Personality may influence behavioral response to cannabis

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ABSTRACT: Effects of cannabis reported by users range from experiences of euphoria and anxiolytic effects to paranoia, anxiety, and increased risk of depression. Previous studies attempting to reconcile the apparent contradictions in user response have not been conclusive. Here, we utilized selectively-bred mice with dominant (Dom) and submissive (Sub) behavioral patterns exhibiting resilience and sensitivity to stress, respectively to elucidate this contradiction. Following short-term, repeated treatment with cannabis oil containing the plant's main psychoactive component delta-9-tetrahydrocannabinol (THC) at two different doses (1.5 mg/kg and 15 mg/kg), Sub mice presented significant place aversion in a conditioned place preference paradigm at a higher dose, whereas Dom mice displayed no place preference or aversion. After 6-week washout period, mice were subjected to several stressor tests. In forced swim test Sub mice displayed a reduction of depressive-like behavior after administration of the lower dose whereas those that got higher dose showed similar levels of depressive-like behavior to the naïve animals. Despite the lack of place preference/aversion, Dom animals receiving 15 mg/kg THC displayed depressive-like behavior similar to the socially-submissive, stress-prone mice. Interestingly, serum corticosterone levels were elevated at the 15 mg/kg dose regardless of the population tested. We conclude here that differences in dominance behavior and stress vulnerability are involved in regulation of cannabis response among users and should be considered when prescribing THC-containing medications to patients.

Introduction:

Cannabis is the most widely-used drug worldwide with an estimated 183 million users, which equates to 3.8% of adults aged 15–64 years (1). Several factors have contributed recently to an improved epidemiological profile of cannabis use. Increase of cannabis use for medical purposes (2-8), higher public levels of interest in cannabis as a research topic (9), and increased research accumulation of scientific data have shifted the public perception of the drug from negative to relatively positive viewpoint. Collectively, this has resulted in growth in the number of recreational users and greater volume of legally reportable, real-world data.

Although cannabis is commonly regarded as an anxiolytic, a broader review of the medical literature attests to a range of both positive and negative responses to cannabis intoxication. For example, many users experience anxiety and paranoia (10-12) , and some studies have also reported an increased risk of developing depression in patients with cannabis chronic use disorder (12-16). Despite attempts to model causative effects of cannabis towards development of anxiety, stress-vulnerability, and depression, no conclusive evidence has emerged to explain the general observation of increased anxiety disorders among cannabis users (10, 17). We postulate that variations in cannabis response reside in differences in personality type, particularly that of stress-resilient and stress-vulnerable populations. To address this issue, we used two strains of mice with markedly differing sensitivities to stress and social dominance behavior, which we attribute to representing personality-like features.

The idea of animal personality, temperament or disposition traits has been extensively investigated (18, 19) and is now considered a valid concept in explaining the inter-individual differences in behavioral response. Behavioral responses to stimuli have been demonstrated to be heritable (20) and also consistent throughout the lifetime of indivuals (19). In the present study, we used

selectively-bred mice that represent opposite extremes of the behavioral spectrum of dominance and submissivness and treated them with cannabis oil, a natural product mixture of cannabinoids with high level of delta-9-tetrahydrocannabinol (THC) to mimick user exposure. Dominant (Dom) and submissive (Sub) mice simulate different types of animal personalities, with Doms displaying elements of manic phenotype and Subs showing depressive-like behaviors (21). In addition, by using different behavioral paradigms, it was shown that Dom and Sub mice exhibit resilience or sensitivity to stress, respectively (22-25). The results we present here suggest that the reaction to stress may dictate the response of individuals to cannabinoids with regards to development of addictive behaviors.

Materials and methods

1. Animal model

Mice were selectively-bred from stock Sabra mice (Harlan Laboratories, Jerusalem, Israel) over 30 generations using a social behavioral paradigm (DSR, see below) which resulted in two animal strains distinct in several measures of social interaction and resource competition: named dominant (Dom) and submissive (Sub) mouse strains (21, 22, 26). Animals were housed in a colony room (12:12 L:D cycle with lights on 07:00–19:00 hrs., 25±2°C, ambient humidity) in groups of five per cage and provided with standard laboratory chow and water, ad libitum. The experiments were conducted in accordance with NIH/USDA guidelines, under the approval of the Ariel University Institutional Animal Care and Use Committee.

2. Test substance

Medical cannabis oil (PharmoCann Ltd, Israel; 25 mg THC/g cannabis oil) was given in doses of 15 mg/kg or 1.5 mg/kg THC i.p.

