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# SOBRE PODER Y PLACER: CB1R COMO NEXO ENTRE LA DOMINANCIA SOCIAL Y LA RECOMPENSA INDUCIDA POR DROGAS

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QUE PARA OPTAR POR EL GRADO DE
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# UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

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# SOBRE PODER Y PLACER:
# CB1R como nexo entre la dominancia social y la recompensa inducida por drogas

**Tesis**
Que para optar por el grado de
**Doctor en Ciencias**

Presenta


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# ON POWER AND PLEASURE

## Cannabinoid 1 receptor as the nexus between social dominance and drug reward

Martin Migliaro

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# Resumen

El trastorno por consumo de sustancias (TCS) representan un importante problema de salud global, afectando a individuos, familias y comunidades. Dado que solo una fracción de los usuarios de drogas desarrollan adicción, comprender los factores que contribuyen a la susceptibilidad a los TCS puede ser útil para adaptar estrategias preventivas. El uso de drogas a está vinculado con conductas agresivas, y se ha identificado la agresividad como un predictor del consumo de drogas. Sin embargo, no está del todo claro por qué estos tipos de comportamientos están interconectados. Se ha presentado evidencia sugiriendo que un mecanismo neurofisiológico común podría explicar ambos tipos de comportamiento. Esta tesis se enfoca específicamente en el sistema endocannabinoide (SEC) expresado en el núcleo accumbens (NAc) y la corteza prefrontal medial (mPFC), cual es fundamental tanto para la búsqueda de recompensas como para el comportamiento social en ambas estructuras.

En diversas especies, la manifestación de la agresión en contextos sociales está intrínsecamente vinculada al estatus de dominio. Por lo tanto, este trabajo de tesis aboga por explorar las relaciones de dominancia en roedores, ofreciendo valiosas perspectivas sobre los fundamentos neurofisiológicos que conectan la agresión con la recompensa de drogas. El estudio inicial incorporado en esta tesis proporciona evidencia que indica que las ratas macho dominantes muestran una mayor sensibilidad a los efectos gratificantes de la d-anfetamina y tienen niveles más bajos de receptores tipo-1 de cannabinoides (CB1R) tanto en el NAc como en el mPFC. En un estudio posterior, el silenciamiento génico de CB1R en la sección dorsal del mPFC, específicamente dentro de la corteza cingulada anterior, fomenta la dominancia social y amplifica la recompensa de drogas. En conjunto, la señalización mediada por CB1R emerge como un factor crucial para promover la dominancia social y la recompensa de drogas, potencialmente sirviendo como biomarcador para la susceptibilidad al uso de sustancias y conductas antisociales.

# Abstract

Substance use disorder (SUD) pose a significant global health problem, impacting individuals, families, and communities. Given that only a fraction of drug users ever become addicted, understanding the factors that contribute to SUD susceptibility can be helpful for tailoring preventive strategies. Drug use is readily linked to aggression, and aggressiveness has been identified as a predictor of drug use. However, it is not all too clear why these types of behaviors are interconnected. Evidence has been put forth suggesting that a common neurophysiological mechanism could account for both types of behavior. This thesis specifically focuses on the endocannabinoid system (ECS) in the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC), integral to both reward-seeking and social behavior in both structures.

Across various species, the manifestation of aggression in social contexts is intricately tied to dominance status. Consequently, this dissertation advocates for an examination of dominance relationships in rodents, offering valuable insights into the neurophysiological foundations that connect aggression to drug reward. The initial study integrated into this thesis furnishes evidence indicating that dominant male rats exhibit heightened sensitivity to the rewarding effects of d-amphetamine and possess lower levels of cannabinoid type-1 receptors (CB1R) in both the NAc and mPFC. In a subsequent study, the in vivo gene-silencing of CB1R in the dorsal section of the mPFC, specifically within the anterior cingulate cortex, fosters social dominance and amplifies drug reward. Collectively, CB1R-mediated signaling emerges as a pivotal factor in promoting social dominance and drug reward, potentially serving as a biomarker for susceptibility to substance use and antisocial behaviors.

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# List of acronyms

| | |
|---|---|
| Δ9-THC | Δ9-Tetrahydrocannabinol |
| 2-AG | 2-arachidonoylglycerol |
| ACC | Anterior cingulate cortex |
| ACEA | Arachidonyl-2'-chloroethylamide |
| ACEA-CPP | ACEA conditioned place preference |
| A-CPP | Amphetamine conditioned place preference |
| AEA | N-arachidonoylethanolamine, anandamide |
| AG | Aggressive grooming |
| AMPH | Dextro-amphetamine |
| Amy | Amygdala |
| BLA | Basolateral amygdala |
| BNST | Bed nucleus of the stria terminalis |
| CAC | Core aggression circuit |
| CB1R | Cannabinoid type-1 receptor |
| CB1R-KD | CB1R knock-down |
| CB2R | Cannabinoid type-2 receptor |
| CBD | Cannabidiol |
| CON | Group infused with control vector |
| copGFP | Green fluorescent protein-like, copepod |
| CPP | Conditioned place preference |
| DA | Dopamine |
| DAGLa | Diacylglycerol lipase alpha |
| $D_{ij}$ | Dyadic dominance index |
| dmPFC | Dorsomedial prefrontal cortex |
| DNN | Deep neural network |
| Dom | Dominant |
| DOM-nv | Naïve dominant |
| DP | Dominance posture |
| dPAG | Dorsal periaqueductal gray |
| eCBs | Endocannabinoids |
| EA | Enclonsed arms (EPM) |
| ECS | Endocannabinoid system |
| EPM | Elevated plus maze |
| FAAH | Fatty acid amino hydrolase |
| GH | Group-housed |
| GPR55 | G-coupled protein receptor 55 |
| HIWL | High-intensity white lighting (EPM) |
| Hyp | Hypothalamus |
| IL | Infralimbic cortex |
| LV | Lentivirus |
| LIRL | Low-intensity red lighting |
| M1 | Muscarinic receptor 1 |
| M3 | Muscarinic receptor 3 |
| MAGL | Monoacylglycerol lipase |
| MD | Maternal deprivation |
| MDS | Modified David's Score |
| mPFC | Medial prefrontal cortex |
| MSNs | Medium spiny neurons |
| NAc | Nucleus accumbens |
| NAcC | Nucleus accumbens core |
| NAcS | Nucleus accumbens shell |
| NAPE | N-arachidonoyl phosphatidyl ethanol |
| NAPE-PLD | NAPE-specific phospholipases |
| OA | Open arms (EPM) |
| PAG | Periaqueductal gray |
| PAM | Peptidylglycine alpha-amidating monooxygenase |
| pCB | Phytocannabinoid |
| PFC | Prefrontal cortex |
| PL | Prelimbic cortex |
| PMv | Premammillary nucleus |
| PPARγ | Peroxisome proliferator-activated receptor gamma |
| PRE | Pre-conditioning session (CPP) |
| RCT | Resource competition test |
| RMC | Reward-motivation circuit |
| SAL | Saline solution |
| shRNA | Small hairpin RNA |
| SI | Social isolation |
| siRNA | Small interference RNA |
| SIT | Social interaction test |
| Sub | Subordinate |
| SUB-nv | Naïve subordinate |
| SUD | Substance abuse disorder |
| TEST | Test session (CPP) |
| TRPV1 | Transient receptor potential vanilloid 1 |
| vHip | Ventral hippocampus |
| VMHvl | Ventromedial hypothalamus |
| VP | Ventral pallidum |
| VTA | Ventral tegmental area |

# Chapter I: Background

| **Chapter highlights** | Drug use and dominance status |
|---|---|
| | Neurobiology of reward-seeking, aggression, and dominance. |
| | The endocannabinoid system in reward and social dominance |

*"The body is a great reason, a plurality with one sense, a war and a peace, a herd and a shepherd. An instrument of your body is also your little reason, my brother, which you call "spirit"— a little instrument and toy of your great reason…*

*…There is more reason in your body than your best wisdom. And who knows why your body needs precisely your best wisdom?"*

-Thus Spoke Zarathustra (1883)
Friedrich W. Nietzsche

### I.1.1. Association between substance use and aggression

Substance abuse disorder (SUD) is a chronic relapsing psychiatric condition characterized by progressive impairment in self-control that culminates in escalated and compulsive consumption of drugs[1]. SUD poses a critical public health concern due to its multifaceted impact on individuals and society. Compulsive drug-seeking behavior disregards adverse consequences, whereby responsibilities are neglected, and social relationships strained. Additionally, substance abuse can hinder personal growth, limit educational and career opportunities, and contribute to legal issues, resulting in a significant decline in overall quality of life for affected individuals. Socially, substance abuse contributes to crime rates, domestic violence, and family disruption, creating a destabilizing effect on communities. Economically, the burden of treatment costs, lost productivity, and increased social welfare expenses weigh heavily on societies. Additionally, the devastating rise in overdose deaths, impaired public safety, and the vulnerability of certain populations highlight the urgency of addressing this issue.

Repeated exposure to drugs in those who are vulnerable leads to long-lasting changes in the brain that contribute to the diminished response to natural rewards, heightened response to drug-related cues, and an impairment in the capacity to self-control[1]. The result is an inability to suppress the urges to consume paired with compulsive drug seeking and drug use regardless of negative consequences[1]. However, SUD doesn't seem to be solely dependent on the reconfiguration of brain functions by chronic drug use. Current estimates show that 1.5%, 9%, 17%, and 27% of individuals with prolonged alcohol, cannabis, cocaine, or heroin use (respectively) transition to addiction[2]. Given that only a fraction of lifetime drug users ever become addicted, the current consensus is that SUD develops in people with certain predispositions or contextual facilitating factors[1,3].

Drug use is readily linked to aggression, and aggressiveness has been identified as a predictor of drug use [4–6]. Using the terminology of Lischinsky and Lin (2020), “aggression is defined as any hostile behavior directed towards a conspecific that has the goal of overpowering” [7]. Recreational consumers of drugs have a more extensive record of aggressive behaviors[8] and aggression among youths is a predictor of early substance use initiation[5]. Individuals exhibiting higher levels of aggressiveness are often more prone to engage in risky behaviors, including experimenting with substances, as they seek immediate gratification and are less concerned about long-term consequences[9]. When accounting for various forms of aggression, recurrent physical assaults emerged as a more influential predictor of early initiation of use [10]. Moreover, externalizing behaviors, including aggression and rule-breaking, are readily correlated with antisocial personality traits[11], which in turn been associated with higher sensitivity to rewards and substance use[12].

It is not all too clear why aggression and drug are co-occurring phenomenon and though several suggestions have been proposed, it remains a topic of ongoing discussion[8]. Rather than drawing

the line of causality between aggression and drug use, evidence indicates that latent phenotypes can facilitate the expression of both types of behavior[4,13–18]. The most notable is trait disinhibition, which is broadly defined as deficient impulse control. This deficiency is characterized by difficulties monitoring and inhibiting behavior, regulating maladaptive emotional responses, and planning for the future[16]. The behavioral disinhibition has been attributed to the interplay of two neurobiological mechanisms working in tandem: a bottom-up process that amplifies reward sensitivity and a concurrent top-down control mechanism that, when compromised, impairs the usual cost-benefit analysis guiding behavior[4,19,20]. Reward sensitivity describes how sensitive an individual is to positive reinforcement and how much pleasure or motivation they derive from rewarding experiences[21]. Top-down control mechanisms of behavior involve the hierarchical organization of neural processes, with higher-level processes guiding and modulating lower-level processes to support adaptive behavior and goal attainment[22]. In the upcoming sections, we will delve in depth into the neural processes underpinning reward sensitivity and the regulation of behavior through top-down control, which will constitute a central theme of our present study.

### I.1.2. Ethology as a tool to refine animal models and frame inquiries

In the quest to understand why certain humans become addicted while others remain unscathed, some intrepid individuals have turned their attention to other animals in the hope of getting their answers. Translational research has the objective of bridging biological and behavioral laboratory findings with psychiatric disorders, though success of this endeavor has been questionable[23–28].

Improving upon the ecological validity of animal models has been a strategy proposed to address the ongoing translational issues in preclinical research, particularly when the objective is to understand the biological function of behavior[24,27,29]. Ecological validity involves considering the environment to which an animal is naturally adapted when designing the experimental test conditions. The concept traces its origins to ethology, the scientific study of animal behavior in its natural habitat, which is the evolutionarily relevant context for interpreting the function of behavior [30,31]. An illustrative example of ecological validity is assessing exploratory or foraging behavior of nocturnal animals during the dark phase, utilizing a recording system capable of capturing video in low-light conditions[32]. Another example is the selection of predator scent as a stimulus for studying stress response in rodents[33]. When aggression is of interest, it matters if the interaction is between unfamiliar rodents meeting in the home-cage of one of the interactants or if the interaction is between cagemates[34]. While the former scenario is indicative of territorial defense, the latter is more relevant for the study of social relationships.

Beyond methodological refinement, ethology provides a framing device for understanding pathophysiology[30,31,35,36]. All organisms, including humans, are a product of evolution by natural selection[37]. Over the course of eons, this process has continuously yielded solutions (i.e., adaptations) to changing biological challenges in a dynamic environment, impacting reproductive

fitness and guaranteeing the inheritance of these solutions[38–40]. From this standpoint, bodies, organs, or individual features are not optimal by design because Nature lacks foresight. Instead, they function with a sufficient level of reliability that enables the preservation of a stable state and the ability to address threats or challenges that may arise. For example, memory is prone to errors, distortions, and forgetfulness, impacting the accuracy of recalling past events. Despite these imperfections, memory formation is generally reliable enough to allow individuals to learn from experiences and adapt to their environment.

Physiology particularly excels in addressing challenges it has encountered over its evolutionary journey, but when the environmental unpredictability exceeds the organism's adaptive capacity, it can lead to mounting pressure on survival. For instance, heightened vigilance and sensitivity to social cues, which may have been adaptive for survival in small social groups, might contribute to social anxiety or depressive symptoms in a larger, more complex society[41]. It has been theorized that chronic mental disorders suffered by humans emerge from unsuccessful implementations of inherited evolutionary solutions to an artificial contemporary environment in which we now live[35,42–47]. On point, addictive substances affect the brain's reward circuit, even though these systems initially evolved to respond to natural stimuli like food and sex[46]. Through serendipity and expounded by ingenuity, humans have discovered and mastered the ability to artificially stimulate this circuit with drugs. Even more, the effects of these substances intense, essentially signaling that they are the kind of stimuli that the organism should be paying attention to and even prioritized over other natural rewards[47].

For the moment, ethology will be put aside so we can focus on the problem at hand. Introduced in the former section and now explicitly stated, the overarching objective of the current study is to understand why aggressiveness and substance use are co-occurring phenomenon. Overall, the available literature suggests that the relationship between drugs and aggression is highly complex and is governed by a combination of both transient and permanent factors[8,48–50]. Individual differences have been implicated in the relationship between drug use and aggressive behavior. As evidenced earlier, individuals who are predisposed to being aggressive are also more likely to consume drugs, which has been interpreted as the result of a common underlying factor. In humans, this hypothesis has been tested and supported using longitudinal, epidemiological studies[51–53]. In a series of animal studies, patterns of aggressive interactions between conspecifics in semi-natural settings has served useful in predicting how individuals consume and seek-out natural rewards[54–56] and several drugs of abuse [57–62]. The discussion of these experiments will be addressed in greater detail in subsequent sections.

