NaCl. All volumes of (i.p.) injection were 10 ml/kg.

2.7. Statistics

Data are shown as mean ± SEM with individual values. Because several data sets did not obey normality rules and/or displayed variance heterogeneities, all data were analysed with non-parametric tests. Except for two series of experiments involving multiple THC doses, all data were compared with a Mann–Whitney test (2-group comparisons). Multiple THC doses were compared by means of Kruskal–Wallis analyses of variance. However, these analyses of variance did not prove significant, hence impeding *post hoc* comparisons. All analyses were achieved using GB-Stat software (version 10.0; Dynamic Microsystems Inc., CA, USA).

3. Results

3.1. Effects of THC on wheel-running preference and performance in T-maze tests

We developed a choice procedure wherein two arms contained at their extremities either a free wheel or a locked wheel (Fig. 1A). This design allowed us to measure (i) the initial latency to reach the wheel and run, (ii) running preference (over a locked wheel), and (iii) running performance during 5-min tests. In contrast to operant conditioning procedures in which the role of CB_1 receptors in the control of running motivation has been established (see above), their role in T-maze behaviours remained to be established. In the first series of experiments, we thus assessed which of the above-mentioned running variables were decreased by CB_1 receptor blockade or by genetic deletion of CB_1 receptors. The CB_1 receptor antagonist SR141716, which was administered at a dose (3 mg/kg) devoid of any intrinsic effect on locomotion (as indicated by total exploration scores; Fig. 1B), did not affect the initial latency to run (Fig. 1C) but decreased free wheel preference (Mann-Whitney test: $z = 2.57$, $p = 0.009$; Fig. 1D) and the running duration per sequence (Mann-Whitney test: $z = 2.24$, $p = 0.025$; Fig. 1E). Compared to CB_1 WT mice, mice lacking CB_1 receptors (CB_1 KO mice) showed similar locomotion (Fig. 1F) but were impaired in the initial latency for the first running sequence (Mann-Whitney test: $z = 2.29$, $p = 0.022$; Fig. 1G), in free wheel preference (Mann-Whitney test: $z = 3.05$, $p = 0.002$; Fig. 1H), and in the mean running duration per running sequence (Mann-Whitney test: $z = 2.63$, $p = 0.008$; Fig. 1I).

As opposed to the effects of CB_1 receptor blockade or deletion, nonselective stimulation of these receptors by THC, at doses lacking intrinsic effects on locomotion (Fig. 1J), was ineffective on T-maze variables (Fig. 1 K-M). These results led us to consider the possibility that THC does not affect running preference when intrinsically high, as expected during the dark phase of the light/dark cycle. Thus, we next tested the effects of THC during the light phase, i.e. when running activity and hence preference is the weakest (see Discussion). Testing during the light phase increased the initial latency to run in vehicle-injected mice,

compared to that measured in vehicle-injected mice tested in the dark phase (Mann-Whitney test: z = 2.48, p = 0.013; Fig. 1O). In addition, it decreased the total running duration during the 5-min test (89.2 ± 19.4 s and 38.4 ± 6.5 s in mice tested under the dark and the light phases, respectively; Mann-Whitney test: z = 2.06, p = 0.039). However, contrarily to our expectations, a 1 mg/kg dose of THC still proved ineffective in the T-maze when tested under the light phase (Fig. 1 N-Q).

3.2. Effects of THC on wheel-running motivation

Using a mouse operant procedure wherein nose poke performance temporarily unlocks a running wheel (Fig. 2A), we trained mice under FR1 and FR3 reinforcement schedules (Fig. 2B), and then administered 0.1-1 mg/kg doses of THC 30 min before a PR session. Indeed, none of these doses affected the maximal number of nose pokes performed during that session (as indicated by Kruskal-Wallis analyses of variance; Fig. 2C) and, hence, breakpoint levels (which ranged from 7.6 ± 1.1 to 10.5 ± 1.3 in THC-injected mice, compared to 9.8 ± 1.2 in vehicle-injected mice). This observation extended to running performances, as assessed by the running duration per rewarded sequence (Fig. 2D).

These negative results might be rooted in the inability of our THC treatment protocols to effectively stimulate CB_1 receptors, and hence affect running motivation. We thus tested the impact of a 1 mg/kg dose of THC on motivation for another reward, namely palatable feeding. Accordingly, mice were tested in a cued-feeding instrumental task wherein food-restricted animals had to nose poke under a PR reinforcement schedule to get access to chocolate-flavoured pellets (Fig. 2E). Following efficient training under FR1 and FR3 schedules of reinforcement (Fig. 2F), mice were treated with 1 mg/kg THC before the PR session. This treatment increased the maximal number of nose pokes performed to get access to food pellets (Mann-Whitney test: z = 2.12, p = 0.034; Fig. 2G), leading to an increased breakpoint level (44 ± 2 and 53.7 ± 3.9 in vehicle- and THC-treated mice, respectively; Mann-Whitney test: z = 1.97, p = 0.049). THC-elicited potentiation of feeding

motivation increased food pellet consumption, albeit to a nonsignificant extent (Mann-Whitney test: z = 1.88, p = 0.06; Fig. 2H). These series of experiments thus suggested that the net impact of THC on motivation for a reward was dependent on the type of reward.

3.3. Effects of THC on wheel-running seeking

Cue-induced reinstatement of reward seeking in animals that have extinguished a reward-reinforced task performance (lever pressing, nose poking) allows us to study craving-like behaviour for that reward (Shaham et al., 2003; Venniro et al., 2016). In the present series of experiments, we thus aimed at investigating whether THC affects exercise craving-like behaviour (Fig. 3A). As for T-maze experiments, we first investigated whether wheel-running seeking after extinction of running-reinforced nose poking is controlled by CB_1 receptors. To selectively assess the role of these receptors during the reinstatement step (thus excluding the use of CB_1 KO mice which display decreased operant responses under FR schedules of reinforcement), naive mice were first exposed to FR reinforcement schedules (Fig. 3B) before being exposed to an extinction period of running-reinforced nose poking (Fig. 3C). Thereafter, mice were pretreated with the CB_1 receptor antagonist SR141716 (or its vehicle) before a cue-induced reinstatement session. Pretreatment with this antagonist decreased the number of nose pokes performed during reinstatement of running seeking (Mann-Whitney test: z = 2.78, p = 0.005; Fig. 3D), indicating that it is controlled by CB_1 receptors. Taking advantage of this result, we aimed at further dissecting the relationships between the endocannabinoid system and cue-induced reinstatement of running seeking. Because frontocortical glutamatergic neurones play a key role in cue-induced reinstatement of reward seeking (Gourley and Taylor, 2016; Shaham et al., 2003), we wondered whether these neurones host the CB_1 receptor population controlling running seeking. As shown previously (Muguruza et al., 2019), the primary reinforcing value of wheel-running was not different between mice lacking CB_1 receptors on cortical glutamatergic neurones (Glu-CB_1 KO mice) and their wild-type (Glu-CB_1 WT) littermates (Fig. 3E). Similar genotype-independent

patterns emerged during either the extinction period (Fig. 3F) or a cue-induced reinstatement session (Fig. 3G).