3. Behavioral experiments:

The assessment and treatment scheme for all groups is summarized in Fig. 1. Following verification of Dom or Sub social behaviors (DSR, see below), mice were subjected to conditioned place preference testing (CPP, see below) with cannabis oil at either low (1.5 mg/kg) or high (15 mg/kg) doses. Following CPP, mice were allowed a 1.5-month wash-out period prior to additional behavioral testing (see RS, TST, and FST below). This was done to ensure that mice were not intoxicated during assessments and that all behavioral responses were the result of stable neurological changes.

Fig. 1. *Behavioral assessment and treatment scheme. (RS: restraint stress; TST: tail-suspension test; FST: forced swim test)*

3.1 Dominant-Submissive Relationship (DSR) Test

The DSR test was used for verifying Dom and Sub strain-specific behaviors as part of selection and colony breeding maintenance. The DSR arena consisted of two identical chambers (l x w x h; 12 x 8.5 x 7 cm) joined by a central, connecting tunnel (27 x 2.5 x 2.5 cm). The competition food target (aqueous solution of 3% milk and 10% sugar) was presented at the tunnel center through a self-refilling well with a small access point to allow feeding by a one mouse in a time only. The end chambers were bordered by removable panels to restrict access to the connecting tunnel and food source until beginning of the test. DSR was conducted for 2 weeks (5 consecutive days/week) with fixed pairs of Dom and Sub mice.

Each testing day, mice were restricted from laboratory chow for 14 hrs., with water provided ad libitum. Dom and Sub mice pairs (6-weeks-old, same gender, each strain) were arranged with individuals of similar weight and placed to the arena for 5 min. Milk drinking time of each animal was manually recorded (21).

3.2 Condition Place Preference (CPP)

A conditioned place preference paradigm was used to measure addictive-like behavior (27) by employing an apparatus which consisted of a plastic box divided into two compartments (l x w x h, 17 x 15 x 37 cm; one with black and white vertical striped walls, one with black walls) with a central grey separation section (9 x 15 x 37 cm).

Compartments were separated by removable dividers. On day one, mice were assessed for 20 min. without chamber dividers to determine their naïve preference to chamber color and location. On days 2-5 (training days), mice were injected (i.p.) twice by: a) treatment with vehicle and

113 placement in the closed, preferred outer compartment for 20 minutes (morning session), followed
114 by b) treatment with cannabis oil and placement in the closed, non-preferred outer compartment for
115 20 minutes (afternoon session). Time between sessions was 4 hours.

116 On day 6, (assessment day) mice were placed in the closed central compartment, without
117 dividers, and dwelling time was recorded for each chamber using an EthoVision 3.1 (Noldus,
118 Holland) system.

119

120 **3.3 Restraint Stress (RS)**

121 Mice were exposed to a restraint stress protocol enabling the differentiation of stress effects upon
122 Dom and Sub mice. Mice of each phenotype (Sub, Dom) underwent restraint stress for 1 hr. using
123 a restriction sleeve, which permitted ease of breathing, but restricted limb movement.

124

125 **3.4 Tail Suspension Test (TST)**

126 The TST is a primary screening test for effects of anti-depressant drugs, which reduce the tail
127 suspension-induced immobility of mice, similar to that observed in the FST . In comparison with
128 the FST, the TST has the advantages of negating the ability of mice to use their natural buoyancy
129 and is considered to be less stress-inducing to the animals (28). Using a sponge-padded clothespin,
130 animals were suspended by their tails for 6 min. from a square stand 30 cm above the table surface.
131 Immobility was recorded manually.

132

133 **3.5 Forced Swim Test (FST)**

134 FST is an acute environmental stressor (22, 29, 30), which measures time mice spend immobile
135 (non-swim time) following immersion in deep water and is meant to reflect behavioral the despair

characteristic of depressed individuals (31, 32). Mice were placed individually into a glass cylinder (30 x 10 cm) filled 25 cm high with water (25 ± 2°C) for 6 min and immobility time was recorded. Mice were removed from the cylinder at 6 min. or earlier if they failed to remain above the water surface. The mice were then dried with paper towels, warmed under a lamp for 10 min, and returned to home cages. In this test, Sub mice display a native, depressive-like phenotype (high immobility time), whereas Dom animals were stress-resistant to this challenge (low immobility time) (22, 29).

3.6 ELISA-based serum corticosterone assay

Blood corticosterone (CORT) levels were measured in serum samples collected from trunk blood taken immediately following euthanasia. Samples were centrifuged at 3500 g for 7 min (24) and supernatants were stored on ice (4°C) for 1 hr. CORT levels were assessed using a commercial ELISA kit (MS E-5400 LDN, Nordhorn, Germany). Mice were gently removed from their home cages with minimal handling prior to blood collection to minimize handling stress. Blood collection was within 2 min. from cage removal.