In light of the aforementioned findings, ethology is useful for setting up the stage. Social animals live in groups and engage in complex and recurring interactions with conspecifics to form organized social structures and exhibit a range of social behaviors[63]. Group-living is an evolutionary strategy readily expressed in mammals, from rodents to humans[64]. The nature and

intricacy of these social interactions can vary widely between different species, from simple aggregations seen in shoals and flocks to highly structured societies with well-defined roles and hierarchies, such as those found in eusocial insect colonies and human cities[63]. For these species, social interactions are crucial for an individual's survival and wellbeing, however, proximal living brings the problem of resource distribution among members[65,66]. Cooperative strategies, particularly among humans, have demonstrated to be essential in addressing the challenge of resource distribution and it has been hypothesized that the size of the neocortex in primates evolved primarily to support these complex social interactions[67,68]. On the other hand, competitive strategies that involve aggression are a ubiquitous phenomenon across the animal kingdom and are readily implemented as a means to assert claim over vital resources and mates [30,68–71]. To minimize the costs of intra-group competition and safeguard a species' overall survival, species must have developed evolutionary stable strategies to constraint destructive aspects of aggression and coordinate behaviors of individual members[68,69,71,72].

The winner and loser effects, observed in vertebrate and invertebrate species, represent the influence of past social interactions on an individual's likelihood of winning or losing future contests[73–75]. Winning a previous interaction, termed the "winner effect," boosts an individual's self-appraisal and drive to overpower opponents, thus increasing the probability of success in subsequent encounters. In contrast, the "loser effect" follows losses, resulting in diminished self-appraisal and an increased reward threshold, which together elevate the prospects of future defeats. Humans that have chronically suffered peer victimization demonstrated dysfunctional reward processing[76] and a reduced neural response measured with fMRI to a monetary reward[77]. Additionally, the presence of a dominant animal decreases the rewarding effect of intracranial self-stimulation in subordinate rodents[78] and monkeys[79]. It is therefore hypothesized that the reduction in the perceived value of incentives (e.g., hedonic stimulus) dissuades an individual that has suffered defeats from engaging in confrontations, preventing further negative outcomes[72]. From this standpoint, we can begin to see how past social interactions shape an individual's likelihood to aggress and approach rewards.

### I.1.3. Social status across animal species

Sociality in humans and other mammals can be understood by the analogy of actors assigned to roles[63]. Thus, *who* one becomes on the proscenium stage will shape *how* one interacts with a particular *other*[63,80–82]. It follows that the word "person" traces its etymological roots back to the Latin "persona", denoting a masked character enacted by an actor. For example, becoming an employee leads to acquiring specific modes of interaction that are molded by the identity of whom one interacts with. When an employee interacts with a superior, the individual is likely to adopt a formal communication style that conveys respect and acknowledges the hierarchical structure of the workspace with the mention of honorific titles[68]. When conflict arises with their boss, employees often approach the matter in a measured way, avoiding direct confrontation and maintaining composure[68]. High-ranking individuals can adopt an authoritative stance that is

expressed using a top-down approach of communication that leaves little room for open dialogue. Another set of behaviors displayed by the powerful may include fear-based tactics, such as intimidation or overt threats, to control their employees[83,84]. A critical reader will easily point out that power dynamics in any assemble of humans cannot be distilled to subjugation, for influence over others can be asserted through more positive means[85,86]. This benevolent power figure is what pop psychology and corporate marketing likes to call “leaders”, even though tyrants also in the business of leading[68]. Nonetheless, these examples are intended as priming fodder for the main event.

The capacity to effectively modify one's behavior in response to peers is rooted in the capability to assess one's own social standing in comparison to others[87]. Social status is a condition of being in relation to conspecifics, rather than an attribute of the individual, that shapes how one behaves towards conspecifics. Henceforth, status necessitates participation of individuals in social relationships for its acquisition and sustenance[88]. By this understanding, status is characterized by its relational quality that emerges from social interactions and comparisons with others. To avoid any confusion, it is worth noting that the definition of status stems from sociology[80,81] and ethology[88], which can diverge from a common understanding of status as an indicator of someone holding a high position that is worthy of renown and respect. Furthermore, social status should not be conflated with the concept socioeconomic status, where the latter is a demographic metric that informs us about an individual's income, education, and occupation without considering direct comparisons between individuals[89].

Actors within a social arena can partake in various modes of interactions that can shape their social standing, such as prestige or dominance[85,86,90,91]. In social dynamics involving prestige, individuals tend to be drawn to and follow peers who possess esteemed knowledge, skills, and personal qualities that are highly regarded within the group. In this sense, non-human animals often follow knowledgeable or motivated individuals who move first to a valuable resource (e.g., food patch, water) or away from danger. Dominance relationships primarily entail submitting to a powerful individual, who is someone that possesses the ability to inflict costs (such as physical injury or jeopardizing one's employment) or withhold benefits (like essential resources or opportunities for career growth)[68,92]. Hence, a powerful and dominant individual has the tendency to influence the behavior of a subordinate through positive and negative punishments.

This leadership style has been coined as tyrannical or despotic but does describe all leaders. In human societies, social status may occasionally derive from dominance but more frequently stems from prestige, facilitated through extensive cooperation and social learning[86]. Notwithstanding, dominance is phylogenetically older and is a readily expressed mode of interaction across taxa[69]. Moreover, fertility is higher in dominant men[93] and other social mammals[94,95], suggesting that dominance-seeking behavior may be influenced by selection pressures in various species[93]. Given these considerations, dominance serves as a suitable

conduit for elucidating the connection between aggression and drug consumption that holds true across mammalian species.

### I.1.4. Social dominance: aggression/submission, status, and rank

The expression "it takes two to tango" is a proverbial saying that suggests that for certain activities to occur or be successful, the involvement of more than one person is required. The expression is readily used to emphasize the idea that a mutual effort is necessary for something to happen effectively or smoothly. As such, social dominance is an emergent mode of interaction, or epiphenomenon, between conspecifics (interactants) that involve patterned aggression and submission[88,96,97]. From the point of view of an outside observer, dominant individuals manifest a greater extent of aggression relative to subordinates, while the latter emit a higher proportion of submissive signals[97]. These patterns in interactions are termed asymmetries. This conceptualization highlights that social dominance is not solely the result of one individual's behavior, but it is rather a *supraindividual* quality of interactants[88,96,97]. Just as a tango requires both partners, the dance of social dominance requires the active participation of both dominant and subordinate.

As previously defined, aggression is “any hostile behavior directed towards a conspecific that has the goal of overpowering an opponent”[7], encompassing both threat displays and physical attacks[7,96,98]. Submission behaviors are displays that convey deference and indicate the absence of a confrontational intent[99]. These behaviors serve to establish a non-threatening demeanor and signal an acknowledgment of the other individual's dominant status. Thus, submission consequently serves as a cost-minimization strategy for lower-ranking individuals when confronted by dominant individuals[69,99]. These behaviors often include adopting a lower or supine posture, emitting ultrasonic vocalizations, avoiding direct eye contact, and freezing in place to minimize movement[100].

The emergence of dominance relationships has been attributed to self-organizing social dynamics in a series of confrontations[101]. Both winners and losers gather information about the resource holding power (RHP) of their adversary, even when the contests involve no physical fighting. RHP is theoretical concept that refers to an individual's ability to gain and maintain control over valuable resources within a given environment or social context. Factors such as fighting ability, physical traits, and prior experience contribute to an interactant´s RHP. Updated information on an opponent to make strategic improvements in subsequent contests. For example, when enough information has been sampled to detect an opponent with a higher RHP, it is most advantageous to yield and avoid serious harm. Consequently, the patterns of aggressive and submissive behaviors embedded in a social relationship are learned modes of interaction[102].

Dominance status does not equate to dominance rank, since the former describes an attribute of social interactions, and the latter informs the position of an individual in a dominance hierarchy[88]. In a strict sense, a dominance hierarchy is a type of social structure that is

characterized by transitive dominance relationships: when individual “α” dominates “β” and “β” dominates “Ω”, then “α” dominates “Ω”[101,103]. Non-transitive structures in small groups have been proposed as additional types of dominance hierarchies[104], however, this is inclusion is of much controversy in the field. In the present dissertation, the precise characterization of dominance hierarchy will be adopted, and the term "rank" will solely be invoked upon substantiating a transitive social arrangement.

### I.1.5. Dominance status and reward-seeking

The consequences of engaging in dominance relationships extend far beyond the social domain, profoundly influencing health, emotional well-being and cognition[105–108]. Consistent with behavioral disinhibition theory, status attainment has been observed to induce risky behavior[109] and aggression[84]. More importantly, social status shapes reward-seeking behavior[110,111]. Participants primed to have a higher sense of power over others or assigned to a high-status role demonstrate an increased anticipated value of reward, while minimizing the perception of potential loss[109,112]. This effect seems to be relevant when high-power individuals don’t have to contend with the looming possibility of losing their status[113]. Moreover, participants higher in personality traits associated with social dominance or participants that were assigned control over resources experienced more positive and less negative emotion, were more likely to perceive social approval, and were less likely to perceive social threats[92]. Additionally, powerful individuals tend to overperceive sexual interest from subordinates, which is thought to be one avenue leading to sexual harassment in the workplace[114].

In a longitudinal and prospective study[53], children were followed for a decade into adulthood to assess how social dominance acts as a mediator in the relationship between testosterone and the risk for developing SUD. Peripheral testosterone was measured in three time periods; ages 10-12, 12-14, and 16. The interest in testosterone arose from previous studies indicating that dominant humans, non-human primates, and rodents have higher circulating testosterone levels than subordinates[106,115,116]. At age 16, evaluation of traits associated with social dominance were conducted, with subsequent assessments of illicit drug consumption at 19 years of age and the development of SUD at the age of 22. Higher testosterone levels in early adolescence predicted social dominance in late adolescence, while also predicting a higher frequency of illicit drug use at 19 years old and the development of SUD. Authors suggested that drug consumption could serve various purposes, such as gaining peer approval, pharmacological disinhibition to facilitate dominant behaviors, but also as a strategy to cope with stress caused by the perception of threats to status loss. These results are consistent with other studies that report high-status individuals tend to use more illicit drugs[117,118].

Evidence derived from studies in rodents further corroborates the notion that social status plays a regulatory role in shaping how individuals engage with hedonic stimuli[119]. When compared to their subordinate counterparts, male dominant rodents are more motivated to work for food

reward[54], self-administer more cocaine[120], and demonstrate a greater preference for contexts associated with cocaine delivery[121]. Δ9-Tetrahydrocannabinol (Δ9-THC), the primary psychoactive component of cannabis, results in conditioned place aversion in subordinate mice when administered at the higher of the two doses. However, it does not have any impact on dominants at either dose[122]. Acquired aversion to Δ9-THC-paired context agrees with studies that demonstrate a biphasic effect of Δ9-THC, where lower doses elicit a hedonic response while aversive/anxiogenic effects are observed at higher doses[123]. Nevertheless, it remains at open question to why dominants are resistant to the aversive effect of Δ9-THC. One possibility is a differential expression of endogenous receptors that mediate the effects of Δ9-THC between dominant sand subordinate individuals. Additionally, recent unpublished (Ostos-Valverde) findings from our laboratory indicate that dominant rats exhibit heightened sensitivity to the rewarding effects of the selective cannabinoid type-1 receptor agonist, arachidonyl-2'-chloroethylamide (ACEA). The lowest dose of ACEA induced drug-seeking only in dominant rats, while a higher dose was able to elicit drug-seeking in both dominant and subordinate rats. An idea derived from previous work[110]; it is possible that dominant individuals are more prone to engaging in drug-seeking due to a higher rewarded sensitivity. Hence, the threshold to which behavior is motivated by a reward-associated stimuli could be lower in dominants, whereby less "convincing" is needed. This gap in knowledge opens an area of opportunity that will be addressed in the current study.

Conflicting findings have been reported in primates. Male dominant cynomolgus macaques have been shown to self- administer cocaine at significantly lower rates and had lower cocaine intakes compared with their subordinate counterparts[62]. The authors concluded that cocaine did not function as a reinforcer for dominants at the doses tested, however, these animals were not avoiding cocaine consumption altogether. In a subsequent study by the same authors[124], this difference between male dominant and subordinates was not observed. Contrary to what has been reported in males, female dominant cynomolgus macaques self-administered cocaine at significantly higher rates than their subordinate counterparts[61]. Considering these varying findings in primate studies, the complex relationship between dominance and cocaine self-administration in non-human primates remains subject of further study.

Anxiety is a factor conducive to drug-seeking and has been suggested as a mediating variable between drug use and dominance status[23,44,59]. Rats displaying high anxiety measured with the elevated plus maze (EPM) have a greater escalation of cocaine self-administration[125] and acquire preference for a cocaine-paired context, whereas low anxiety rats do not[126]. On the other hand, subordination has been described as anxiogenic[127,128], however, findings in rodents are not consistent[129]. Certain studies suggest that individuals in subordinate status exhibit the highest levels of anxiety[54], whereas contrasting research findings indicate the contrary or show no discernible distinction[129].Housing conditions are likely to explain discrepancies between studies, since same-sex housing, unlimited food availability and a simplistic laboratory environment are

known to reduce agonistic confrontations, potentially attenuating the anxiogenic effects of social conflict[129,130]. In support of this idea, subordinate male rats housed in mix-sex colonies consumed more alcohol than their higher-ranking conspecifics, which was interpreted by the authors as a consequence of social stress[60]. The intricate relationship between anxiety, dominance status, and drug-seeking behavior emphasizes the requirement for more investigation, a consideration that forms a crucial aspect of the present dissertation.

### I.1.6. Section conclusion

SUD is a public health concern that plagues societies across the world, nevertheless, not every consumer becomes an addict. For this reason, the current consensus is that addiction develops in vulnerable individuals that are subject to inherent and contextual risk factors. Behavioral predictors are effective in predicting how individuals interact with substances of abuse. Social status, understood as a learned mode of interaction, contributes to an individual's susceptibility for SUD, which is likely due to how status shapes behavioral disinhibition and reward sensitivity. Due to its wide expression across animal species, the study of dominance relationships in rodents stands out as a viable animal model to explore neurophysiological underpinnings that link aggression to drug reward.