Having established that CB_1 receptors control running seeking (independently of cortical glutamatergic processes), we then tested the effect of a 1 mg/kg dose of THC. Administration of this dose in mice that underwent prior FR training (Fig. 3H) and extinction (Fig. 3I) phases did not change the amplitude of running seeking (Fig. 3J). Taken together, these data indicated that although CB_1 receptors control running seeking, their stimulation by THC does not affect this behaviour.

3.4. Effects of JZL184 on wheel-running motivation and seeking

The above operant conditioning experiments indicated that THC does not stimulate running motivation or running seeking, which both require tonic CB_1 receptor stimulation (see Discussion). In turn, this suggested that the exogenous overstimulation of CB_1 receptors was ineffective on either running variable, hence questioning the generalisation of this ineffectiveness to the endogenous overstimulation of CB_1 receptors. Prior evidence for 2-AG being the endocannabinoid through which CB_1 receptors control reward processes (Covey et al., 2017) led us to examine whether JZL184 boosts running motivation. JZL184 is a selective inhibitor of MAGL (Long et al., 2009), the enzyme that degrades 2-AG molecules at the presynaptic level. Administration of JZL184 thus potentiates 2-AG-elicited stimulation of CB_1 receptors. Mice conditioned to run (Fig. 4A) under FR1 and FR3 reinforcement schedules (Fig. 4B) were thus tested in a PR session 2 h after being administered 8 mg/kg JZL184 (or its vehicle). Indeed, JZL184-treated animals displayed running motivation scores (Fig. 4C) and running performances during each rewarded sequence (Fig. 4D) that were both similar to those measured in vehicle-injected animals. To examine whether MAGL inhibition affected exercise craving-like behaviour, mice that had undergone FR1/3 (Fig. 4E) and extinction (Fig.4F) sessions were administered JZL184 before a cue-induced reinstatement. As for running motivation, MAGL inhibition did not change the intensity of running seeking

(Fig. 4G). These data thus suggested that both running motivation and running seeking were unaffected by the endogenous overstimulation of CB_1 receptors.

4. Discussion

Self-reports suggest that cannabis usage prior to exercise is mainly aimed at increasing exercise pleasure whilst facilitating post-exercise recovery (Gillman et al., 2015; Huestis et al., 2011; Kennedy, 2017; Ware et al., 2018). In some cases, cannabis usage might also increase exercise motivation, and to a lesser extent, performance (YorkWilliams et al., 2019), although these effects might occur in a sport discipline-dependent manner (Lorente et al., 2005). This information, however, relies on survey-based beliefs for which scientific grounds are still lacking. This study has therefore examined in mice the respective impacts of cannabis' main psychoactive ingredient, namely THC, on running performance, preference, and motivation, and extended this investigation to running seeking. Although CB_1 receptors exert a tonic control on running motivation, their stimulation by THC boosted neither running motivation nor running performance. Conversely, THC increased palatable feeding motivation, suggesting that THC might stimulate reward motivation in a reinforcer-dependent manner. The inability of THC to stimulate running motivation extended to exercise craving-like behaviour, as assessed by a cue-induced reinstatement of running seeking. The finding that similar results were observed when 2-AG degradation was impeded suggests that running motivation and performance are insensitive to the acute endogenous/exogenous overstimulation of CB_1 receptors.

In the first series of experiments, we aimed at investigating whether THC affects running preference and performance. To do so, we could have used classical conditioned place preference tests whereby neurobiological bases for wheel-running preference have been established (Fernandes et al., 2015; Lett et al., 2001). However, these tests actually measure after-running, rather than running, preference, and it has been reported that running and after-running might depend on different processes (Belke and Wagner, 2005). This led us to use a different paradigm. We thus developed a T-maze procedure wherein mice could choose between a free running wheel and a locked wheel (in order to control for unspecific reward preferences linked to wheel shape or texture). We first observed that CB_1 receptors

exert a tonic control on running preference and performance. Whether these receptors are those shown to control running motivation under operant conditioning procedures (Muguruza et al., 2019) is presently unknown. In this context, it is relevant to mention that the T-maze has been proposed to provide a motivation index by means of the initial latency to reach the reward (Robinson et al., 2005). This suggestion is supported by the observation that dopamine transients in the nucleus accumbens (to which project VTA dopaminergic neurones) progressively increase with the approach to the reward at the arm extremity (Howes et al., 2013). Our finding that the genetic deletion of CB_1 receptors increased the initial latency to reach the reward therefore might suggest that running motivation, whether measured in the T-maze or under PR reinforcement schedules, is controlled by one unique CB_1 receptor population (located on GABAergic terminals; Muguruza et al., 2019). The additional observation that neither SR141716 pretreatment nor genetic deletion of the CB_1 receptor gene affected total locomotion confirmed our previous suggestion that CB_1 receptor-dependent controls of locomotor and running activities rely on distinct processes (Chaouloff et al., 2011). As opposed to the acute blockade of CB_1 receptors, their acute stimulation by THC failed to affect running preference or running performance. The latter result is in keeping with our previous observation that at doses up to 1 mg/kg THC does not modify free wheel-running performance (Dubreucq et al., 2013). Several explanations might be provided for the inability of THC to affect T-maze behaviours. Besides that based on a balance between rewarding and aversive effects of THC (Han et al., 2017; see below), one possible explanation is that due to the partial agonistic property of THC (Pertwee, 2008), THC can behave as a CB_1 receptor antagonist when this receptor is weakly expressed. However, the observation that SR141716 was effective in the T-maze renders this possibility unlikely. Alternatively, the failure of THC to affect T-maze behaviours could be explained by the inability of the cannabinoid, at the doses used herein, to effectively stimulate CB_1 receptors. Besides previous evidence for 1 mg/kg THC being effective on other CB_1 receptor-dependent functions in mice, including fasting-induced refeeding (Bellocchio et al., 2010) and mediated aversion in reality testing paradigms (Busquets-Garcia et al., 2017), our present observation