4. Statistical analysis.

Multiple comparisons were performed by one-way ANOVA without matching (with Bonferroni correction for means). Means separation test for multiple comparisons were conducted with a Tukey test. Unpaired Student t-test was used for discrete comparisons. Threshold for significance was $\alpha=0.05$.

Results

158 Dom and Sub mice differentially responded to cannabis oil exposure in the CPP test. No stress- or
159 drug-induced place preference was noted in Doms or Subs injected with 1.5 mg/kg cannabis oil
160 (Fig. 2A). Dom mice injected with 15 mg/kg cannabis oil also exhibited no place preference. In
161 contrast, their counterparts developed strong aversion to the drug side, which indicates that the
162 response to cannabis may be dose-dependent by personality type or stress sensitivity (Fig. 2B).
163 Surprisingly, acute stress did not influence the development of place preference/aversion.
164
165 ***Fig. 2.*** *Condition Place Preference (CPP) drug-paired delta time (n=5 per treatment).* ***A:*** *No place*
166 *preference in Sub and Dom groups injected with 1.5 mg/kg dose both before (No Stress) and after*
167 *(Stress) acute stress.* ***B:*** *Sub mice, but not Dom mice, injected with 15 mg/kg dose developed strong*
168 *aversion to drug both before (No Stress: unpaired t(5)=4.212, *p=0.0084) and after (Stress:*
169 *unpaired t(5)=3.277, *p=0.0220) acute stress. Data are presented as delta ± SEM.*
170
171 Results from FST demonstrated that immobility time was markedly higher in Dom mice
172 treated with 15 mg/kg cannabis oil than in 1.5 mg/kg cannabis oil ($p<0.001$; Fig. 3) or naïve Dom
173 groups, suggesting that the high cannabis dose increases depressive-like behavior in socially-
174 dominant individuals. However, no significant difference was observed between the 15 mg/kg Dom
175 group and 15 mg/kg, 1.5 mg/kg and naïve Sub group. Results of TST did not indicate any treatment
176 effects at the doses of cannabis oil given here (data not shown).
177
178 ***Figure 3.*** *Acute effect of Forced Swim Test (FST) with total immobility time (seconds) in cannabis*
179 *oil-injected mice compared with Naïve groups. Within-strain effects analyzed by one-way ANOVA:*
180 *Sub: F[2,12]=7.18, p=0.0089; Dom: F[2,9]=17.53, p=0.0008. Letters represent results of Tukey*
181 *means separation test within mouse strain. Naïve Sub vs. Naïve Dom: unpaired t(7)=8.437,*

p<0.0001; 1.5 mg/kg Sub vs. 1.5 mg/kg Dom: unpaired t(8)=3.501, p=0.0081. Data are presented as delta ± SEM.

To better understand the observed behavioral changes after cannabis oil injections, we measured the effect of low and high dose treatments on hypothalamic-pituitary-adrenal (HPA) axis activity by measuring the serum corticosterone (CORT) levels. CORT levels were not altered in Dom and Sub mice after injection of 1.5 mg/kg cannabis oil as compared with naïve mice. However, injection of the higher, 15 mg/kg cannabis oil dose resulted in significant elevation of CORT levels both in Dom and Sub groups (Fig. 4).

Figure 4. *Serum CORT levels following acute stress and second CPP test. One-way ANOVA with Bonferroni means-separation test. Data are presented as mean ± SEM. Within-strain effects analyzed by one-way ANOVA: Sub: F[2,10]=6.242, p=0.0174; Dom: F[2,9]=7.993, p=0.0101. Tukey means separation test within mouse strain: *, p<0.05.*

Discussion

In this study, we measured long-term outcomes of cannabis oil exposure on Dom and Sub mice to examine the effect of personality and stress on addictive-like behavior. Our previous studies with Dom and Sub mice have established marked differences between strains in response to various stressors (22-25). Submissive behavior has been linked to increased susceptibility to stress and, presumably, addictive behaviors, whereas resiliency to stress may be associated with dominant personalities (22, 25). Previous work with Dom and Sub mice demonstrates that these animals react differentially to stressogenic factors, antidepressants, and mood stabilizing agents (22, 23, 33, 34).

In addition, the two strains exhibit differing aging-related cognitive impairments and demonstrate significant differences in short- and long-term synaptic plasticity (35). Here, we show that Dom (stress-resilient) and Sub (stress-sensitive) mice react differently following exposure to a high and low doses of cannabis, implicating the role of social dominance behaviors and/or stress sensitivity in the response to drug exposure.