### I.2.1. Neurobiology of reward-seeking

A reason why people use psychoactive substances is for their intensely gratifying sensations[131]. These drugs act upon phylogenetic ancient neuronal systems that evolved to code for natural rewards (e.g., food and sex) and promote adaptive behavioral patterns[132]. Reward is an inherent mechanism by which the brain establishes associations between diverse stimuli (e.g., substances, situations, or events) and a desirable outcome or pleasurable sensation[133]. This conditioning process leads to modifications in behavior, compelling individuals to actively approach or pursue rewarding stimuli. Reward-seeking is a category of goal-directed, motivated behavior that includes all actions that organisms implement to regulate the probability, proximity, and availability of a stimuli[134]. In essence, delving deeper into the neurobiology of reward not only grants us insights into the fundamental mechanisms driving reward-seeking behavior, but also holds the potential to significantly enhance our comprehension of addiction and its underlying processes[3,135].

The mesolimbic dopaminergic system is a complex network of structures that plays a crucial role in the experience of pleasure and reinforcement (Illus. 1). Herein, we will refer to this system as the reward-motivation circuit (RMC), nonetheless, it should not be overlooked that this circuit has also been shown to be involved in the active avoidance of aversive stimuli or states[1,136]. Central to this circuitry is the nucleus accumbens (NAc), located within the ventral striatum, which serves as a hub for relaying information from both dopaminergic and glutamatergic inputs onto the basal ganglia motor circuits[137]. An encounter with a motivationally salient stimulus leads to the release of dopamine (DA) from the ventral tegmental area (VTA) onto the NAc to promote

the initiation and continuation of approach or avoidance behaviors[1,137]. The GABA-releasing medium spiny neurons (MSNs) are the principal population of the NAc and serve as the primary means of output signaling from the structure. Direct and indirect projections (via the ventral pallidum) from MSNs back to the VTA have been both implicated in approach and avoidance[138]. Rather than serving opposing roles, it seems that these pathways can drive both reward and aversion, depending on their neuronal stimulation pattern[138,139].

The firing of medium spiny neurons (MSNs) is not directly induced by DA receptor binding[137], rather the activity from DA receptors alters cell excitability through positive and negative influences on membrane conductance[137]. The former effect is attributed to $G_s$-coupled D1-like (D1 and D5) receptors, while the latter can be elicited by $G_i$-coupled D2-like (D2, D3, and D4) receptors[1]. Lesion of the dopaminergic VTA-NAc pathway does not affect the behavioral hedonic response to reward, or "liking", but rather impairs the willingness to perform work to obtain a reward, or "wanting"[140,141]. As such, it has been proposed that DA release in the NAc encodes "incentive salience" and mediates motivation of approach behaviors[142]. Furthermore, DA release plays a role in the learning process that drives the acquisition of reward-seeking. Dopaminergic neurons fire when an individual is exposed to a discriminative stimulus that precede drug delivery, hence serving as a predictor of reward and motivator for action[1]. The generation of action potentials in NAc MSNs relies on excitatory input from glutamatergic projections arising from the VTA, the prefrontal cortex (PFC), basolateral amygdala (BLA), and the ventral hippocampus (vHip)[137]. Optic intracranial self-stimulation of NAc inputs from either PFC, BLA, or vHip is reinforcing[143,144]. Nonetheless, it seems that anatomically distinct glutamatergic inputs

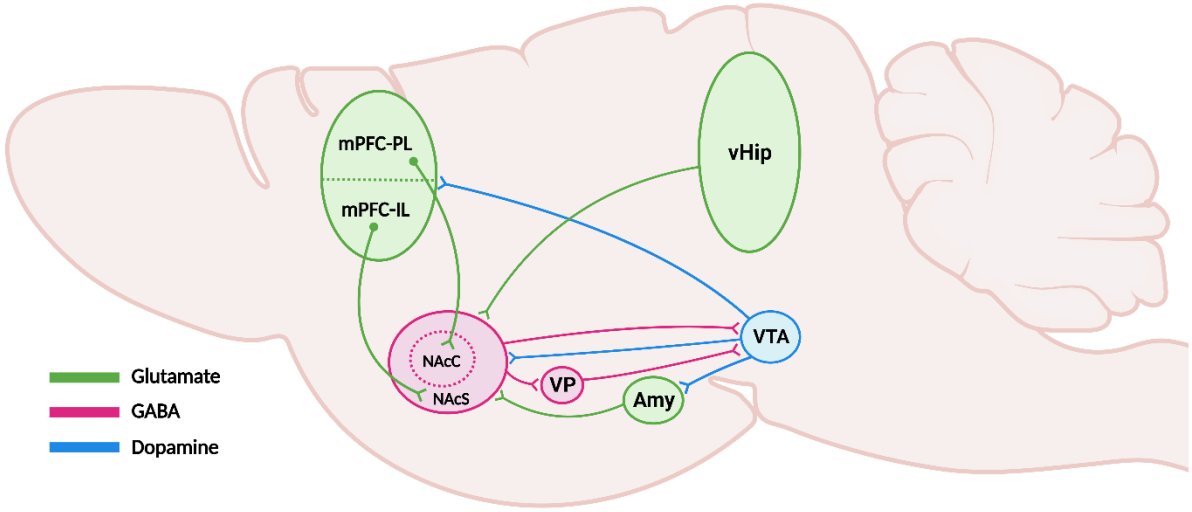


**Figure 1.** *Reward-motivation circuit in the rodent brain.* Located in the ventral portion of the striatum, the nucleus accumbens (NAc) plays a pivotal role integrating reward-related information from dopaminergic and glutamatergic afferents. Glutamatergic signals originating in the mPFC target distinct regions within the NAc. Activation of projections from the PL region fosters reward-seeking behavior, while activation of projections from the IL region suppresses reward-seeking tendencies. Lastly, GABAergic output from the NAc can modulate activity of VTA cells trough a direct path and an indirect path. Abbreviations: prelimbic subregion of the medial prefrontal cortex (mPFC-PL), infralimbic subregion of the medial prefrontal cortex (mPFC-IL), nucleus accumbens core (NAcC), nucleus accumbens shell (NAcS), ventral pallidum (VP), amygdala (Amy), ventral tegmental area (VTA), and ventral hippocampus (vHip). References supporting the concepts in this figure are in the text. Illustration Created with BioRender.com.

covey different types of reward-related information: the vHip encodes contextual information, the BLA relays emotionally salient events, and the PFC outputs the results of cost-benefit analysis[137]. Regarding the inhibition of reward-seeking, evidence suggests the necessity of coordinated activity among multiple NAc inputs, rather than opposing processes between these circuit components[145]. Regardless, genetically and functionally distinct subpopulations of glutamatergic inputs to the NAc arising from the same projection region can differentially affect behavior. For example, optic activation of excitatory inputs from a population of neurons projecting from the BLA and expressing Calcium/calmodulin-dependent protein kinase type II subunit alpha (CaMKIIα) is reinforcing[146]; whereas another non-overlapping population also from the BLA expressing the neuropeptide cholecystokinin (CCK) reduces the reinforcing effects of a hedonic stimulus[147].

The PFC is conserved structure across mammals, sharing functional similarities in guiding behavior in relation to reward and punishment[148]. The rodent medial PFC (mPFC) is considered to be functionally homologous to the human dorsolateral PFC[148], though this is a contentious topic of ongoing exploration[149,150]. The mPFC can be further subdivided into different subregions: the infralimbic cortex (IL), the prelimbic cortex (PL), and the anterior cingulate cortex (ACC)[150]. The IL is positioned ventrally and is adjacent to the PL, which in turn is positioned ventrally to the ACC. The mPFC consists of diverse neuronal subtypes, primarily categorized into excitatory pyramidal neurons and inhibitory interneurons. The excitatory/inhibitory balance within the structure is subject to inputs from distant structures; including DA innervating from the VTA, noradrenaline from locus coeruleus, acetylcholine from the basal forebrain, and serotonin from the raphe nucleus[148].

The IL and PL have been shown to play dissociable roles reward-seeking behavior[151]. Activation of the PL promotes cocaine seeking while stimulation of the IL suppresses relapse after extinction[151]. Both subdivisions of the mPFC send projections to the NAc, albeit targeting distinct areas. The NAc core (NAcC) receives input primarily from the PL, while the NAc shell (NacS) from the IL[152]. Optical intracranial self-stimulation of PL terminals in the NAc reinforcing[144] and the promoting role of the PL-NAcC pathway on cocaine seeking is dependent on DA release in the PL, with its probable source being the VTA[148,153]. On the other hand, optogenetic activation of IL-NAcS inhibits ongoing drug-seeking[154], whereas the inhibition this pathway impairs the extinction of cocaine self-administration[155]. Taken together, the distinct functions of mPFC in reward-seeking behavior are evident through their respective impacts on drug seeking, their projection patterns to distinct NAc regions, and the modulatory roles played by these pathways in eliciting and inhibiting drug-seeking behaviors.

### I.2.2. Neurobiology of aggression and dominance

Upon the detection of aggression-provoking a stimulus (e.g., olfactory cues, auditory input, or behavioral displays), a cascading neural process occurs, involving the transfer of sensory information to what has been termed the core aggression circuit (CAC)[7]. The CAC comprises a subcortical network of interconnected nuclei, including the amygdala, bed nucleus of the stria terminalis (BNST), ventrolateral region of the ventromedial hypothalamus (VMHvl), and ventral

segment of the premammillary nucleus (PMv) of the hypothalamus. The amygdala and BNST are believed to contribute with the assessment of a social context and threat detection, which are conducive to aggression[156,157]. BNST in particular seems to play crucial role in the expression of defensive and submissive behaviors during social interaction[156]. The VMHvl receives projections from both amygdala and BNST[158], and activation the VMHvl has been readily reported to elicits attacks[159,160]. It has been suggested the activity from VMHvl cells contributive to aggressive arousal and the probability of an attack when an aggression-provoking cue is detected[7]. The PMv provides excitatory projections to the VMHvl and may play a role in regulating the intensity of aggression[161]. Similarly, the vHip also sends glutamatergic projections to the VMH, and the activation of this neurons has been reported to induce attack behavior under stressful conditions[162]. Lastly, the periaqueductal gray (PAG) area, situated in the midbrain, relays information from the CAC[158] and in turn sends projections to motor-control neurons in the spinal cord[163], promoting the expression of aggressive behaviors.

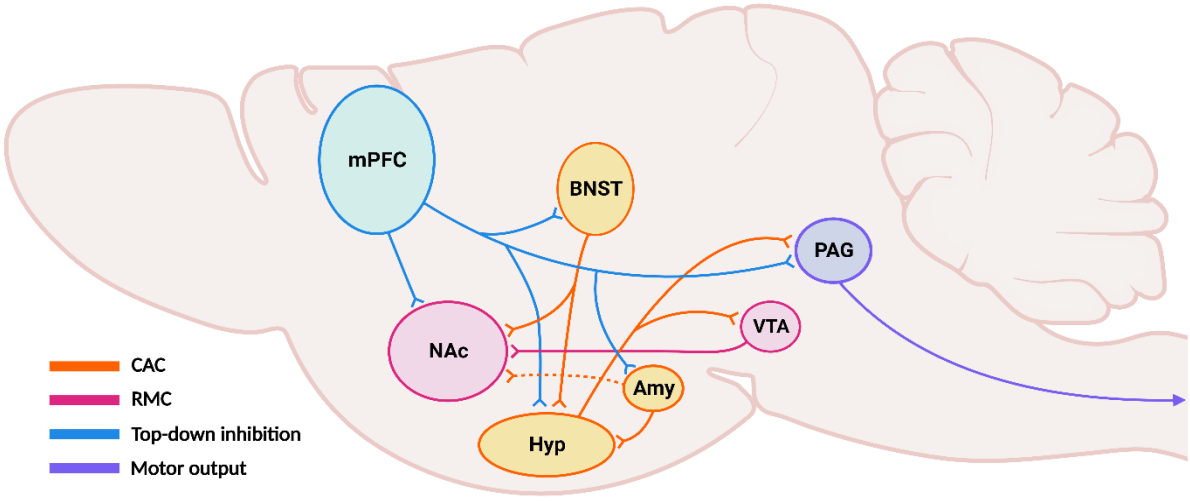


**Figure 2.** *Pathways and structures involved in aggression in the rodent brain.* The neurobiology of aggression consists of a complex interplay of various brain regions and neural pathways. A core substrate known as core aggression circuit (CAC) is responsible for encoding aggressive arousal and facilitating aggressive actions through projection to the midbrain premotor area. Additionally, the interaction with the reward-motivation circuit (RMC) facilitates the acquisition of learned adaptations concerning the execution of aggression, thereby allowing for the fine-tuning and modification of aggression based on contextual cues, reinforcement, and reward mechanisms. Due to the significant risks associated with aggressive encounters, the CAC is closely regulated by inhibitory top-down control mechanisms. Abbreviations: media prefrontal cortex (mPFC), nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), hypothalamus (Hyp), amygdala (Amy), ventral tegmental area (VTA), and periaqueductal gray (PAG). References supporting the concepts in this figure are in the text. Illustration Created with BioRender.com. Illustration was published in Migliaro et al. (2023).

Aggression can be reinforcing, given that individuals can learn to perform an arbitrary task for the opportunity to attack a conspecific[160]. More specifically, aggression is reinforcing when the

probability of overpowering is favorable (i.e. weaker, castrated, and submissive opponents)[160]. This conditionality suggests that victory, rather than aggression itself[164,165]. Additionally, consecutive experiences of winning can be conditioned to a context, whereby subjects can develop a learned preference for a previously neutral set of stimuli[166], suggesting that dominance is rewarding. Of note, reinforcer and reward are distinct constructs in behavioral science, whereby the former refers to the capacity of a stimulus to increase the future occurrence of a particular behavior, while the latter informs about the pleasure-inducing capacity of a stimulus.

Consistently, aggression has been shown to engage the mesolimbic dopaminergic system[111,164]. DA levels in the NAc increase in aggressor rats after a confrontation[167], whereas the pharmacological blockade of DA receptors after an encounter attenuated the aggression of victors in subsequent confrotations[168]. Additional, optical stimulation of DA neurons increase competitiveness over a food reward[169]. The CAC and the RMC are highly interconnected; for example, the VTA has bidirectional connections with the VMHvl[158], whereas NAc receives projections from both amygdala[146] and BNST[170]. Taken together, striatal dopaminergic signaling is a likely mechanism by which the successful execution of aggression is reinforced to facilitate adaptive behavioral responses in social interactions[164].

Fighting can be an injurious and sometimes, a fatal affair. For this reason, it is likely why inhibitory mechanisms for aggression are present in numerous species, allowing individuals to back down from a fight or prevent its insurgence. The mPFC has been shown to provide a top-down control of aggression. For example, increasing activity in the mPFC through transcranial direct-current stimulation has been found to reduce self-reported aggressiveness and the intentions to commit aggressive acts in violent offenders[171,172]. In mice, the optogenetic activation of the mPFC is associated with a downregulation of aggression, whereas inhibition has the opposite effect [173]. It has been speculated that mPFC provides a cost-benefit analysis related to the consequences of engaging in a fight, whereby a perceived loss reduces the expression of aggressive behaviors [7,174]. There are several projection targets by which the mPFC could be exercising a top-down control of aggression, including the lateral hypothalamus[175], amygdala[176], and BNST[156]. Another potential influence of the mPFC on aggressive behavior is through interaction with the RMC [177,178], whereby adjusting the willingness to participate in aggressive interactions[164]. Finally, the direct projections from the mPFC to the dorsal PAG (dPAG) impact social behavior in aggressive encounters. Specifically, pharmacogenetic inhibition the mPFC-dPAG projections led to an increase of behaviors associated with social avoidance and submission [179].