that this THC dose increased motivation for palatable feeding (see below) permits us to reject this possibility. Another explanation lies in our experimental conditions. Mice were tested during the dark phase of the light/dark cycle, i.e. when animals are the most active and hence the most motivated for running. Confirmingly, laboratory rodents voluntarily perform most, if not all, of their daily wheel-running activity during the dark phase of the diurnal cycle (see Dubreucq et al., 2013 for an illustration). Indeed, there is evidence for a circadian regulation of mesocorticolimbic VTA dopaminergic neuronal activities (Mendoza and Challet, 2014; Sidor et al., 2015). Accordingly, we could not discard the possibility that running preference, and hence performance, reached their maximal levels when tests were performed, thus impeding stimulatory impacts of THC on these variables. To examine this possibility, we then tested THC effects under the light phase of the light/dark cycle, i.e. when the reinforcing value of wheel-running is at its lowest level. As expected, the initial latencies to reach the free wheel were increased whilst running performances were decreased, compared to the values measured during the dark phase. These differences were not accounted for by putative differences in training efficiencies because mice from both series of experiments were trained under the dark phase, and hence showed similar scores during the training process. THC still proved ineffective on running preference and performance when tested during the light phase, indicating that the inability of THC to boost these variables is independent of baseline reinforcing values of wheel-running. However, we cannot exclude that mice felt the light as stressful, which might have introduced a bias in our analysis of THC effects under low running motivation.

Operant responding for a reward under PR reinforcement schedules allows for a selective estimation of the drive for that reward (Hodos, 1961). By means of this procedure, we have shown that CB_1 receptors present in the VTA are both necessary and sufficient for running motivation (Muguruza et al., 2019). This receptor population, located on GABAergic terminals, is likely the one shown to control running performance under no-cost conditions, i.e. when mice have free access to the wheel (Dubreucq et al., 2013). The finding that running motivation levels, as measured under PR reinforcement schedules, correlate with the

firing rates of VTA dopaminergic neurones (Muguruza et al., 2019) strengthens the hypothesis that CB_1 receptors controlling running motivation are located on GABAergic terminals exerting a tonic inhibitory control of VTA dopaminergic neurones (Covey et al., 2017; Lupica and Riegel, 2005; Melis et al., 2012). Indeed, disinhibition of dopaminergic neurones, as expected from the stimulation of this CB_1 receptor population, generates high-frequency bursts in these neurones (Lobb et al., 2010), hence allowing reward processing (Corre et al., 2018; Van Zessen et al., 2012). Acute THC increases VTA dopaminergic activity and dopamine release at projection sites (Chen et al., 1993, French et al., 1997; Tanda et al., 1997), including in humans (Bossong et al., 2015), and does so likely through VTA CB_1 receptor-expressing GABAergic neurones (Covey et al., 2017; Lupica and Riegel, 2005; but see Good and Lupica, 2010). These data thus strongly suggested that THC might actually amplify running motivation; however, doses up to 1 mg/kg were found to be ineffective. This result could not be explained by the (5 %) ethanol solution in which THC was dissolved as breakpoint levels and running performances were respectively similar in vehicle-injected mice and in mice injected with JZL184 vehicle (which was ethanol-free). Taken with the above mentioned observation that a 1 mg/kg dose of THC increased palatable feeding motivation (in agreement with Barbano et al., 2009), this last result indicates that THC stimulates motivation for one reinforcer but not for another. This differential effect of THC might be accounted for by the findings that running motivation and motivation for palatable feeding are controlled by different CB_1 receptor populations. Thus, whilst CB_1 receptors on GABAergic neurones exert a tight control on running motivation, these receptors are not involved in the CB_1 receptor-mediated control of the motivation for palatable feeding (Muguruza et al., 2019). On the other hand, CB_1 receptors located on cortical glutamatergic neurones lack influence on running motivation (Muguruza et al., 2019) but control in a tonic manner motivation for palatable feeding (Domingo-Rodriguez et al., 2020). These findings, which illustrate how the endocannabinoid system controls motivation in a reward-specific manner, suggest that THC might then preferentially stimulate CB_1 receptors located on cortical glutamatergic neurones when offered palatable food whilst it might preferentially

stimulate CB_1 receptors located on GABAergic neurones when offered wheel-running. In addition to this qualitative (CB_1 receptor population-dependent) control of reward motivation, THC might also exert a quantitative (CB_1 receptor population-dependent) control of reward consumption (and possibly motivation). Thus, mouse fasting-refeeding experiments have indicated that the respective hyperphagic and hypophagic effects of 1 mg/kg and 2.5 mg/kg doses of THC are mediated by distinct CB_1 receptor populations. Thus, THC-induced hyperphagia depends on CB_1 receptors located on glutamatergic neurones whilst the hypophagic effect of THC requires CB_1 receptors located on GABAergic neurones (Bellocchio et al., 2010). Whether this differential control finds its origins at the motivation level remains however to be determined. Another possibility relates to the finding that GABA-mediated reinforcing effects of THC, when present, might be opposed by the aversive consequences of THC stimulation of CB_1 receptors on VTA (Vglut2-expressing) glutamatergic neurones (Han et al., 2017) and/or CB_2 receptors on dopaminergic neurones (Zhang et al., 2014). Although we cannot reject the hypothesis that such opposing actions of THC occur when animals want to run, but not to feed, two observations suggest that this might not be the case. First, THC doses lower than 3 mg/kg did not target CB_1 receptors on VTA glutamatergic neurones in mice (Han et al., 2017). Second, neither a selective CB_2 receptor agonist nor a CB_2 receptor antagonist modified wheel-running performance under no-cost conditions (Dubreucq et al., 2013), suggesting that running motivation and/or intrinsic running performance are insensitive to CB_2 receptor stimulation. One last mechanism that possibly underlies the differential effects of THC on palatable feeding motivation and running motivation involves the use of food restriction, as opposed to *ad libitum* feeding, in palatable feeding tests. Indeed, VTA dopaminergic neurones – through which THC affects reward processes (see above) – are highly sensitive to chronic food restriction. As an example, amphetamine- and cocaine-elicited increases in accumbal extracellular dopamine levels are amplified by chronic food restriction (Cadoni et al., 2003; Rougé-Pont et al., 1995; Stuber et al., 2002). Moreover, the burst firing activity of dopaminergic neurones is increased by prior food restriction (Branch et al., 2013). Apart from intrinsic impacts on VTA dopaminergic

neurones, food restriction also elicits CB_1 receptor-dependent changes in synaptic plasticity (Thoeni et al., 2020), which might have contributed to the aforementioned differential effects of THC on motivation for feeding and running.