According to recent studies, chronic use of medical cannabis can lead to various neurological adverse effects depending on the dose of THC and THC-like cannabinoids (36). The list of neurological symptoms observed after chronic THC exposure is wide and includes seizures, epileptic seizures, headache – the same symptoms that medical cannabis is alleged to cure (36). Moreover, not only chronic, but acute administration of THC may lead to various psychiatric experiences including anxiety, transient hallucinatory and delusional experiences (37-41). In one of the first human studies, D'souza et al. (2004) (42) administrated intravenous THC in 2 doses (2.5 mg and 5 mg) to 22 healthy adults in a double-blind, placebo-controlled design. They found that THC induced a psychosis-like experience including symptoms such as: perceptual alterations, anxiety, euphoria, and attention difficulties. In a similar study, Morrison et al. (2009) reported similar effects produced by lower dose of THC (43).

Recent studies also revealed various long-term negative effects of cannabis on mental health including impairment of attention, psychomotor task ability, short-term memory, increased risk of psychoses, depression, and anxiety disorders (44-48). Our FST results in mice are in agreement with the depression and anxiety aspects observed in human studies. We showed here that mouse behavioral patterns were highly impacted by cannabis exposure and the effect was long-term and manifested even after a long period of wash-out. As expected, comparisons with naïve mice showed significantly elevated levels of immobility in Sub mice, indicating more prominent depressive-like

behavior. This result is consistent with our previous studies and is considered a marker associated with dominant and submissive behavior (22). Depressive-like behavior was reduced after administration of lower dose of cannabis in Subs, demonstrating drug stress-relieving and relaxing properties. The lack of significance in Dom naïve vs Dom 1.5 groups can be explained by general very low levels of immobility in Dom naïve group. However, the most prominent response to cannabis was observed specifically in 15 mg/kg Dom group where the immobility time reached the level of Sub mice, indicating development of depressive-like behavior in Dom individuals despite Dom mice displaying no place preference response to drug as shown in CPP test. These findings are in a good agreement with human studies showing that THC affects individuals differently: some individuals experience relaxation, whereas others develop psychotic states (49, 50).

We postulated that differences in behavioral patterns of Dom and Sub mice after cannabis oil injections may reside in their differing sensitivities to stress, therefore we measured the levels of serum CORT, an indicator of HPA-axis activity. The relationship between elevated serum cortisol and depressive behavior has been established in several studies: Johnson et al. (51) demonstrated that repeated injections of CORT increase depressive-like behavior in rats; in human studies, cortisol levels were also elevated in response to THC administration, which presumably could result in depressive behaviors in sensitive individuals. In our study, CORT levels were elevated both in Doms and Subs after exposure to 15 mg/kg cannabis oil, yet were unchanged in 1.5 mg/kg group, indicating that higher doses of cannabis may contribute to the development of depressive-like behavior observed in FST. Stimulation of HPA-axis by cannabis oil and release of CORT occurs presumably via brain cannabinoid (CB-1) receptors located in the brainstem, namely the locus coeruleus (LC) and the nucleus of the solitary tract (NTS). Activation of CB-1 receptors by cannabinoids in these regions may modulate noradrenergic activity, resulting in norepinephrine

251 release that has long been known to play a prominent excitatory role in the regulation of the HPA
252 axis. This in turn leads to elevated activity of neurons releasing corticotropin-releasing hormone
253 and, hence, elevated corticotropin levels. Furthermore, THC may directly activate paraventricular
254 nuclei of the hypothalamus where CB-1 receptors and CRH mRNA are co-expressed (52).

255 Another experiment conducted in the frame of the current study also revealed personality-
256 based alterations in response to cannabis. Using the CPP paradigm, we demonstrated that Sub, but
257 not Dom mice, developed strong aversion to cannabis exposure. CPP tests may demonstrate either
258 place preference or aversion after cannabinoid exposure in test animals, which depends on
259 administered drug dosage (53-57). . Development of aversion to THC in mice may indicate that
260 endogenous cannabinoids are involved in activation of counter-reward pathways that trigger
261 aversion and anxiety. For instance, Bhattacharyya et al. confirmed the anxiogenic role of
262 cannabinoids that was mediated by the modulation of amygdala function through CB-1 receptors
263 (58). Contrary to our expectations, no effect of acute stress was observed following cannabinoid
264 treatment in both mice groups. One option explaining this phenomenon is that acute stress is not
265 sufficient to affect pathways regulating the development of place preference/aversion in mice after
266 drug exposure, whereas we employed three acute stressors (RS, TST and FST).

267 Herein, we propose that individual behavioral traits or "personality" are involved in regulation
268 of the response to cannabis exposure, mediated by the balance between activation of reward/counter-
269 reward pathways. We also suggest that there are two independent pathways - predisposing (Sub
270 mice) and evoked (Dom mice) – that trigger the manifestation of depressive-like behavior.
271 Moreover, since Dom mice with depressive-like behavior show a different drug-aversive effect from
272 Subs, the mechanism to develop depressive-like behavior may be shared with pathways responsible
273 for THC effects.