The mPFC control over aggression has been attributed as a result cost-benefits computations related to the consequences of engaging in a fight, whereby an anticipated defeat reduces the probability of attacking[7,174]. In primates and rodents, the ACC has been implicated in goal-directed choice in value-based decision making, where information on value and contingencies are integrated to form predictive models that guide decision making under uncertainty.[180–182]. The value of this capability is magnified in complex social settings, affording individuals the ability to foresee potential risks and rewards, thus leading to adaptive behavior[183]. A series of studies

have demonstrated that the rodent dorsomedial prefrontal cortex (dmPFC), which incorporates the ventral portion of the ACC and the dorsal portion of the PL, plays a crucial role controlling aggression[173], conflict resolution[184], and the establishment of dominance relationships[56,185,186]. For example, chemogenetic inhibition of pyramidal neurons in the dmPFC induces rank descension in cohabiting mice in competitive encounters, while optogenetic activation promotes rank ascension by increasing effortful behaviors aimed at displacing an opponent[185]. Moreover, neuronal ensembles in the dmPFC have been found to code for the aggressive behaviors of oneself and the opponent in a competitive setting[186]. Collectively, the ACC plays a central role as a neural foundation that propels adaptive responses within the intricacies of social dynamics.

### I.2.3. Section conclusion: conjunction between the neurobiology of drug-seeking and aggression

The execution of aggression embedded in a social relationship and drug-seeking are both learned patterns of behavior, modulated by overlapping circuits that underlie reward signaling and top-down control mechanism. Notably, compelling evidence underscores the pivotal roles of the NAc and the mPFC in this dynamic interplay. Consequently, this thesis will dedicate significant attention to elucidating the contributions of these key brain structures, offering a deeper understanding of how they jointly influence and potentially interact in shaping both aggression and drug-seeking.

### I.3.1. The endocannabinoid system

In the ensuing sections, endocannabinoid signaling will be presented as pivot, orchestrating the interplay between social dominance and drug-seeking. This hypothesis finds its roots in the previously discussed behavioral disinhibition theory, which suggests the existence of a shared mechanism underpinning both aggressive behaviors and the pursuit of drugs. This hypothesis gains credence from the emerging understanding of the endocannabinoid system's intricate involvement in the NAc and mPFC.

The ECS consists of a group of endogenous ligands (endocannabinoids, eCBs), the enzymes involved in their synthesis and degradation, and their respective receptors[187]. Activation of particular G protein-coupled receptors (e.g., metabotropic glutamate receptor type 5, mGluR5; muscarinic receptors, M1/M3) or depolarization triggers the on-demand synthesis and release of eCBs[137]. These messengers are lipophilic molecules of small size derived from cell membrane precursors[188], being N-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) the two most widely studied[189]. Even though most attention has been focused on AEA and 2-AG, the list of identified eCBs is much more extensive[189]. Notably, cis-9-10-octadecenoamide (oleamide, ODA) is an endogenous fatty acid amide like AEA, which rose to prominence for its sleep-inducing properties[190].

Despite sharing a common precursor, AEA and 2-AG are synthesized and degraded by distinct pathways. Multiple routes have been proposed for the synthesis of AEA, but it appears that the predominant source driven by the hydrolysis of N-arachidonoyl phosphatidyl ethanol (NAPE) by a NAPE-specific phospholipases (NAPE-PLD)[191]. The primary enzymes responsible for AEA degradation are fatty acid amino hydrolase (FAAH)[192]. It is worth noting that FAAH also appears to be involved in the degradation of 2-AG[193] and ODA[194]. Diacylglycerol lipase (DAGL) is responsible for the hydrolysis of diacylglycerol, leading to the synthesis of 2-AG[195]. Meanwhile, monoacylglycerol lipase (MAGL), functioning as a hydrolytic enzyme, is considered to be the main contributor to the degradation of 2-AG in the brain[196]. Lastly, ODA is synthesized from oleoylglycine by the neuropeptide processing enzyme peptidylglycine alpha-amidating monooxygenase (PAM), or alternatively by the direct amidation of oleic acid via oleoyl coenzyme A by cytochrome c using ammonia as the nitrogen source[197].

Classic cannabinoid receptors, of which there are two main types (CB1R and CB2R), are metabotropic receptors coupled to $G_{i/0}$ proteins in neurons[198]. Adenylyl cyclase and specific voltage-dependent calcium channels are inhibited upon the activation of CB1R or CB2R, while multiple MAP kinases and inwardly rectifying potassium channels are concurrently activated[187]. CB1Rs are highly expressed in the central nervous system, with the majority being positioned on axon terminals[187]. The activation of presynaptic CB1Rs inhibits the release of various neurotransmitters, including GABA and glutamate, leading to short and long-term changes in neuronal activity[137,199]. In several brain regions, CB1R is expressed at higher levels on GABAergic terminals than glutamatergic neurons[200,201]. In astrocytes, CB1R is strongly coupled to $G_q$ and the activation of the receptor leads to an increased intracellular calcium, triggering glial release of glutamate, ATP, or adenosine[202]. While CB2Rs are primarily found on microglia, which are resident immune cells in the brain[203], functional receptors have also been reported in neurons[204,205]. Similar to CB1R receptors, CB2R in presynaptic terminals also inhibit neurotransmitter release through a $G_i$-dependent mechanism[206].

Most of our combined knowledge about the ECS's involvement in behavior stems from pharmacological studies. Accordingly, it is crucial to introduce the pharmacological repertoire that has been utilized in this field of study. Phytocannabinoids (pCBs) are a class of naturally occurring compounds found in the Cannabis plant and share pharmacological similarities to eCBs [207]. The most well-known pCB is Δ9-tetrahydrocannabinol (Δ9-THC), which primarily binds to CB1R, albeit as a partial agonist [208]. In addition, Δ9-THC also has affinity and activity for the transient receptor potential vanilloid 1 (TRPV1); the orphan G-coupled protein receptor (GPR55); and the peroxisome proliferator-activated receptor gamma (PPARγ) [209]. Another important pCB is cannabidiol (CBD), which can act as a CB1R negative allosteric modulator [210]. CBD can also inhibit FAAH, consequently increasing bioavailability of AEA, possibly eliciting an indirect activation of cannabinoid receptors [211,212]. There is experimental evidence indicating that CBD activates the serotonin receptor 1a (5-HT1a) and TRPV1, among others [211,213]. Synthetic

cannabinoids are manufactured compounds that offer a precise control over potency and can be used to selectively activate or inhibit different subtypes of cannabinoid receptors [198]. Another class of synthetic compounds are enzyme inhibitors, which interfere with the activity of FAAH or MAGL and consequently increase the bioavailability of eCBs [214,215].

Now, we will turn our attention to acknowledging the caveats associated with pharmacological manipulation of the ECS. As mentioned in a previous section, cannabinoid receptors, particularly CB1R, are widely distributed throughout the brain, but their expression levels vary across different cell types and brain regions [202]. This variability suggests region-specific and cell-specific effects of eCB signaling. Additionally, eCBs and their receptors interact with numerous signaling systems, creating intricate crosstalk and modulation of neuronal communication [202]. As a result, the pharmacological activation or blockade of these receptors leads to a diverse range of effects

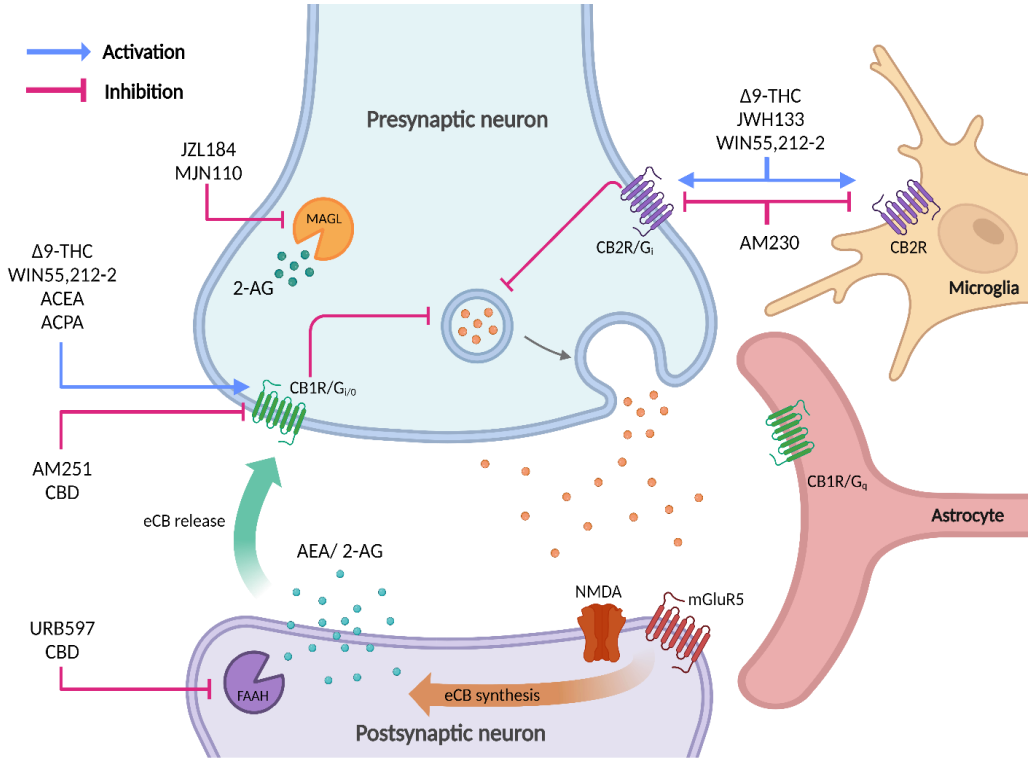


**Figure 3.** *Endocannabinoid signaling in the central nervous system and pharmacological agents that act on the ECS.* The synthesis of anandamide (AEA) or 2-arachidonoylglycerol (2-AG) is triggered by GPCR activation (e.g., mGlu5) or depolarization. Retrograde release of eCBs act on presynaptic cannabinoid receptors, which inhibit neurotransmitter release. Δ9-THC and synthetic cannabinoids (WIN55,212-2, ACEA, ACPA, & JWH133) act on cannabinoid receptors (CB1R & CB2R) expressed in neurons, astrocytes, and microglia. The main degradation pathway of AEA is mainly carried out by fatty acid amino hydrolase (FAAH), while monoacylglycerol lipase (MAGL) is principally involved in the degradation of 2-AG. Cannabinoid receptor agonists are represented as blue arrows, while the CB1R antagonist/inverse agonist (AM251), the CB2R antagonist (AM230), FAAH inhibitors (URB597& CBD) and MAGL inhibitors (JZL184, & MJN110) are represented as red lines. References supporting the concepts in this figure are in the text. Adapted from "Synapse with Astrocyte and Pathway (Layout)", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates. Illustration was published in Migliaro et al. (2023).

that synergistically contribute to an observable impact in behavior, which precludes us from pinning down their effects to a discrete physiological mechanism. This problem is especially notorious with systemic administrations of pCBs due to their target promiscuity [209]. To add another layer of complexity, eCBs can act on targets other than CB1R and CB2R. For example, AEA can function as an agonist for TRPV1 and PPARγ, whereas 2-AG can activate TRPV1 and the $GABA_A$ receptor [189] Consequently, the effects of hydrolysis inhibitors may be mediated by the interplay of multiple target receptors. In summary, while the pharmacological manipulation of the ECS offers a valuable avenue to explore the neurobiology of aggression, its implementation is not without challenges, as the diverse array of effects generated by this approach raises further inquiries about the underlying physiological mechanisms driving behavior. Thus, to derive meaningful conclusions about the involvement of eCB signaling in aggression, results from pharmacological studies should be pondered with the aforementioned limitations.

### I.3.2. ECS in drug-reward

Cannabinoid signaling in the NAc in known to enhance the hedonic response, or "liking" of natural rewards and drugs[131]. Intra-accumbal infusions of CB1R antagonists induces aversion to cocaine-associated contextual cues[216], reduces methamphetamine self-administration[217], and attenuates NAc DA release that responds to appetitive cues[218]. Additionally, NAc DA release by d-amphetamine was blocked by the systemic administration of rimonabant, a CB1R inverse agonist[219].The activation of CB2R dose-dependently reduces DA release in the NAc and induces conditioned place aversion[220] Intra-NAc infusion of DAGL inhibitor, which reduces the biosynthesis of 2-AG, was shown to reduce cocaine-seeking behavior[221]. Taken together, structure-wide reduction in CB1R signaling seems to reduce the rewarding impact of stimuli, likely by a reduction in DA release. Nonetheless, experimental approaches that target CB1R in specific afferents in the NAc demonstrate a more complicated reality.

Dopaminergic terminals innervating the NAc do not expresses CB1R, rather the receptor modulates terminal DA release by shaping the activity patterns of MSNs and their excitatory inputs[137]. CB1R is expressed in afferents from the mPFC, BLA, and vHip[144,147,222], as well as in fast-spiking interneurons (FSIs)[223]. Intra-NAc infusions of a CB1R agonist reduced intracranial optical self-stimulation of the excitatory PL-NAc pathway, suggesting that CB1R activation could be participating in a bottom-up downregulation of reward signaling originating in the mPFC[144]. Afferents from the BLA that project to D2 MSNs and express CCK have been reported to reduce the rewarding effects of sucrose and infusion of a CB1R agonist ameliorates this effect[147]. Thus, CB1R activation in the NAc can have distinct roles on reward depending on origin of input signaling. FSIs that express CB1R participate in feedforward inhibitory circuit which regulates output of NAc MSNs[223]. $CB1R^+$ FSI-mediated feedforward inhibition was robust when compared to lateral inhibition from MSN collaterals, which was sparce and weak[223]. Interestingly, CB1R

activation preferentially suppressed CB1+ FSI-mediated feedforward inhibition, while leaving lateral inhibition intact[223]. It remains an open question as to how this mechanism participates in behavior.

In rodents, intra-PL administrations of rimonabant in conditioned place preference (CPP) test increased cocaine-seeking[224]. Additionally, PL infusions of AM251 during the conditioning phase with a sub-threshold dose of morphine increased reward-seeking, while infusions of WIN55,212-2 induced aversion[225]. In healthy human participants, the magnitude of DA release in the NAc in response to amphetamine treatment was negatively correlated to the availability of CB1R in the ACC[226]. Furthermore, authors reported that the direct effect of mesolimbic DA release on the self-reported hedonic response was mediated by CB1R availability in the ACC, indicating its involvement in reward. Interestingly, CB1R availability was not a mediating variable wanting another treatment with amphetamine. The ACC's engagement in reward processing provides a control over behavior to maximize rewards and minimize punishments[227]. Even more, CB1R activity in the ACC has been implicated in effort related cost-benefit analysis[228], possibly by modulating the functional connectivity between the ACC and the NAcC[229]. Taken together, these results suggest that dampening CB1R signaling in the mPFC increases reward response to several drugs of abuse.