There is overwhelming evidence for CB_1 receptors exerting a control on reinstatement of drug-seeking following drug-free periods. For example, SR141716 has been shown to block reinstatement for heroin triggered by a priming injection of the opioid (Fattore et al., 2003). Similarly, CB_1 receptor blockade prevents cocaine-elicited reinstatement of cocaine seeking (De Vries et al., 2001). When reinstatement is promoted by the exposure to the cues paired with reward self-administration, SR141716 decreases reinstatement for drugs such as cocaine (De Vries et al., 2001), methamphetamine (Anggadiredja et al., 2004), heroin, nicotine, and alcohol (De Vries et al., 2005) and for a natural reward such as palatable food (De Vries et al., 2005; Ward et al., 2007). In line with these results, SR141716 decreased cue-induced reinstatement of running seeking, suggesting that CB_1 receptors control this behaviour. However, because we could not test CB_1 receptor knock-out mice due to the lower reinforcing value of wheel-running in these mice, as evidenced under FR reinforcement schedules (Muguruza et al., 2019), it is unknown whether this negative impact of SR141716 was accounted for by its CB1 receptor blocking properties or by its inverse agonist actions at these receptors (Bouaboula et al., 1997). On the other hand, it is unlikely that SR141716 decreased running seeking through its blockade of mu-opioid receptors (Seely et al., 2012) because *in vivo* evidence for such a blockade in mice was based on a high (10 mg/kg) dose of SR141716. In this context, it is relevant to note that naloxone, at a dose (3 mg/kg) decreasing fasting-induced refeeding by more than 60%, failed to alter wheel-running motivation (as assessed under PR reinforcement schedules; unpublished observations), an observation in line with a previous report in rats (Rasmussen and Hillman, 2011). The observation that the CB_1 receptor population controlling cue-induced reinstatement of running seeking is not located on cortical glutamatergic neurones contrasts with the finding that cue-induced reinstatement of cocaine seeking is increased in Glu-CB_1 KO mice compared to Glu-CB_1 WT mice (Martin-Garcia et al., 2016). Because the deletion of CB_1

receptors from GABAergic neurones diminishes motivation for running (Muguruza et al., 2019), but increases that for cocaine (Martin-Garcia et al., 2016), our results reinforce the above-mentioned suggestion that the mechanisms through which the endocannabinoid system controls reward processes are reward-dependent. Studies aimed at examining the effects of THC in reinstatement protocols have provided mixed results. Thus, THC failed to affect drug priming-elicited reinstatement for heroin (Fattore et al., 2003) or for cocaine (Schenk and Partridge, 1999), reduced reinstatement for methamphetamine (but increased that elicited by exposure to the conditioning cues: Anggadiredja et al., 2004), and increased alcohol seeking (McGregor et al., 2005). The present study indicates that THC does not modify wheel-running seeking, as modelled by a cue-induced reinstatement protocol. However, whether a similar result would have been observed if the animals had been primed by a preliminary free access to the wheel remains to be explored.

Taken together, the present observations indicate that acute THC administration does not affect running preference, performance, or motivation, suggesting in a more general manner that CB_1 receptor stimulation does not bear an effect on running. Although there is biochemical (Diez-Alarcia et al., 2016) and behavioural (Panagis et al., 2014) evidence for functional differences between THC and prototypical CB_1 receptor agonists, our results suggest that stimulation of CB_1 receptors cannot boost running when these receptors are already endogenously stimulated by endocannabinoids. If true, it is then expected that overstimulating these receptors, e.g. by blocking endocannabinoid degradation, would be without impact on running variables. In keeping with the key role of 2-AG in the control of VTA dopaminergic activity (and hence reward processes) by the endocannabinoid system (Covey et al., 2018; Oleson et al., 2014; Oleson et al., 2016; but see Wiebelhaus et al., 2015), we thus tested the impact of the MAGL inhibitor JZL184. As observed with THC, a mouse treatment regimen (8 mg/kg, 2 h beforehand) shown to increase tissue 2-AG levels (Busquets-Garcia et al., 2011; Long et al., 2009a) and to increase motivation for alcohol (Gianessi et al., 2020), affected neither running motivation nor running seeking. Our results suggest that potentiating the endocannabinoidergic tone (and hence CB_1 receptor

stimulation) through inhibition of 2-AG degradation does not further increase the drive for running. On the other hand, this raises the possibility that increasing the endocannabinoidergic tone through degradation of the other major endocannabinoid, namely anandamide (AEA), might have led to a different result. This suggestion is at first sight supported by the observation that acute exercise increases circulating levels of AEA, but not 2-AG, in humans (Hillard, 2018). However, except for one study which also observed an increase in AEA levels (Fuss et al., 2015), the other analyses of blood endocannabinoid levels in trained rodents exposed to acute wheel-running sessions did not detect changes in endocannabinoid levels (Chaouloff et al., 2012; Thompson et al., 2017). Moreover, studies aimed at examining the impact of acute wheel-running on brain endocannabinoids failed to detect significant increases (Chaouloff et al., 2012; Fuss et al., 2015; Thompson et al., 2017), a result which might be accounted for by the time lag between brain sampling and exercise onset and/or the likeliness that changes in endocannabinoid release with exercise are too discrete with regard to their location to be observed in gross tissue samples. Although we cannot discard the possibility that increasing AEA levels or both AEA and 2-AG levels might boost running motivation and/or seeking, the following observations are noteworthy. First, systemic administration of a dual inhibitor of MAGL and of the AEA-degrading enzyme, fatty acid amide hydrolase (FAAH), namely JZL195 (Long et al., 2009b), decreased wheel-running performance (Dubreucq et al., 2013). When locally perfused in the VTA, JZL195 lacked impact on wheel-running performance (Dubreucq et al., 2013). Lastly, administration of URB597, a selective FAAH inhibitor (Kathuria et al., 2003), using an effective protocol in mice (1-3 mg/kg, 1 h beforehand; Busquets-Garcia et al., 2011) also failed to alter wheel-running performance (unpublished observations). Although these observations might suggest that inhibition of AEA degradation does not boost running motivation, a direct examination of this suggestion will require JZL195- and/or URB597-treated mice exposed to PR reinforcement schedules.