The finding that the cannabis effect is dependent on individual personality may also warrant consideration of cannabis use in relation to medical treatment. We suggest that personality-based behavioral differences should be considered as an essential element of medical cannabis treatment and should be taken into consideration when prescribing and selecting the right dose of THC-containing medications to patients.

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425

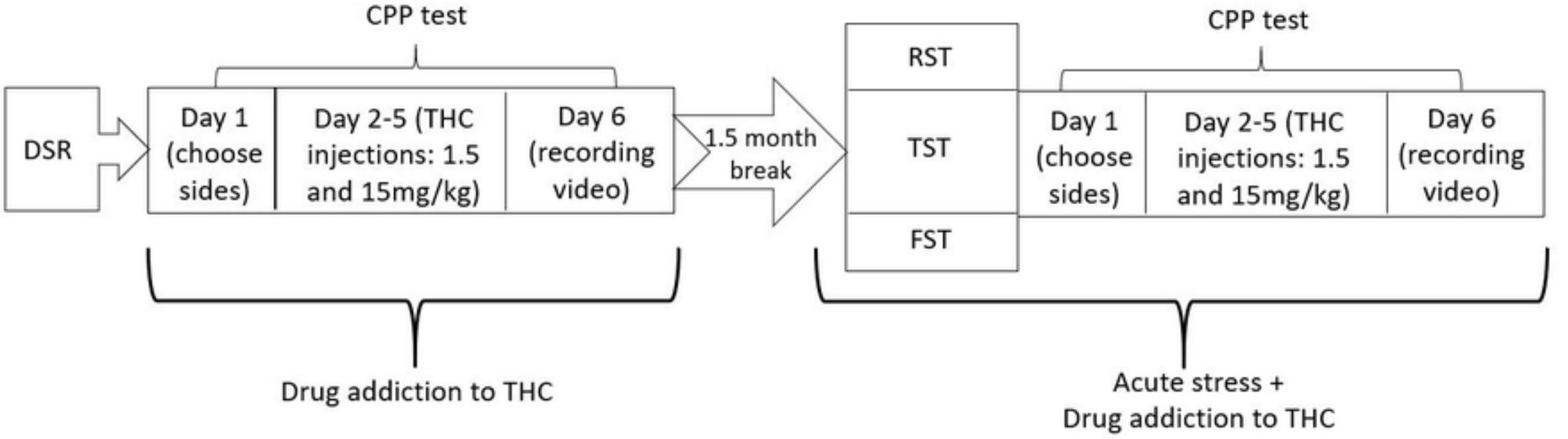
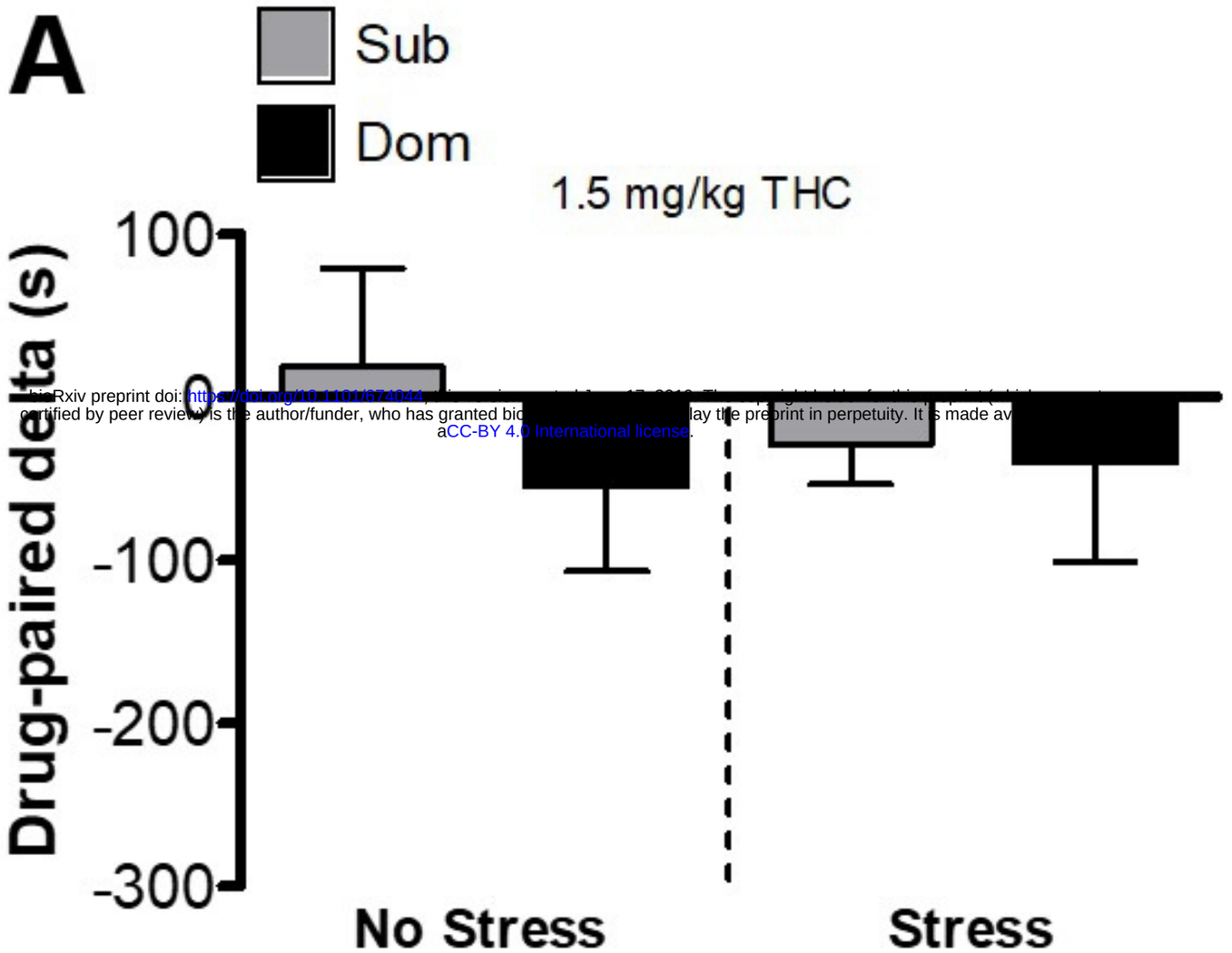