### I.3.3. Endocannabinoid signaling as an endogenous regulator of dominance

The literature linking the ECS to aggression is extensive[34]. For this reason, we will start by discussing how modulation of the ECS affects aggression as a platform for discussing social dominance. Historically, the study of cannabinoids and aggression has been a subject of contention for much of the last century and rages on today[8,49,50,230,231]. Among the reasons cited for the prohibition of marihuana was the belief that its intoxication directly provoked violence[230]. Studies exploring the link between aggression and cannabis use have discovered a nuanced relationship, which is shaped by both the individual's age of consumption initiation and the presence of underlying mental health conditions[48,232,233]. Notwithstanding, multiple investigations have confirmed that the acute administration of pCBs, selective CB1R/CB2R agonists, or FAAH/MAGL inhibitors has a significant impact on decreasing aggressive behavior in several animal models of aggression presented. Δ9-THC has been the most researched substance, and the vast majority of studies have found an aggression-reducing effect in several species[34]. A similar effect has also been reported in humans, where cannabis consumption reduced the use of hostile words[234] or self-reported aggression[235].

The evidence supporting the involvement of cannabinoid receptors in the regulation of aggression is substantial[34]. First, selective CB1R agonists reduce aggression[236,237]. The reduction of aggression by CB1R agonists[237], CBD[238], or by the pharmacological enhancement of endocannabinoid signaling[237], is attenuated by AM251. However, contradictory findings have been reported with administration of AM251 on its own[237–239]. Holding this in mind, the function of AM251 as an antagonist has been put into question, since the substance can act as a CB1R inverse agonist[240] and a GPR55 agonist[241], which could contribute as a confounding variable.

Higher aggressiveness observed in mutant mice lacking CB1R[236,242,243] or mice with decreased CB1R expression[237] strengthens the notion that CB1R activation by endogenous ligands could be contributing mechanism to the regulation of aggressive behaviors. Although CB2R is considered to play a role in aggression similar to CB1R[244], the current body of research investigating its function in this context remains limited. Further studies are needed to unravel the full extent of CB2R's involvement and its implications for aggression. Circling back to pCBs, their anti-aggressive effects are likely driven by the activation of multiple cannabinoid and non-cannabinoid receptors, such as the 5-HT1A[238]. Nevertheless, no research has tested if the effects of Δ9-THC on aggression are blocked/attenuated by a CB1R/CB2R antagonist[34].

Currently, there is a dearth of research elucidating the mechanistic intricacies of how eCB signaling regulates aggression[34]. In one study, the participation of eCB signaling in the vHip was presented as a mechanism by which CB1R could be downregulating aggression[237]. However, these findings were obtained using a double-hit stress model meant to simulate impulsive aggression, where individuals were subjected to postweaning social isolation (SI) and foot shocks. Evidence provided in this study demonstrates that this mechanism becomes less relevant in group-housed (GH) mice, thus constraining the applicability to explaining abnormal forms of aggression. Complementarity, social stressors known to induce aggression, like SI and maternal deprivation (MD), have been reported to lower CB1R expression in the mPFC[245,246], suggesting that changes in eCB signaling could be playing a role in the formation of abnormal aggression.

### I.3.4. Alteration of cannabinoids on social interaction within dominance hierarchies

Aggressive encounters are a dynamic and coordinated effort between interactants, whereby they exchange information about their relative fitness and adjust behavior to minimize the risk of injury[247]. Consistent with this notion, aggressive behavior of an untreated interactant increases in the presence of an opponent drugged with hashish extract, Δ9-THC, or MAGL inhibitor[239,248,249]. Specifically, this effect was observed in untreated subjects with the most propensity to fight (i.e., dominant, resident, or "approaching"). Miczek reported in Δ9-THC-treated subordinates a reduction in behaviors that signaled submission, which are known to play a vital role in de-escalating fights and reducing the risk of injuries in various animal species[99]. Consequently, it is reasonable to hypothesize that the increased aggression observed in untreated animals could be linked to impaired submission signaling in their drug-administered partner.

In a series of experiments[250], colonies of mice consisting of 5 males and 12 females were housed in an ecologically designed housing complex. For each experiment, Δ9-THC was administered to a single subject per colony, and territorial behaviors were assessed for five days after drug treatment. The top-ranking male mice in the hierarchy experienced a rank decline within 24 hours following the intravenous administration of 20 mg/kg of THC. Interestingly, this effect was only observed in colonies that demonstrated greater intragroup conflict, i.e., lower asymmetry in aggressive interactions. In despotic hierarchies where only one individual dominates the others, Δ9-THC administration did not affect the rank of the dominant subjects. Additionally, the administration of Δ9-THC to subordinate mice in a despotic hierarchy also did not impact their rank. When using a lower dose of Δ9-THC (0.5 mg/kg; i.v.), it was found that dominant subjects

(regardless of group structure) displayed less aggression directed toward an intruder in the group without affecting their rank.

Taken together, the interference by cannabinoids on aggressive/submissive behaviors can have distinct consequences that depend on the status of the treated individual. Thus, cannabinoids may increase the asymmetry between an untreated dominant and a treated subordinate by altering the expression of behaviors that signal submission. Conversely, the decline in rank among treated dominants in unstable hierarchies can be attributed to their reduced aggressiveness, which is perceived as a diminished threat by untreated rivals, ultimately leading to their overthrow.

### I.3.5. Section conclusion: ECS at a crossroad

Gaining insight into the intricate neurobiological mechanisms that underlie the relationship between drug-seeking behaviors and social dominance holds paramount importance as it has the potential to illuminate the vulnerabilities that make individuals more prone to SUD. In earlier sections, evidence indicates that the ECS within the mPFC and NAc is a potential linchpin between drug-seeking and aggression.

### I.3.6. Disclosure of section contents

A portion of text in the current chapter has been published in the review article Migliaro et al. (2023), which Martin Migliaro wrote and revised. Furthermore, M.M. is credited as the first and corresponding author.

# Chapter II: Thesis problem statement, question, and hypothesis

## II. Thesis research scope

### *II.1. Problem statement*

SUD presents a pervasive public health challenge, with addiction development associated with inherent and contextual risk factors in vulnerable individuals. Interested in understanding factors that predispose individuals to consuming drugs of abuse, we focused in understanding why dominance status and the rewarding effects of drugs of abuse are linked behavioral phenomenon[32,53,120,121]. It has been hypothesized that a common neurophysiological mechanism could explain the relationship between the manifestation of aggressive behaviors and drug seeking, as both patterns may be indicative of reward processing[4,110]. However, there has been no research conducted to investigate whether a shared mechanism can give rise to both behavioral profiles.

Literature suggests that reward sensitivity could account for the district drug-seeking profiles between dominant and subordinate individuals, though this hypothesis has not been tested outright. Notably, cocaine exhibits heightened reinforcing and rewarding effects for dominant rodents. This thesis will focus on the role of CB1R in two brain structures involved in reward-seeking behavior and aggression, the NAc and mPFC. Existing evidence suggests that blocking CB1R in the mPFC increases cocaine-seeking behavior, while blocking CB1R in the NAc induces aversion to cocaine. Furthermore, CB1R-KO increases aggression, while activating CB1R decreases aggression. Nonetheless, it is unknown if the ECS differs between dominant and subordinate in brain structures that underlying aggression and reward-seeking behavior. This study seeks to provide deeper insights into the intricate neurobiological mechanisms underlying SUD vulnerabilities, specifically investigating if CB1R in the mPFC and NAc acts as a link between social dominance and drug-induced reward, contributing to our understanding of the complex interplay between social dominance and drug-seeking behavior.

#### *II.2.1. Primary question and hypotheses*

- $Q_G$: Is there a connection between social dominance and drug-induced reward through the involvement of the CB1R receptor in the mPFC and NAc?
    - $H_{G1}$: If dominant rats exhibit greater drug-seeking behavior, then it is expected that they have a distinct endogenous expression of CB1R compared to SUB rats: lower expression of CB1R-in mPFC and higher in-NAc.
    - $H_{G2}$: If CB1R serves as a link between social dominance and reward, it is anticipated that artificially reducing the receptor should favor the acquisition of dominant status and drug-seeking behavior.

### *II.2.2. Specific questions, hypotheses, and objectives of first study*

- $Q_1$: Considering that dominant rodents consume and seek more cocaine, are dominant rats more sensitive to drug reward?
    - $H_1$: If dominant rats are more sensitive to the rewarding effects of AMPH, then it is expected that dominant rats would require a lower dose of AMPH to demonstrate reward-seeking behavior.
    - $O_1$: To determine drug reward sensitivity, conditioned place preference will be assessed in dominant and subordinate rats with multiple doses of amphetamine.

- $Q_2$: Considering that anxiety is linked with drug consumption and the robust drug-seeking pattern of dominant rodents, are dominant rats more anxious?
    - $H_2$: If anxiety is associated with increased drug-seeking, then dominant rats will demonstrate more anxiety-like behavior.
    - $O_2$: To assess anxiety-like behavior, the exploratory behavior of dominant and subordinate rats will be evaluated in the elevated plus maze.

- $Q_3$: Considering that role of CB1R in drug reward (more detail below) and the robust drug-seeking pattern of dominant rodents, is the endogenous expression of CB1R and other ECS components in the mPFC and NAc different between dominant and subordinate rats?
    - $H_{3a}$: Previous studies show that blocking CB1R in the mPFC increases drug reward. If lower activity of mPFC-CB1R is associated with augmented drug reward, then a lower level of mPFC-CB1R is expected in dominant rats.
    - $H_{3b}$: Previous studies show that blocking CB1R in NAc decreases drug reward. If lower activity of NAc-CB1R is associated with decreased drug reward, then a higher level of NAc-CB1R is expected in dominant rats.
    - $O_3$: To evaluate the endogenous levels of CB1R and other ECS components in the mPFC and NAc, western blot will be used to assess protein expression from samples of each structure taken dominant and subordinate rats.

### *II.2.3. Specific questions, hypotheses, and objectives of second study*

- $P_1$: Considering that dominant rats have lower expression of CB1R in the mPFC, does the expression ACC-CB1R play a causal role in determining dominant status among rats?
    - $H_1$: If lower expression of ACC-CB1R plays a causal role in social dominance, then it is expected that an artificial reduction in ACC-CB1R will favor the acquisition of dominant status.

- $O_1$: Dominance acquisition will be evaluated in rats that received an ACC-infusion of a viral vector coding a shRNA that blocks the production of CB1R or a control viral vector.

- $P_2$: Considering that privileged access to resources is highly correlated with dominance status, does the expression ACC-CB1R increase resource competition as well?
    - $H_2$: If ACC-CB1R is expected to favor the acquisition of dominant status, then it is expected that an artificial reduction in ACC-CB1R will also increase competitiveness for a vital resource.
    - $O_2$: Performance in the resource competition test will be evaluated in rats that received an ACC-infusion of a viral vector coding a shRNA that blocks the production of CB1R or a control viral vector.

- $P_3$: Considering that CB1R in the mPFC has been associated with anxiety and anxiety is linked with drug consumption, could the artificial reduction in ACC-CB1R elicit anxiety-like behavior?
    - $H_3$: If ACC-CB1R is linked to the expression of anxiety, then it is expected that an artificial reduction in ACC-CB1R will alter anxiety-like behavior.
    - $O_3$: Exploratory behavior in the elevated plus maze will be evaluated in rats that received an ACC-infusion of a viral vector coding a shRNA that blocks the production of CB1R or a control viral vector.

- $P_4$: Considering that dominant rats are more sensitive to the rewarding effects of ACEA and have a lower expression of CB1R in the mPFC, does the expression ACC-CB1R play a causal role in drug-induced reward?
    - $H_4$: If lower expression of ACC-CB1R plays a causal role in drug reward, it is expected that a reduction in ACC-CB1R would favor the seeking of ACEA.
    - $O_4$: Drug reward, as measured with conditioned place preference with ACEA, will be evaluated in rats that received an ACC-infusion of a viral vector coding a shRNA that blocks the production of CB1R or a control viral vector.

### II.3. On the structure of the thesis

The current dissertation is composed of two sequential studies and their results have each assigned to different chapters. The first of these studies is published and served an exploratory purpose, establishing the groundwork consequentially built upon by the second study. Lastly, the general discussion and conclusions are addressed in the last two chapters of the thesis.

# Chapter III: Methods and materials

### III.1. Subjects

Ninety (n =150) male Wistar rats (weighing 300–350 g) were obtained from the Animal Facility at the Faculty of Medicine, Universidad Nacional Autónoma de Mexico. Access to food (Rodent Diet 5001, LabDiet) and water was unrestrained. Rats were maintained in a 12:12 h. reversed light schedule (lights off from 8:00 am to 8:00 pm) with constant ambient temperature (22C◦±2) and humidity (52 % ±2). All animals were weaned at 30 days of age and cohabited with same sex littermates until they were 10–11 weeks old. When the appropriate weight was reached, rats from different litters were weight-matched and assigned to a triad or dyad, depending on the experiment. Each group cohabited in an acrylic home-box (50 × 40 ×20 cm) for 10 days before any experimental procedure. Tails were marked with a distinctive label made with permanent marker to keep track of individual identity. Experiments were carried out during the active period of the animals, between 10:00 a.m. and 4:00 p.m. All experimental and animal husbandry procedures adhered strictly to the Official Mexican Regulation on "Technical specifications for the production, care and use of laboratory animals" (NOM-062-ZOO-1999) and were in agreement with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003); and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996; and in compliance with the ARRIVE (Animal Research: Reporting in Vivo Experiments) guidelines (www.nc3rs.org. uk/arrive-guidelines); and were approved by the Ethics and Scientific Committees of the Faculty of Medicine, UNAM (061-2019/014-CIC- 2019).

### III.2. Viral vector for in vivo gene silencing of CB1R and control vectors

Lentiviral particles with CB1R shRNA small-hairpin RNA (shRNA, sc-270168-V) that target the rat CNR1 gene, control shRNA (sc-108080), or green fluorescent protein-like from copepod (copGFP, sc-108084) were purchased from Santa Cruz. 1 µl volume of $1 \times 10^6$ infectious units of lentivirus was delivered into the ACC according to the manufacturer's instructions and previous study[237]. To standardize coordinates in the ACC, rats that had received infusions of copGFP control lentiviral particles were subjected to transcardial perfusion following two weeks of incubation. Brains were extracted and sliced into 35 µm coronal sections using a cryostat. Finally, slices mounted with VECTASHIELD medium with DAPI (Vector Laboratories) and an epifluorescence microscope was used to verify injection sites.