Although this study is the first to dissect the relationship between THC and running drive, its relevance to human exercise is hampered by several limitations. Firstly, we exclusively

used THC even though cannabis is composed of hundreds of ingredients, including cannabidiol (CBD), which shares anxiolytic and analgesic properties with THC and modulates the negative impacts of THC (Curran et al., 2016; Elsaid et al., 2019). Indeed, while acute THC administration to humans decreases motivation – as assessed by an effort task – to earn money, this amotivation effect is buffered when CBD is added to THC (Lawn et al., 2016). Moreover, it is the combination of THC and CBD, as compared to either compound alone, that is the most frequently linked to well-being effects in sport (Zeiger et al., 2019). Although these studies illustrate the need to include CBD with THC in animal studies aimed at deciphering the effects of human cannabis, it is noteworthy that the THC content of street cannabis has recently increased at the expense of CBD content. Because THC mediates the rewarding value of cannabis (Curran et al., 2016) and hence its addictogenic properties, it is thus likely that THC is the main cannabinoid that mediates cannabis usage by sportspeople. The second limit lies in the acute use of THC in animals never exposed to the cannabinoid beforehand. This contrasts with the human situation wherein sportspeople using cannabis before and/or after exercise are chronic cannabis consumers. One consequence of such a chronic usage is the observation that exercise increases THC circulating levels (Wong et al., 2013) following THC long-term storage in, and release from, fat tissues (Kreuz and Axelrod, 1973). Accordingly, the kinetics of THC entry into the brain should differ from those triggered by its acute administration in naive individuals, with possible impacts on running. Another limit of the acute use of THC relates to the intrinsic impact of prior chronic cannabis/THC ingestion on motivation processes. As indicated above, chronic cannabis usage leads to amotivation, in line with the negative effects of chronic use on mesocorticolimbic dopaminergic activity (Bloomfield et al., 2016). However, this might not include motivation for exercise owing to the significant number of cannabis users who are regular exercisers. The paucity of animal data on that issue does not help to solve this question as, to our knowledge, only three studies examined the consequences of repeated THC administration on wheel-running. In fact, different doses of THC proved ineffective on wheel-running performance in fed rats (Scherma et al., 2017). Moreover, repeated THC

treatment to food-restricted animals increased food intake whilst decreasing (Verty et al., 2011) or not effecting (Lewis and Brett, 2010) wheel-running performance. Taken together, these data suggest that repeated THC administration does not boost running performance.

One last limit of the present study is linked to our noncontingent THC administration protocol. Indeed, self-administration of drugs associated with specific cues and contexts is more relevant to human drug usage. For example, cued-cocaine self-administration has longer-lasting synaptic impacts on VTA dopaminergic neurones, compared with noncontingent cocaine administration (Chen et al., 2008). This is also true for wheel-running as the amplitude of the acute running-elicited potentiation of excitatory inputs to VTA dopaminergic neurones is higher when running is cued, compared to free running (Medrano et al., in press). However, although THC self-administration is observed in monkeys (Justinova et al., 2005), this procedure has proven to be difficult to introduce in laboratory rodents (but see: Melis et al., 2017; Smoker et al., 2019; Spencer et al., 2018; Zangen et al., 2006). This difficulty is mainly due to the poor reinforcing properties of THC in these species (Panagis et al., 2004) and the main use of the intravenous, as opposed to the inhalation, route of administration (Melis et al., 2017). Recently, a study reported the successful development of a cued-THC (or CBD) self-administration procedure wherein rats are willing to exert effort to inhale either of these cannabis ingredients under FR and PR reinforcement schedules (Freels et al., 2020). The use of this paradigm should thus prove useful to dissect the relationships between the respective drives for cannabis and exercise.

5. Conclusion

This study is the first to examine the consequences of acute THC administration on running preference and performance in a T-maze task, and on running motivation in a cued-running instrumental task. Although running preference and motivation are tonically controlled by CB_1 receptors, THC proved ineffective on these two variables. This ineffectiveness contrasted with the stimulating impact of THC on palatable feeding

motivation. Lastly, THC also proved unable to affect cue-induced reinstatement of running seeking. Future works using chronic THC treatment regimens with or without other cannabis ingredients such as CBD should help define cannabis' effects in human sportspeople.

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Ethical statement

Animal procedures, which complied with the French (Décret 2013-118) and European (2010/63/EU) rules on animal experimentation, were approved by the local Ethic Committee (Comité d'Ethique 50) with agreement numbers 33-063-69 and 22435 (F.C.) and A33-063-098 (animal facilities) provided under authority of the Préfecture de Gironde and the French Ministry of Agriculture.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Authors contribution

I.H., C.M., B.R., G.M., and F.C. contributed to the conception and design of the study, I.H., C.M., B.R., and F.C. participated in acquisition and analyses of the data, F.C. drafted the article, I.H., C.M., B.R., G.M., and F.C. revised the article and approved its final version.

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Legends to the Figures

Fig. 1. Wheel-running preference and performance are insensitive to THC. (A) T-maze set-up with free and locked running wheels at arm extremities. Except for one series of experiments (N-Q), all tests were ran during the dark phase of the light/dark cycle. Acute CB_1 receptor blockade by SR141716 (3 mg/kg; n = 6) 30 min beforehand affects neither total locomotion (B) nor the initial latency to run (C) but reduces free wheel preference (D) and running duration per running sequence (E), compared to its vehicle (n = 6). Total locomotion is similar in mice with a genetic deletion of CB_1 receptors (CB_1 KO; n = 15), compared to their wild-type (CB_1 WT; n = 19) littermates (F). CB_1 KO animals display an increased initial latency to run (G), decreased wheel preference (H), and decreased running performance per running sequence (I), compared to CB_1 WT mice. Administration of 0.1 or 1 mg/kg THC (n = 8 per dose) 30 min beforehand does not affect T-maze behaviours, compared to vehicle treatment (n = 8; J-M). Administration of 1 mg/kg THC (n = 9) during the light phase of the light/dark cycle does not alter T-maze behaviours, compared to vehicle administration (n = 11; N-Q). All data are shown as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ (Mann-Whitney tests).