CPP test
DSR
Day 1 (choose sides)
Day 2-5 (THC injections: 1.5 and 15mg/kg)
Day 6 (recording video)
1.5 month break
RST
TST
FST
CPP test
Day 1 (choose sides)
Day 2-5 (THC injections: 1.5 and 15mg/kg)
Day 6 (recording video)
Drug addiction to THC
Acute stress + Drug addiction to THC

Figure 1



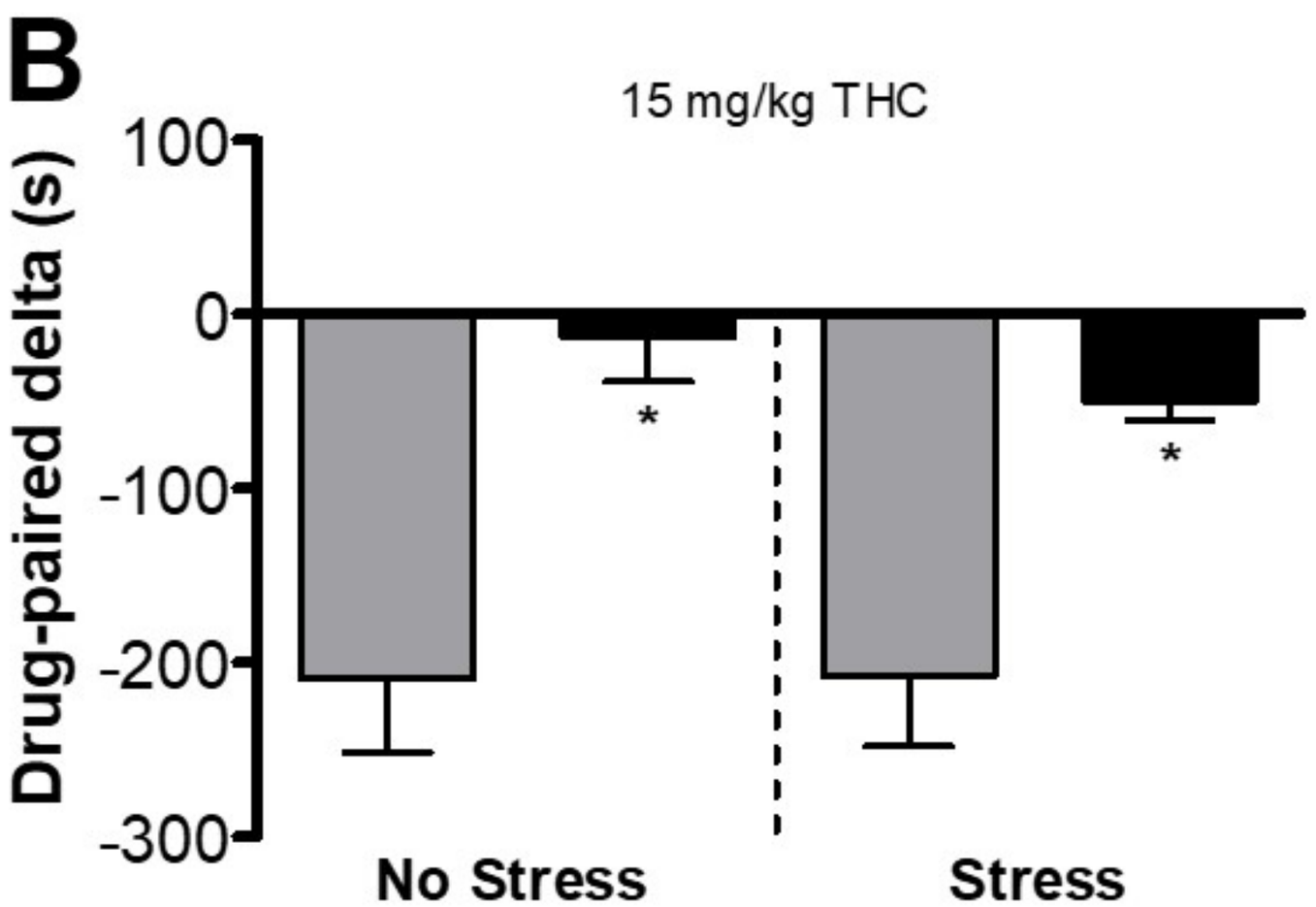


Figure 2

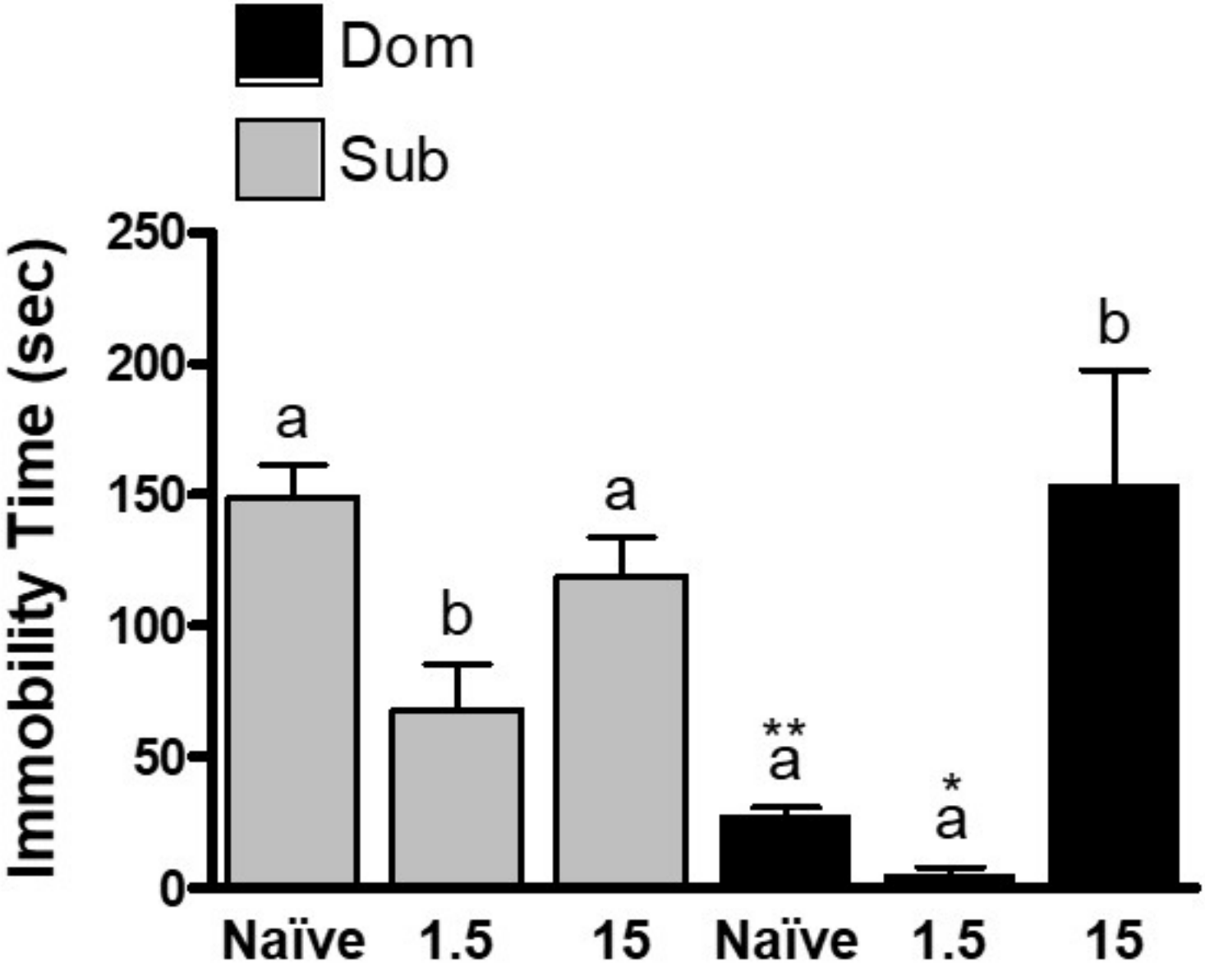

Dom
Sub
Immobility Time (sec)
250
200
150
100
50
0
a
b
a
**
a
*
a
b
Naïve
1.5
15
Naïve
1.5
15