### III.3. Stereotaxic surgery, micro-infusions, and recovery

Animals were anesthetized with ketamine/xylazine (75/5 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf). One of three viruses was bilaterally infused into the ACC (anterioposterior, 2.7 mm; mediolateral, ±0.5 mm; dorsoventral from meninges, −2.4 mm)[251] at a rate of 0.1 µl /min. as previously reported[237] (Fig. 5A). During the incubation period, rats with CB1R shRNA or control shRNA were allowed to recover and cohabit in dyads in modified home-boxes. These enclosures featured a perforated acrylic central divider along its width separating each subject in equally sized compartments that served to prevent physical contact while still enabling the exchange of visual, auditory, and olfactory cues.

### III.4. Social interaction

The analysis of dyadic agonistic interactions in same sex-groups is a robust and replicable ethological model of dominance that retains the complexity of group-dynamics[129,252–256]. Aggressive grooming (AG) and dominance posture (DP) are stereotypical behaviors readily expressed in rats and are both classified as aggressive behaviors[100]. An effective indicator of dominance is when aggression is concurrently expressed with recipient submission. Accordingly, AG involves a series of bites in rapid succession directed to the neck or nape while the recipient stays immobile. Meanwhile, DP (also referred to as pinning) occurs when one rat is on top of another that concurrently displays a supine posture[100].

To record agonistic interactions (aggression/submission) in freely interacting triads or dyads, each home box (50 × 40 ×20 cm) was assigned to a custom-built observation station fitted with an overhead near-infrared camera (Provision HD Analog IR Bullet Camera, I1-390AHDE36+, 30 FPS). To have an unobstructed camara view, the wire lid from each home box was removed and replaced with a wooden frame (50 ×40 cm) that extended wall height by 50 cm. Following the 10-day or 14-day cohabitation period for triads or dyads respectively, spontaneous behavior was recorded in one-hour long videos for three consecutive days. On the last day of recording, weight was measured. To account for the circadian pattern in agonistic behavior[257], rats were recorded during the dark phase under dim red-light conditions (~15 lx). To account for the circadian pattern in agonistic behavior[257], rats were recorded during the dark phase under dim, red-lights (~15 lx). The duration and directionality aggressive/submissive dyadic interactions (i.e., AG and DP;) were manually scored offline.

### III.5. Dyad assignment and social interaction test (SIT)

Six weeks after viral vector infusion, animals were assigned to groups with mismatching viral treatments. The first encounter of the newly formed dyads was recorded in a neutral arena for a duration of two hours. Aggressive/submissive interactions (duration of AG and DP) were scored offline. These neutral arenas later became their home box.

### III.6. Determination of social dominance in dyads

The rats' dominance was established by analyzing their spontaneous social interactions (SoIn), as detailed in the previous chapter[32]. SoIn was recorded in each home box (50 × 40 ×20 cm) assigned to a custom-built observation station fitted with an overhead near-infrared camera (Provision HD Analog IR Bullet Camera, I1-390AHDE36+, 30 FPS). Following the 14-day cohabitation period after dyad assignment, aggressive/submissive behavior was recorded in two-hour long videos for three consecutive days. During these recording sessions, animals did not have access to either food or water. To evaluate the stability of dominance status through time, this protocol was repeated for a second time 14-days after the first day of recording. To distinguish between these two sets of SoIn sessions, the first was referred to as Round 1 (SoIn Rnd1) and the latter as Round 2 (SoIn Rnd2).

The dyadic dominance index ($D_{ij}$)[258] was calculated for each individual from the duration of agonistic interactions. To account for the magnitude of events, the duration of agonistic interactions was transformed so that each second of the encounter was entered into the $D_{ij}$ equation as a single event[32,259].An individuals' $D_{ij}$ score reveals their likelihood of winning in pairwise interactions. Higher $D_{ij}$ values, those approaching 1, suggest that an individual tends to be the primary initiator or dominant figure in aggressive exchanges. Conversely, lower $D_{ij}$ values, nearing 0, indicate that an individual is more likely to be the recipient or subordinate party in such encounters. To distinguish between symmetrical and asymmetrical relations, the chi-squared test was used[63], where the null hypothesis states that there is no asymmetry in dyadic interactions and $p < 0.05$ was considered sufficient to reject the null hypothesis. In asymmetric relationships, individuals possessing the higher $D_{ij}$ were categorized as dominant, while those with lower scores were classified as subordinate.

**III.7. Determination of social dominance and social structure in triads**

Modified David's Score (MDS) is a measure of an individual's overall success in competitive encounters within social groups and is used as an indicator of dominance[258,260–263]. David's score is based on unweighted and weighted proportions of victories and defeats in dyadic interactions, where overall success considers the relative strength and weakness of interactants. This method of scoring was designed specifically for unbalanced paired-comparison data[264], which is a characteristic of agonistic interactions. The modification of the David's score[258] refers to the inclusion of a Bayesian estimator that corrects for chance and improves prediction. The value of MDS in triads ranges from 3 to 3, where positive values indicate that an individual is predominantly an actor in agonistic encounters. Conversely, negative values indicate that an individual is predominantly a recipient in agonistic encounters. To account for the magnitude of each interaction, the duration of agonistic interactions was transformed so that each second of the encounter was entered into the MDS equation as a single event[259].

The formation of discrete categories from dominance scores is a common and effective strategy to simplify the study of social dominance in relation to other behavioral and physiological variables[57,60,256,265]. Binary dominance status and ordinal ranking are two readily used classifications schemes based on dominance scores. The former classification segregates individuals into two categories according to the proportion of victories to defeats, where individuals that disproportionally win agonistic encounters are categorized as "Dominant" and those that disproportionally lose are categorized as "Subordinate"[60–62,124]. Dominance scores, like MDS, report this proportion and a discrimination threshold defined by the experimenter is used to assign an individual to a category.

Alternatively, the latter classification scheme ranks the dominance scores of individuals belonging to the same social group and assigns each individual to a distinct ordinal category (e.g., "α", "β", or "Ω"; alternatively, "Dominant", "Subdominant", or "Subordinate")[259,266]. Even though both classification schemes inform about the social dominance of individuals, ordinal ranking indicates an individual's position in a transitive dominance hierarchy[258,267]. Confusion in rank assignment arises when dominance scores from members of a group approximate and this

problem is prevalent in non-transitive dominance structures[261,267]. Therefore, proof of a transitive social structure is a prerequisite for the proper implementation of ordinal ranking[268].

Network architecture analysis was used to verify transitivity[104,269]. In brief, dominance relations in a group can be represented as a directed network, where individuals are nodes and edges inform about the directionality of dominance. Unidirectional and bidirectional edges were used to represent asymmetrical (i.e., dominance) and symmetrical dyads, respectively. Null dyads, or the absence of an edge between two nodes, were used to indicate the absence of interaction. $D_{ij}$[258] for each member of a triad was calculated and used to determine the directionality of dominance. $D_{ij}$ for each individual reports the probability of victories in a dyadic relation, where values that approximate 1 indicate that an individual is predominantly the actor in agonistic encounters. Conversely, values of $D_{ij}$ that approximate 0 indicate that an individual is predominantly the recipient of agonistic encounters. To distinguish between symmetrical and asymmetrical relations, the chi-squared test was used[63], where the null hypothesis states that there is no asymmetry in dyadic interactions and $p < 0.05$ was considered sufficient to reject the null hypothesis. A binarized dominance matrix was generated, where the dominant individual received a 1 in its row, and the subordinate received a 0. If an asymmetric relation cannot be proven (null hypothesis is not rejected), both individuals received a 1. This dominance matrix is analogous to an unweighted, directed adjacency matrix from where out- and in-degrees can be determined to identify the structure of each triad[266]. A transitive social structure, for example, demonstrates a feedforward motif with 3 unidirectional edges.

**III.8. Resource competition test (RCT) in dyads**

There is a strong correlation between dominance status and the privileged access to essential and palatable resources[129,255,270]. In the interest of having a supplementary behavioral metric to gauge an individual's dominance status, we implemented a resource competition assay. The apparatus involves a modified acrylic home box, featuring an acrylic partition that divides the arena into a common holding area and a narrow, short corridor that has a mounter water bottle. The entrance to the corridor is controlled by a guillotine door, which is lifted to allow access to the waterspout for only one animal.

This behavioral assessment comprised three sessions, one conducted each day, immediately following the recording of SoIn Rnd2. All sessions were carried out under the same lighting conditions as in SoIn. The first two sessions were conducted to familiarize individuals with the apparatus. During these sessions, one member of the dyad was placed alone with unrestricted access to the drinking area for 30 min (guillotine door lifted). Incentive to drink water is provided by the prior restriction during the social interaction recording session. After the last SoIn session of a round, both dyad members were prevented access to water for two more hours, thus totaling to 4 hours of water restriction. On the last session of RCT, rats did not have access to water for an additional two hours after SoIn, resulting in a total of 4 hours of water restriction. Subsequently, both members of the dyad were placed within the apparatus while the guillotine door was lowered. After a duration of 1 minute, the guillotine door was lifted, and the session

was recorded with the near-infrared camara for 10 minutes. Time spent drinking was manually scored offline.

### III.9. Elevated plus maze (EPM)

EPM provides an assessment of anxiety-like behavior by measuring the exploration of open (unprotected) arms compared to closed (protected by high walls) arms. The cross-shaped maze stood 90 cm above the ground and each arm measured 50 ×12 cm extended from a central platform (12 ×12 cm). The walls of the closed arms had a height of 50 cm. Subjects were placed in the center of the maze and allowed to explore for 5 min and were recorded with an overhead near-infrared camara (Provision HD Analog IR Bullet Camera, I1-390AHDE36+, 30 FPS). In triads, maze exploration was evaluated in a low-stress (low-intensity red lighting, LIRL; ~15 lx,) and a high-stress situation (high-intensity white lighting, HIWL; ~500 lx) to test the reactivity of dominants and subordinates to contextual stressors[243]. A counter- balanced experimental design was chosen with a one-week (7 days) separation between sessions, where half of the subjects were exposed to one of the stress conditions and the complementary condition was tested in the second session. In dyads, maze exploration was evaluated under HIWL in two sessions, 14 days apart.

A deep neural network (DNN) was trained to recognize body points using the open-source software DeepLabCut (version 2.2.1.1), which enabled the markerless tracking of animal movement in recorded EPM videos[271,272]. The DNN was trained and tested using 1000 labeled frames (190 ×220 pixels) of rats exploring the maze under both light conditions. A subset of labeled frames was held-out from training and used to evaluate the performance of the DNN (25 %). An NVIDIA GeForce RTX 3070 Laptop GPU (CUDA supported) was used to train the DNN for 200,000 iterations and analyze videos. Simplified Behavioral Analysis (SimBA)[273] was used to process pose estimation data into a region of interest analysis, which reported total movement (cm) and the duration (s) in open arms (OA) and enclosed arms (EA).

### III.10. Drugs

Dextroamphetamine sulfate (AMPH) was purchased from Sigma-Aldrich Inc (51-63-8). and was dissolved in sterile saline solution (0.9 % NaCl). ACEA was purchased from Cayman Chemical (91054). ACEA stock was dissolved in sterile saline solution (SS, 0.9 % NaCl) until it reached a concentration of 10 µg/kg/ml.

### III.11. Amphetamine/ACEA conditioned place preference (A-CPP)

A-CPP provides a measure of approach behavior towards a drug- associated environment that is incentivized by the rewarding effects of the drug[274]. The protocol implemented here has been described elsewhere[275]. The A-CPP chambers consisted of two visually distinct compartments (25 × 30 ×30 cm, each) connected by a central corridor (10 ×30 ×30 cm). In the pre-conditioning session (PRE), rats were allowed free access to all compartments for 15 min. This session was recorded and duration in each compartment was manually scored to determine the unconditioned preference to a particular compartment. The conditioning phase lasted 10 days

and in alternating days, an intraperitoneal injection of saline or AMPH (3.5, 5.0, or 6.5 mg/kg) or ACEA (10 μg/kg) was given before confining the subject in a distinct compartment for 30 min. The least preferred compartment in the PRE session was paired with AMPH or ACEA, while the most preferred compartment was paired with saline. The test session (TEST) took place on the last day, where rats were allowed free exploration for 15 min once more. This session was recorded and duration in each compartment was manually scored offline. CPP score was calculated by subtracting the time spent in the compartment associated to saline from the time spent in the compartment associated to AMPH or ACEA in both PRE and TEST sessions. AMPH doses were chosen based on another study from our laboratory[276]. ACEA dose was chosen based on unpublished data from our lab that tested several doses and reported that with this dose that only dominants demonstrated place preference.

### III.12. Tissue extraction and Western blot

The NAc (shell and core), and the mPFC (ACC, PL, and IL)[251] were extracted and suspended in a lysis solution containing 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Tris-buffered saline (TBS), pH 7.4, 10 mM phenylmethylsulfonyl fluoride (PMSF) and a protease and phosphatase cocktail inhibitor cOmplete (1 tablet/ 50 mL PBS) (Roche Diagnostics). Tissue samples were homogenized by sonication for 5 s (Cole Parmer 4710). Then, the homogenates were centrifuged (1000 g, 10 min, 4 ◦C) and the supernatant was collected and stored at 20 ◦C. From each sample, 50 μg of protein was electrophoretically separated and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore), which was then blocked overnight at 4 ◦C with nonfat dry milk (5 %, Bio-Rad) dissolved in TBS with Tween (TBS-T; 20 mM Tris·HCl; 136 mM NaCl; 0.1 % Tween-20; bovine serum albumin (BSA) 1 %, pH 7.4). One section of each PVDF membrane was incubated overnight at 4 ◦C with a rabbit polyclonal antibody against CB1R (1:500; Abcam, ab23703), CB2R (1:1000; Santa Cruz, sc-25494), FAAH1 (1:500; Abcam, ab54615), or DAGLa (1:500; Santa Cruz, sc-292130) diluted in TBS-T with 1 % BSA. The remaining portion was incubated overnight at 4 ◦C with a mouse polyclonal antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:15000; Chemicon International, MAB374) or vinculin (1:10000; Sigma-Aldrich, V9131) diluted in TBS-T with 1 % BSA. Membranes were then washed three times (10 min. each) with TBS-T and probed for 2 h. at room temperature with biotinylated donkey anti-rabbit or donkey anti-mouse secondary antibody (1:500; Jackson ImmunoResearch, AB-2340593 & AB-2307438) diluted in TBS- T with 1 % BSA. Later, membranes were washed and incubated for 2 h. at room temperature with avidin-biotin complex (1:500; Vector Labs) diluted in TBS-T with 1 % BSA. Immunoreactivities were visualized using 0.01 % diaminobenzidine, 0.05 % nickel ammonium sulfate, and 0.01 % hydrogen peroxide for 5 min. Blot analysis was done with ImageJ, where optic density of immunoreactivity of each protein of interest was normalized to a load control protein in the same sample.