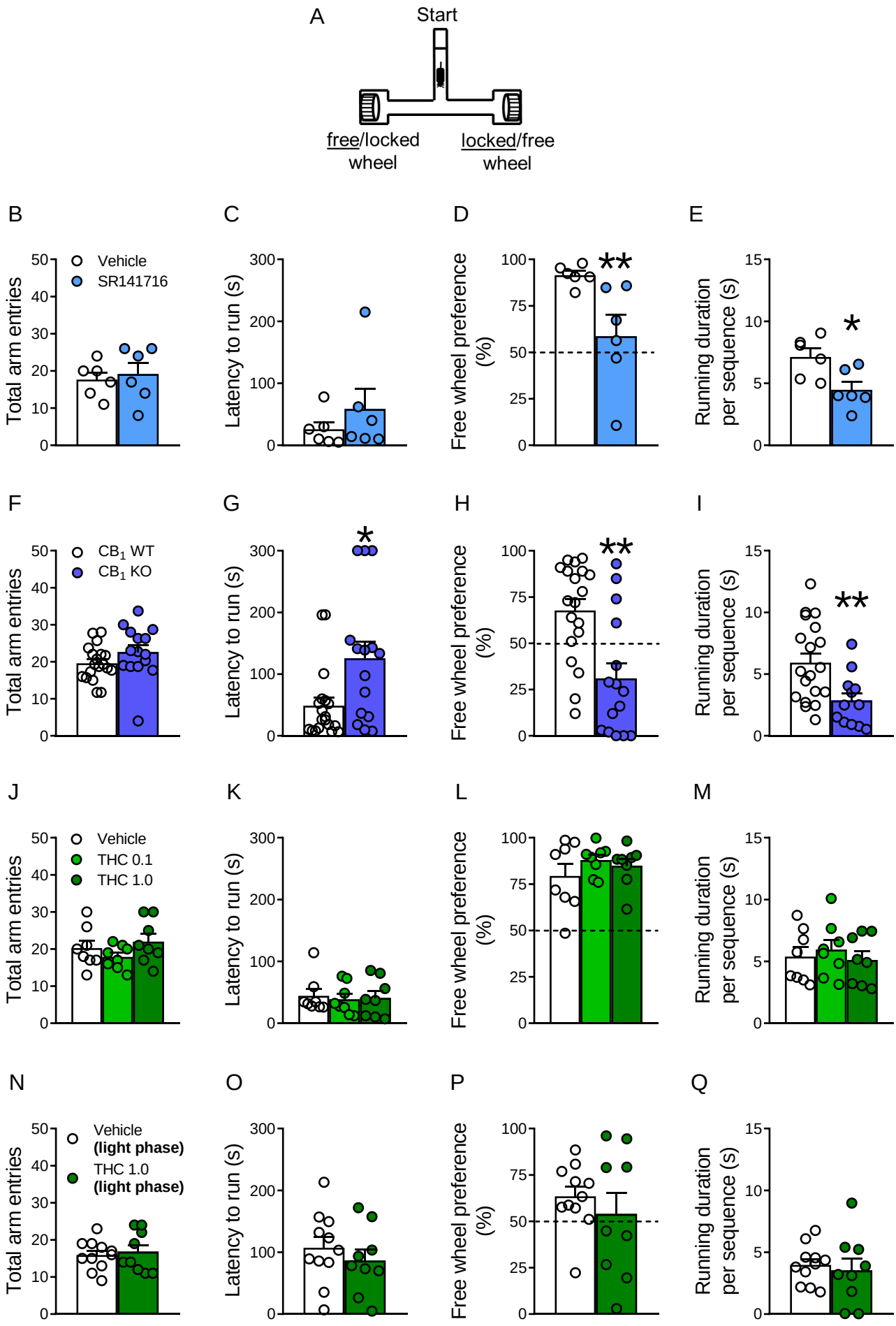
Fig. 2. THC boosts motivation for palatable feeding, but not for wheel-running. (A) Operant chamber set-up for the study of wheel-running motivation. (B) Performances of active and inactive nose pokes during the conditioning phase of wheel-running (12 sessions) under FR1 and FR3 schedules of reinforcement (n = 41). (C and D) Administration of 0.1-1 mg/kg doses of THC (n = 10-11 per dose) 30 min beforehand does not affect either the maximal number of nose pokes performed (C) or the running duration per rewarded sequence (D) under a PR reinforcement schedule. (E) Operant chamber set-up for the study of palatable food motivation. (F) Performances of active and inactive nose pokes during the conditioning phase of feeding (12 sessions) under FR1 and FR3 schedules of reinforcement (n = 18). (G

and H) Administration of 1 mg/kg THC (n = 10) 30 min beforehand increases the maximal number of nose pokes performed (G) but not the number of food pellets consumed (D) under a PR reinforcement schedule, compared to vehicle (n = 8). All data are shown as mean ± SEM. * $p < 0.05$ (Mann-Whitney tests).

Fig. 3. Cue-induced reinstatement of wheel-running seeking is insensitive to THC. (A) Operant chamber set-up for the study of wheel-running seeking. (B and C) Active and inactive nose pokes for wheel-running under FR1 and FR3 schedules of reinforcement (B) and during extinction sessions (n = 19; C). (D) Pretreatment with the CB_1 receptor antagonist SR141716 (3 mg/kg; n = 9) 30 min beforehand decreases active nose poke performance during a cue-induced reinstatement session, compared with its vehicle (n = 10). (E and F) Active and inactive nose pokes for wheel-running under FR1 and FR3 schedules of reinforcement (E) and during extinction sessions (F) in mice lacking CB_1 receptors on cortical glutamatergic neurones (Glu-CB_1 KO; n = 16) and in their wild-type (Glu-CB_1 WT; n = 17) littermates. (G) Cue-induced reinstatement of wheel-running seeking is not different between Glu-CB_1 KO and Glu-CB_1 WT mice. (H and I) Active and inactive nose pokes for wheel-running under FR1 and FR3 schedules of reinforcement (H) and during extinction sessions (n = 27; I). (J) Active nose poke performance during a cue-induced reinstatement session is insensitive to a pretreatment 30 min beforehand with a 1 mg/kg dose of THC (n = 13), compared to vehicle pretreatment (n = 14). All data are shown as mean ± SEM. ** $p < 0.01$ (Mann-Whitney tests).

Fig. 4. Wheel-running motivation and seeking are insensitive to MAGL inhibition. (A) Operant chamber set-up for the study of running motivation and craving-like behaviour. (B) Performances of active and inactive nose pokes during the conditioning phase of wheel-running (12 sessions) under FR1 and FR3 schedules of reinforcement (n = 24). (C and D) Administration of 8 mg/kg JZL184 (n = 12) 2 h beforehand does not change the maximal number of nose pokes performed (C) or the running duration per rewarded sequence (D)

under a PR reinforcement schedule, compared to vehicle administration (n = 12). (E and F) Active and inactive nose pokes for wheel-running under FR1 and FR3 schedules of reinforcement (E) and during extinction sessions (n = 32; F). (G) Active nose poke performance during a cue-induced reinstatement session is insensitive to a pretreatment 2 h beforehand with an 8 mg/kg dose of JZL184 (n = 16), compared to vehicle pretreatment (n = 16). All data are shown as mean ± SEM.