Figure 3

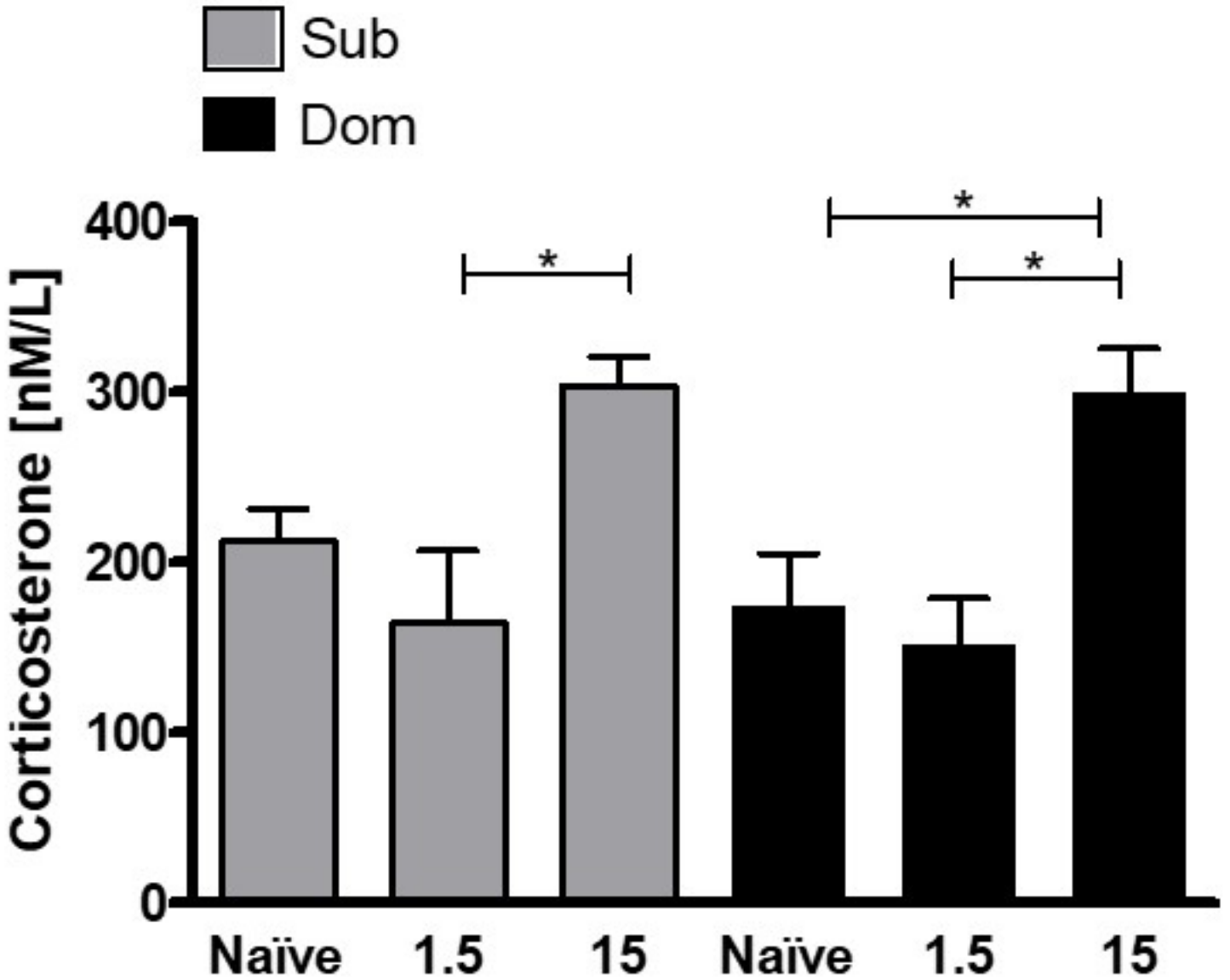

Sub
Dom
Corticosterone [nM/L]
400
300
200
100
0
*
*
*
Naïve
1.5
15
Naïve
1.5
15

Figure 4