### III.13. Statistical analysis

For all experiments, values are expressed as MEAN ±SEM. Differences between two groups were analyzed using an unpaired Student's t-test. A two-way ANOVA with repeated measures followed by a Tukey post-hoc test for multiple comparisons was used to compare data between groups

while accounting for multiple measurements taken on the same subjects across sessions or within a session. One-way ANOVA was used to compare total drinking time in RCT between groups. Spearman's rank-order correlation and linear regression were used to analyze the relationship between MDS and O.D. of eCBS components. To satisfy the requirements of linear regression, O.D. data was normalized with the following equation: log2(y +1). All statistical analyses were performed using jamovi (version 1.6) based on R (R CoreTeam. 2020) and $p < 0.05$ was considered significant.

# Chapter IV: Dominance status is associated with a variation in CB1R expression and amphetamine reward

**Chapter highlights**

- Dominant rats are more sensitive to the rewarding effects of d-amphetamine.
- Higher sensitivity of dominants to the rewarding effects of d-amphetamine cannot be attributed to anxiety
- Dominant rats have a lower endogenous expression of CB1R in the mPFC and the NAc

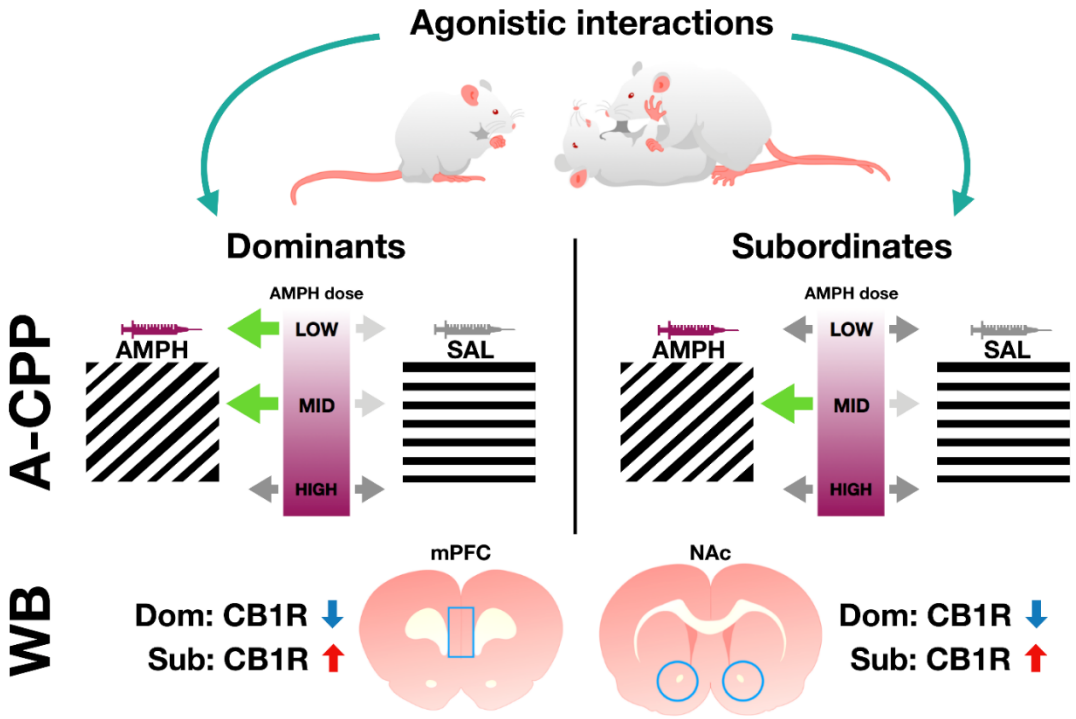


*Figure 4.* Visual abstract detailing main findings from first study. Male rats were classified as dominant or subordinate according to patterns in social interactions. Results from CPP show that dominant rats are more sensitive to the rewarding effects of AMPH. Anxiety-like behavior did not differ between dominant and subordinate rats, thus it is unlikely that anxiety incited drug-seeking in dominant individuals. Protein levels of CB1R were evaluated with Western Blot and showed that dominant rats have a lower endogenous expression of CB1R in the mPFC and the NAc. Design of figure is attributed to Dr. Eva Carolina Soto Tinoco.

IV.3. Study results

*IV.3.1. Dominance status, actor agonism, and weight*. A network architecture analysis was used to determine the group structure of each triad and showed that 57 % of triads (17/30) had a transitive social structure, while the remaining triads (13/30) demonstrated non-transitive dominance structures (Fig. 1E). No null dyads were identified in any group. We opted for a binary dominance status classification scheme (see Methods, Section 5.2.2) because ordinal ranking was inadequate for a substantial number of individuals (n =39). Considering that a modified David's Score (MDS) above zero indicates that an interactant wins more than it is defeated, any subject with a MDS above this threshold was assigned to the Dominant (Dom) group. On the other hand, individuals with a score below 0 were assigned to the Subordinate (Sub) group. This classification scheme revealed a total of 48 Dom and 42 Sub.

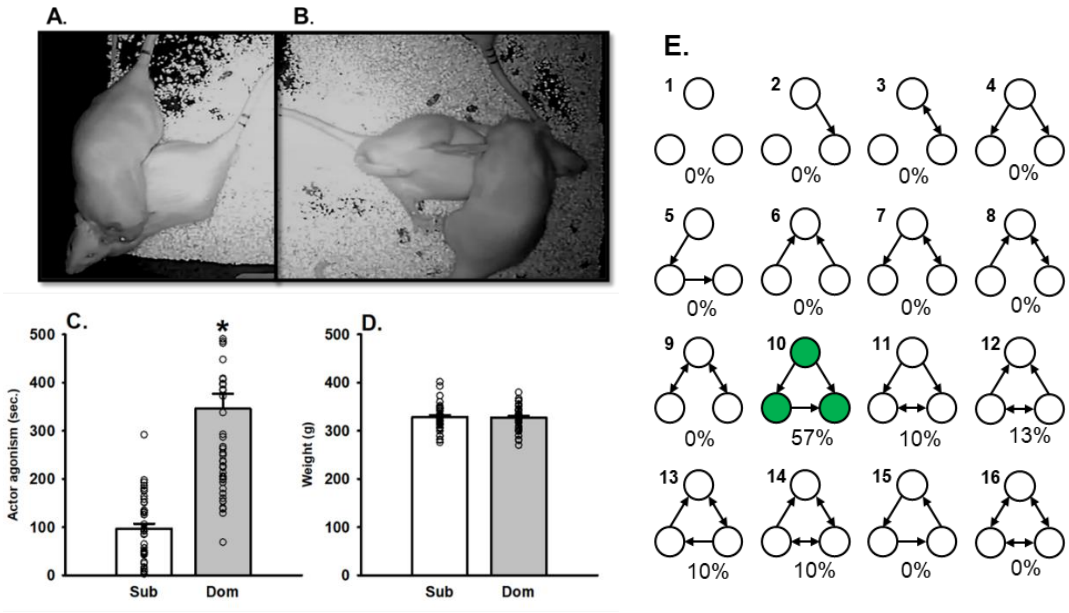


*Figure* 5. (A & B) Photographic examples of aggressive grooming and dominance posture. (C & D) Duration of actor agonism and weight of dominant ($n$ = 48) and subordinate ($n$ = 42) rats. *$p$ < 0.05. (E.) Network representations of all theoretically possible group arrangements and their prevalence among 30 rat triads Transitive dominance hierarchy is highlighted in green, which was expressed in 57% triads. Figure was published in Migliaro et al. (2022).

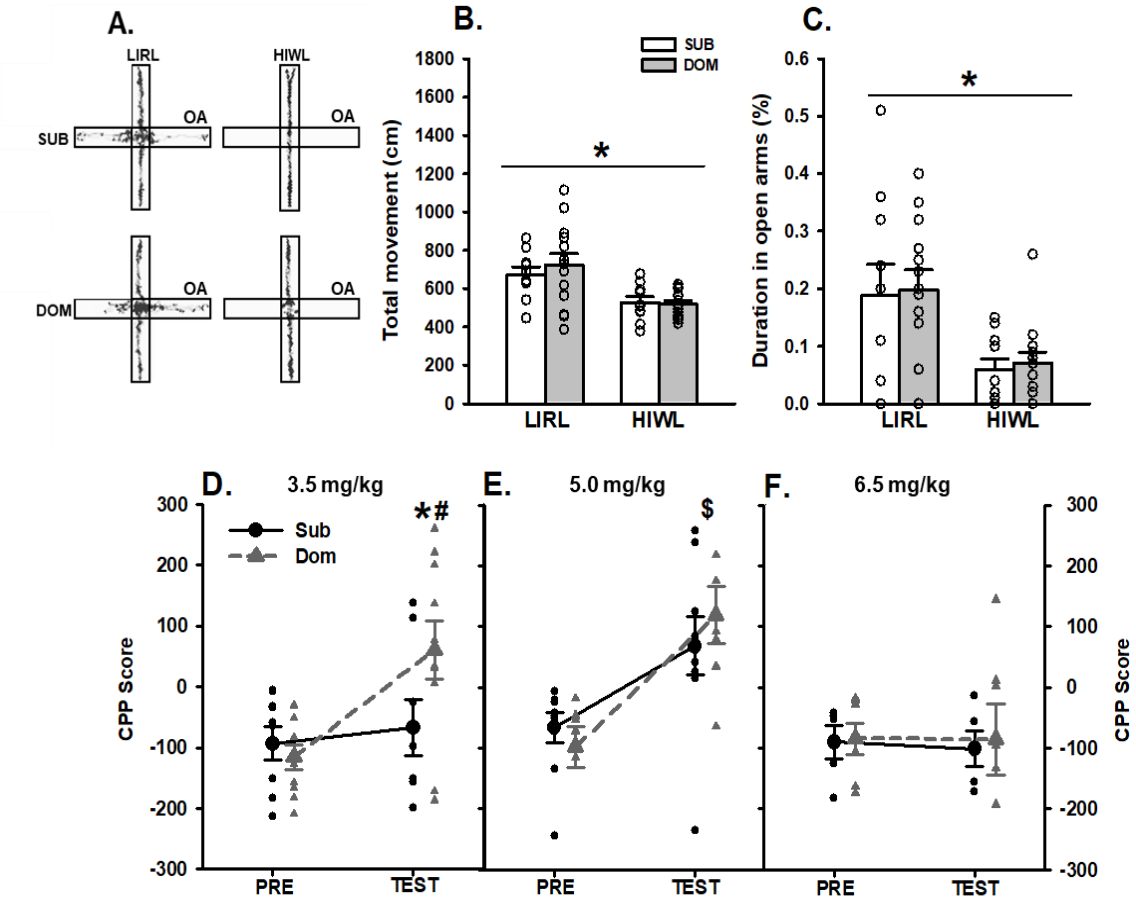


*Figure* 6. (A) Exploration of EPM of a SUB and a DOM under two lighting conditions: low-intensity red lighting (LIRL) and high intensity white lighting (HIWL). (B & C) EPM. Total movement (cm) and duration in open arms as a percentage of total time (n Sub = 10, n Dom = 13). *p < 0.05 main effect by the lighting condition. (D–F) CPP Score (time spent on the AMPH side [minus] time spent on the SAL side) at different doses of AMPH (3.5, 5.0, and 6.5 mg/kg) during the pre-conditioning session and test session. A-CPP 3.5 mg/kg (n Sub = 8, n Dom = 10); A-CPP 5.0 mg/kg kg (n Sub = 9, n Dom = 9); A-CPP 6.5 mg/kg kg (n Sub = 5, n Dom = 7). *p < 0.05 DOM vs SUB. #p < 0.05 DOM-_TEST vs DOM_PRE. #p < 0.05 TEST vs PRE. Figure was published in Migliaro et al. (2022).

Duration of actor agonism (time spent imposing any agonistic behavior, Fig. 1A &1B) was used to further verify the grouping of subjects. Dom individuals spent significantly more time ($t_{[88]}$ = 7.155, p <0.001) being actors of agonistic behaviors than Sub; about 4-times more, (Fig. 1C) and no weight differences ($t_{[88]}$ =0.167, p =0.868) were identified between Dom and Sub (Fig. 1D).

*IV.3.2. Anxiety-like behavior and dominance status.* To determine if anxiety was a variable that could explain any potential association between dominance status and drug reward, exploration of the elevated plus maze by Dom (n =13) and Sub (n =10) was evaluated under low-intensity red lighting (LIRL) and high-intensity white lighting (HIWL). Mean distance travelled was found to be lower in HIWL and the statistical analysis reported a significant main effect by the lighting condition($F_{[1,20]}$ =15.055, p <0.001); however, no differences were observed between Dom and Sub (Fig. 2B). Furthermore, percentage of duration in the open arms was lower in HIWL white lighting ($F_{[1,20]}$ =13.426, p =0.002) regardless of dominance status (Fig. 2C).

*IV.3.3. Rewarding effects of d-amphetamine and dominance status.* Drug reward was tested in three independent sets of Dom and Sub with increasing doses of AMPH. At the lower dose of 3.5 mg/kg (n Sub = 8, n Dom =10, Fig. 2D), the statistical analysis reported a significant main effect by Session ($F_{[1,16]}$ =9.728, p =0.007) and interaction between the factors DomStatXSession ($F_{[1,16]}$ =5.328, p =0.035), where only Dom demonstrated a preference for the AMPH-paired compartment (p <0.05, TEST vs. PRE). At the intermediate dose of 5.0 mg/kg (n Sub =9, n Dom =9, Fig. 2E), a main effect of Session was observed ($F_{[1,16]}$ = 13.990, p =0.002), where both dominants and subordinates developed a preference for the AMPH-paired compartment. Lastly, neither group (n Sub =5, n Dom =7, Fig. 2F) demonstrated an acquired preference for AMPH-paired compartment at 6.5 mg/kg.