A
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free/locked wheel
locked/free wheel
B
C
D
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Vehicle
SR141716
Latency to run (s)
Free wheel preference (%)
Running duration per sequence (s)
F
G
H
I
CB1 WT
CB1 KO
J
K
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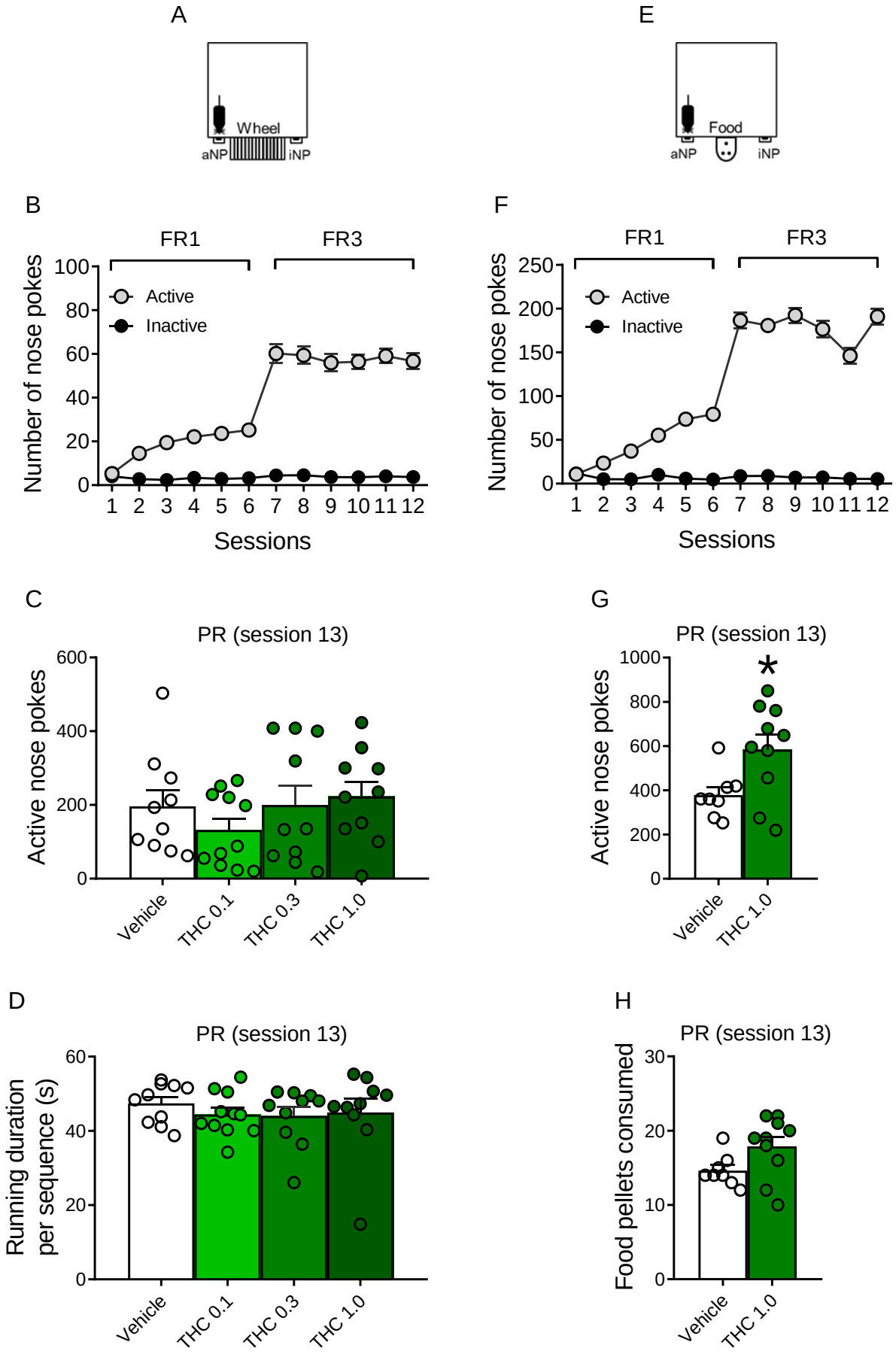
Vehicle (light phase)
THC 1.0 (light phase)

Figure 1


A
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Food
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Number of nose pokes
Active
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Sessions
F
FR1
FR3
Number of nose pokes
Active
Inactive
Sessions
C
PR (session 13)
Active nose pokes
Vehicle
THC 0.1
THC 0.3
THC 1.0
G
PR (session 13)
Active nose pokes
*
Vehicle
THC 1.0
D
PR (session 13)
Running duration per sequence (s)
Vehicle
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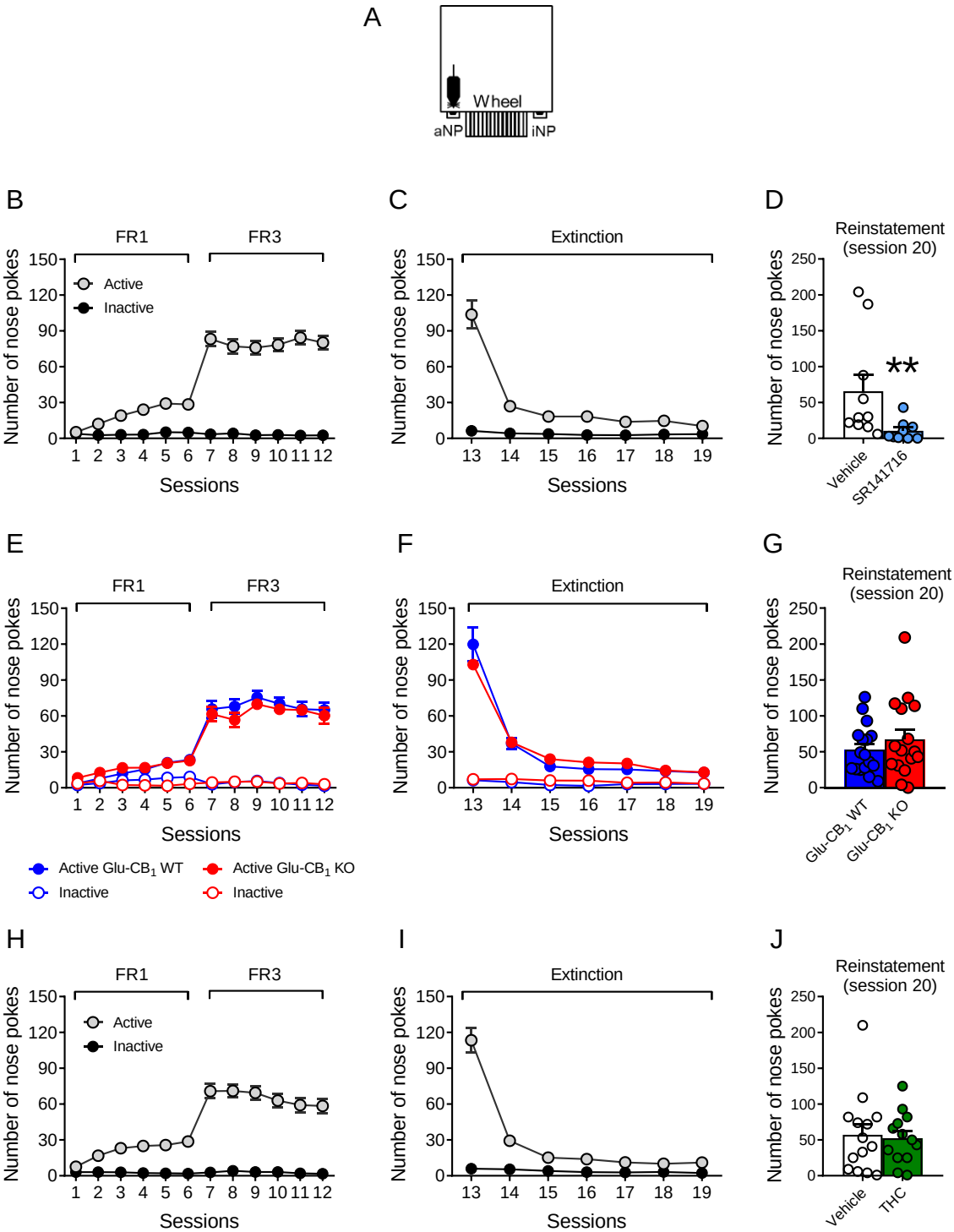
THC 0.3
THC 1.0
H
PR (session 13)
Food pellets consumed
Vehicle
THC 1.0

Figure 2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D
Reinstatement
(session 20)
Number of nose pokes
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Vehicle
SR141716
E
FR1
FR3
Number of nose pokes
Sessions
Active Glu-CB1 WT
Inactive
Active Glu-CB1 KO
Inactive
F
Extinction
Number of nose pokes
Sessions
G
Reinstatement
(session 20)
Number of nose pokes
Glu-CB1 WT
Glu-CB1 KO
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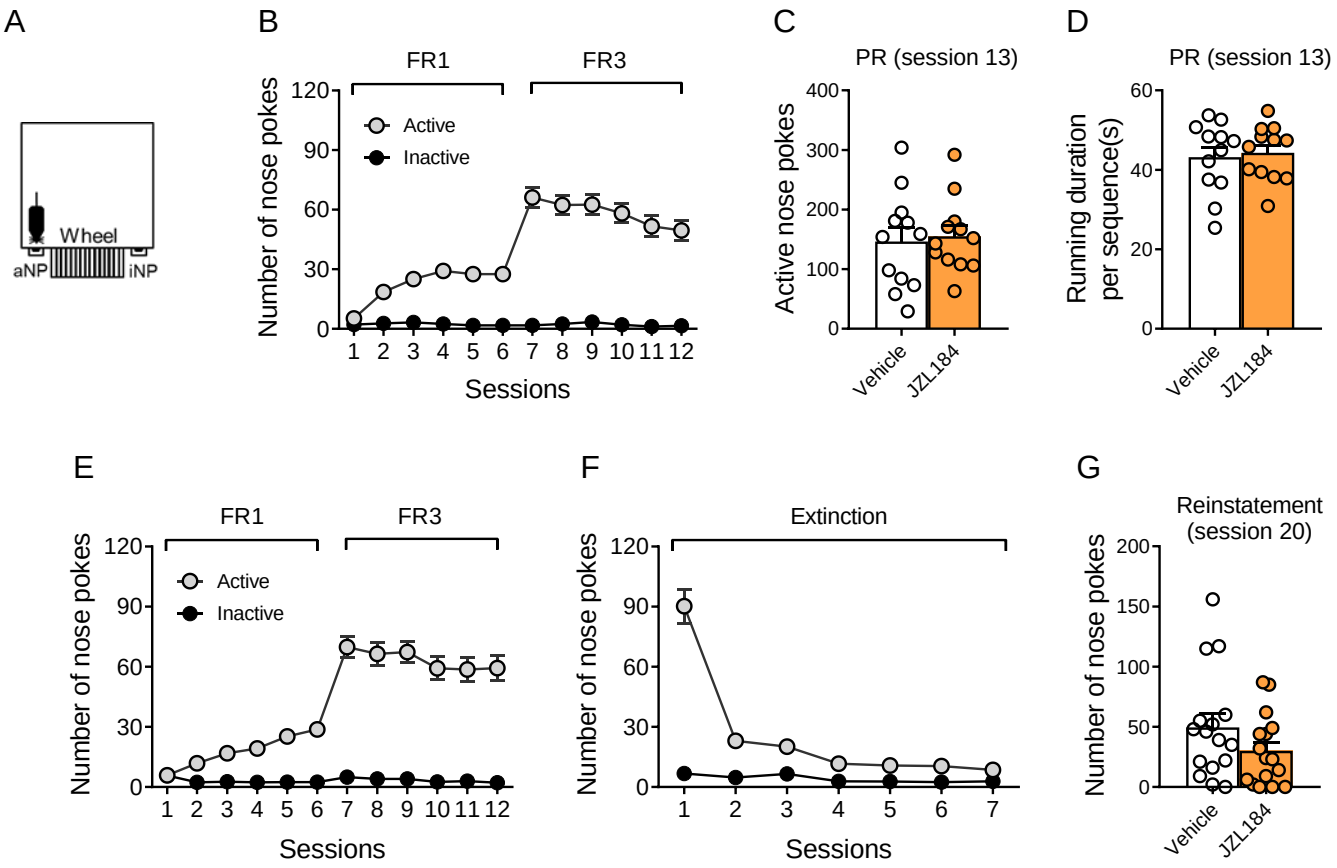
Reinstatement
(session 20)
Number of nose pokes
Vehicle
THC

Figure 3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Number of nose pokes
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PR (session 13)
Active nose pokes
Vehicle
JZL184
D
PR (session 13)
Running duration per sequence(s)
Vehicle
JZL184
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FR3
Active
Inactive
Number of nose pokes
Sessions
F
Extinction
Number of nose pokes
Sessions
G
Reinstatement (session 20)
Number of nose pokes
Vehicle
JZL184

Figure 4