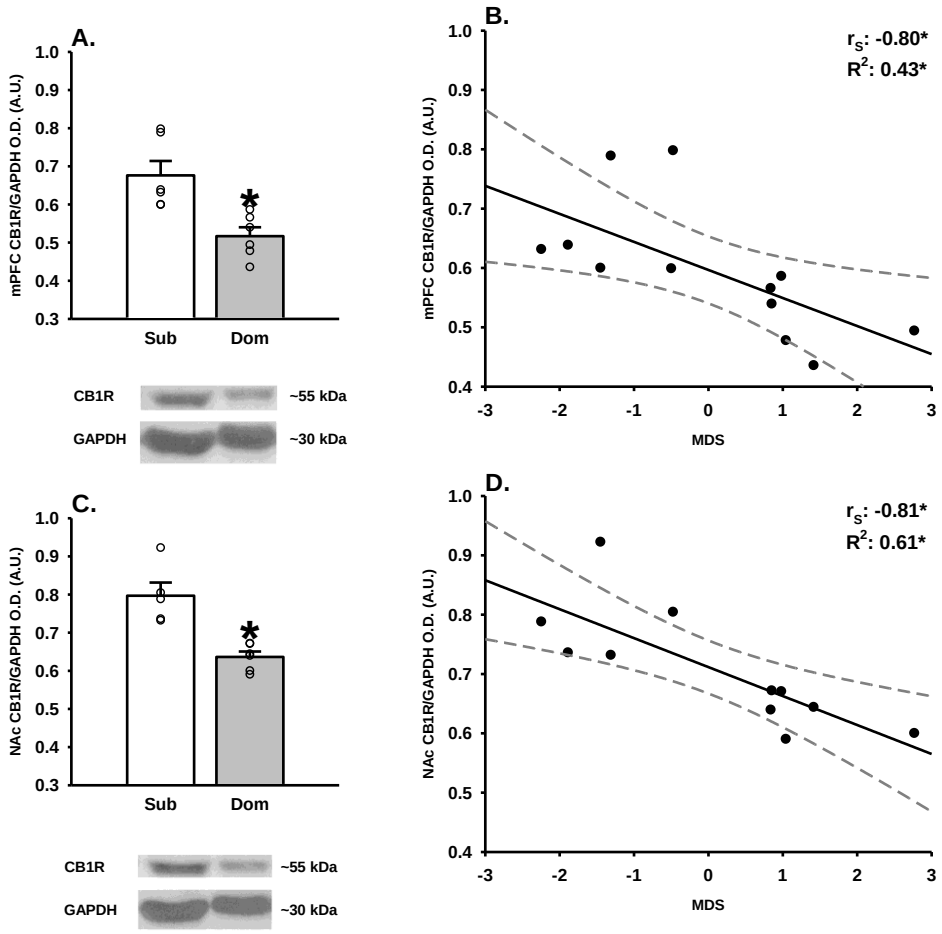


*Figure* 7. (A) Expression of CB1R in the mPFC (n Sub = 6, n Dom = 6) and (C) NAc (n Sub = 5, n Dom = 6). Analysis of correlation between MDS and CB1R expression in (B) mPFC and (D) NAc. *$p < 0.05$. Figure was published in Migliaro et al. (2022).

*IV.3.4. Association between the endocannabinoid system and dominance status.* Brain samples from a total of 6 Dom and 6 Sub that were not subjected to CPP nor EPM were used to determine protein expression of several components of the endocannabinoid system. Due to mishandling, one sample of NAc (Sub) was removed from the analysis. Sub rats had a higher expression of

CB1R than Dom in the mPFC and NAc ($t_{[10]}$ = 3.488, p =0.006; $t_{[9]}$ =4.403, p =0.002, Fig. 3A & C). Furthermore, MDS was inversely correlated with CB1R in mPFC (rS = 0.80, p < 0.001) and explained a significant proportion of the variance ($R^2$ =0.43, $F_{[1,10]}$ =7.543, p =0.020, Fig. 3B). In NAc, MDS was also inversely correlated with CB1R (rS = 0.81, p <0.001) and explained a significant proportion of the variance ($R^2$ =0.61, $F_{[1,9]}$ =14.300, p =0.004, Fig. 3D).

CB2R expression did not differ between dominants and subordinates in mPFC ($t_{[10]}$ =1.215, p =0.252, Fig. 4A) and NAc ($t_{[9]}$ =0.965, p = 0.360, Fig. 4D). Furthermore, the expression of FAAH1 (mPFC: $t_{[10]}$ = 0.184, p =0.858; NAc: $t_{[9]}$ = 1.149, p =0.280, Fig. 4B & 3E) and DAGLa (mPFC: $t_{[10]}$ =0.379, p =0.713; NAc: $t_{[9]}$ =0.387, p =0.708, Fig. 4C & F) did not differ. No correlation was observed between MDS and CB2R, FAAH1 nor DAGla.

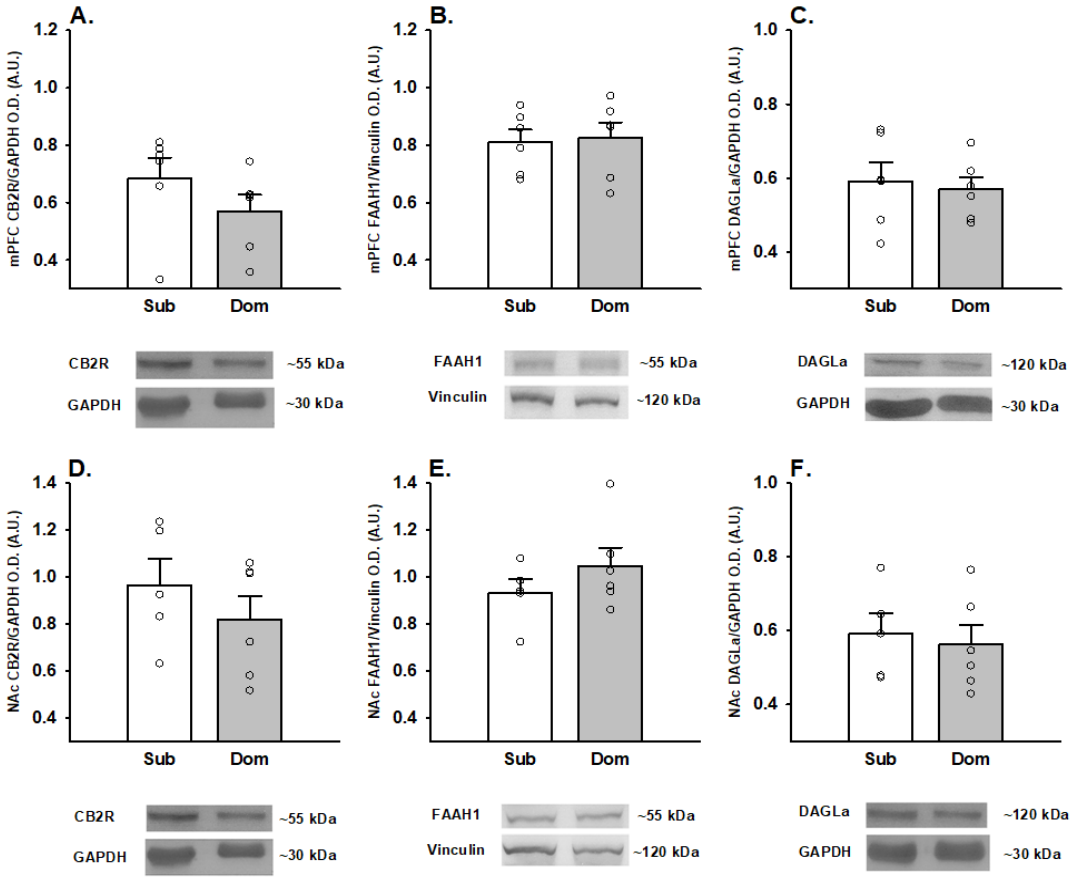


*Figure* 8. (A & D) Expression of CB2R, (B & E) FAAH1, and (C & F) DAGLa in the mPFC (n Sub = 6, n Dom = 6) and NAc (n Sub = 5, n Dom = 6). Figure was published in Migliaro et al. (2022).

### IV.4. Disclosure of section contents

Results from the current chapter have been published in the article Migliaro et al. (2022), which Martin Migliaro wrote and revised. Furthermore, M.M. is credited as the first author.

# Chapter V: CB1R of the anterior cingulate cortex links drug reward and dominance status

**Chapter highlights**

- ACC CB1R-KD favors social dominance without an abnormal expression of aggression.
- ACC CB1R-KD does not alter competitiveness for a vital resource.
- ACC CB1R-KD increases drug-reward while not affecting anxiety.

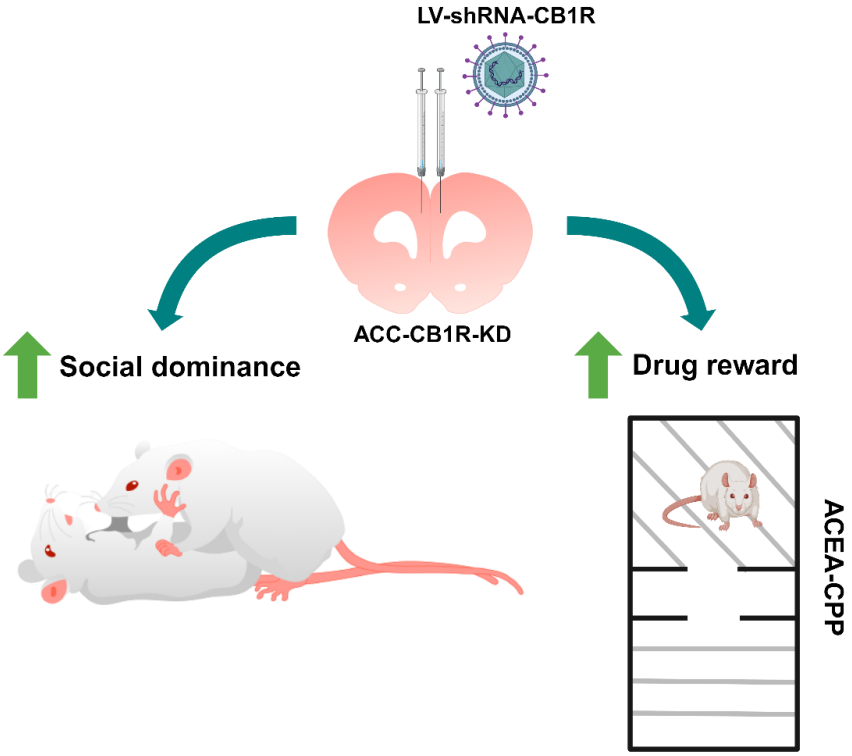


*Figure 9.* Visual abstract detailing main findings from first study. Male rats were infused in the ACC with lentivirus particles containing an shRNA that silences the CNR1 gene or control. CB1R-KD favors social dominance without an abnormal expression of aggression. Furthermore, ACC CB1R-KD increases drug-reward while not affecting anxiety.

## V.2. Study protocol

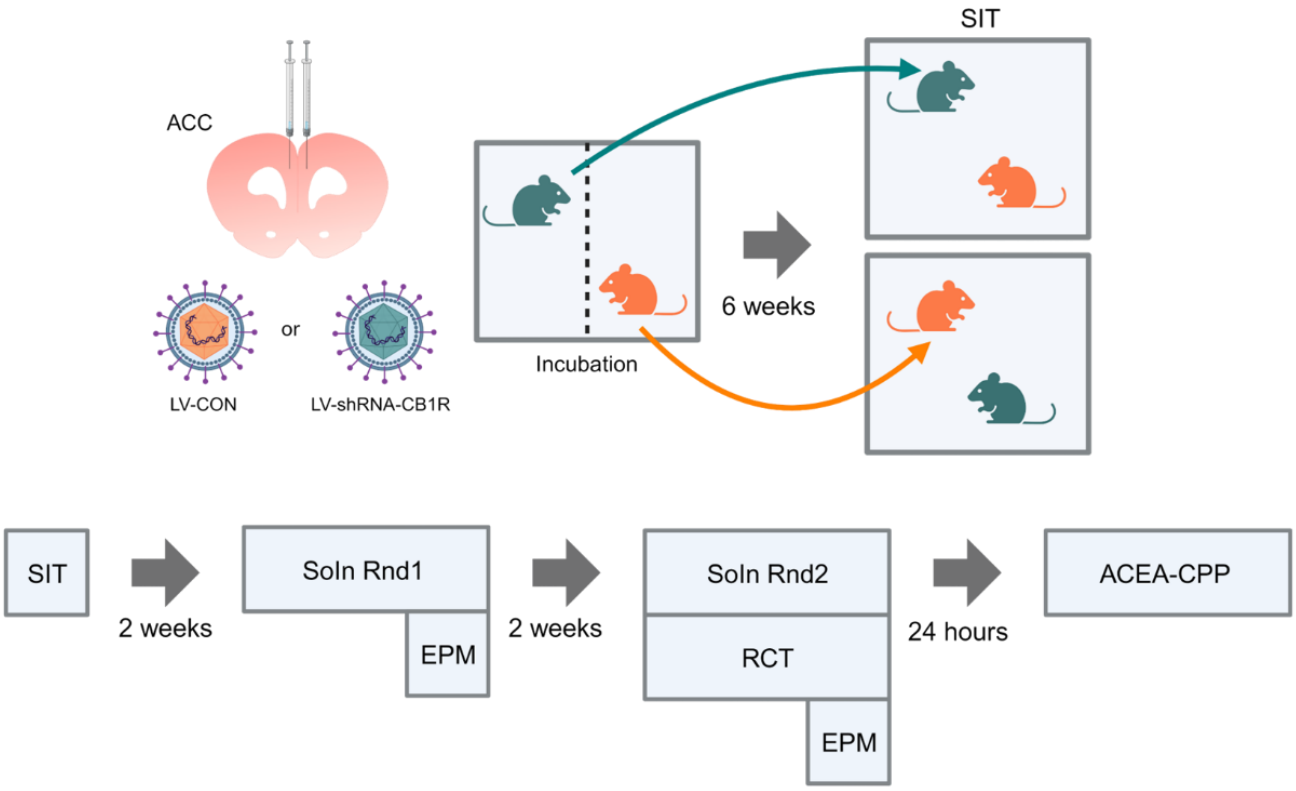


*Figure 10.* **(A)** Schematic of intra-ACC infusion of control lentiviral particles (LV-CON) or lentiviral particles for CB1R gene-silencing (LV-shRNA-CB1R). **(B)** In a six-week incubation period, animals were housed in dyads separated by a perforated acrylic central divider. After the end of the incubation period, rats were assigned to new dyads of mismatching viral treatments and the first encounter was recorded (SIT). **(C)** Social interaction was recorded two and four weeks after SIT, where each set of session was termed SoIn Rnd1 and SoIn Rnd2 respectively. SIT, SoIn Rnd1 and Rnd2 consisted of 2 hour-long sessions of spontaneous social interaction that were scored offline. SIT only consisted of a singular session, while SoIn rounds has three session, one session per day. Anxiety-like behavior was assessed with EPM in both SoIn rounds. To assess privileged water access, a RCT was conducted following each session of SoIn Rnd2. RCT was comprised of two habituation sessions and one test session, each held on separate days. ACEA-CPP protocol commenced twenty-four hours after start of the last session of SoIn Rnd2.

## V.3. Study results

*V.3.1. ACC CB1R-KD favors social dominance without an abnormal expression of aggression.* Two unacquainted rats, each one infused with LV-CON or LV-shRNA-CB1R in the ACC (Fig. 5A-B), were assigned to a dyad. A total of fifteen dyads were formed. The initial social interaction of the newly established dyads was assessed in a subgroup of eleven dyads, and actor aggression levels did not exhibit variance among animals infused with distinct viral vectors (Fig. 5C). The social interaction among these eleven dyads, in addition to four more dyads, was analyzed two and four weeks after their initial pairing (referred to as SoIn Rnd1 and Rnd2). No significant variations in aggression levels between the two groups was observed, though CON rats tended to decrease by SoIn Rnd2 (Fig. 5D). The dominance index of CON and CB1R-KD rats showed no significant difference in SoIn Rnd1 (Fig. 5E) and acquisition of dominance status was evenly distributed between CON and CB1R-KD (n=7, n=6), while four dyads were unable to establish a dominance relationship (Fig. 5F). In the subsequent round, CB1R-KD rats displayed an increase

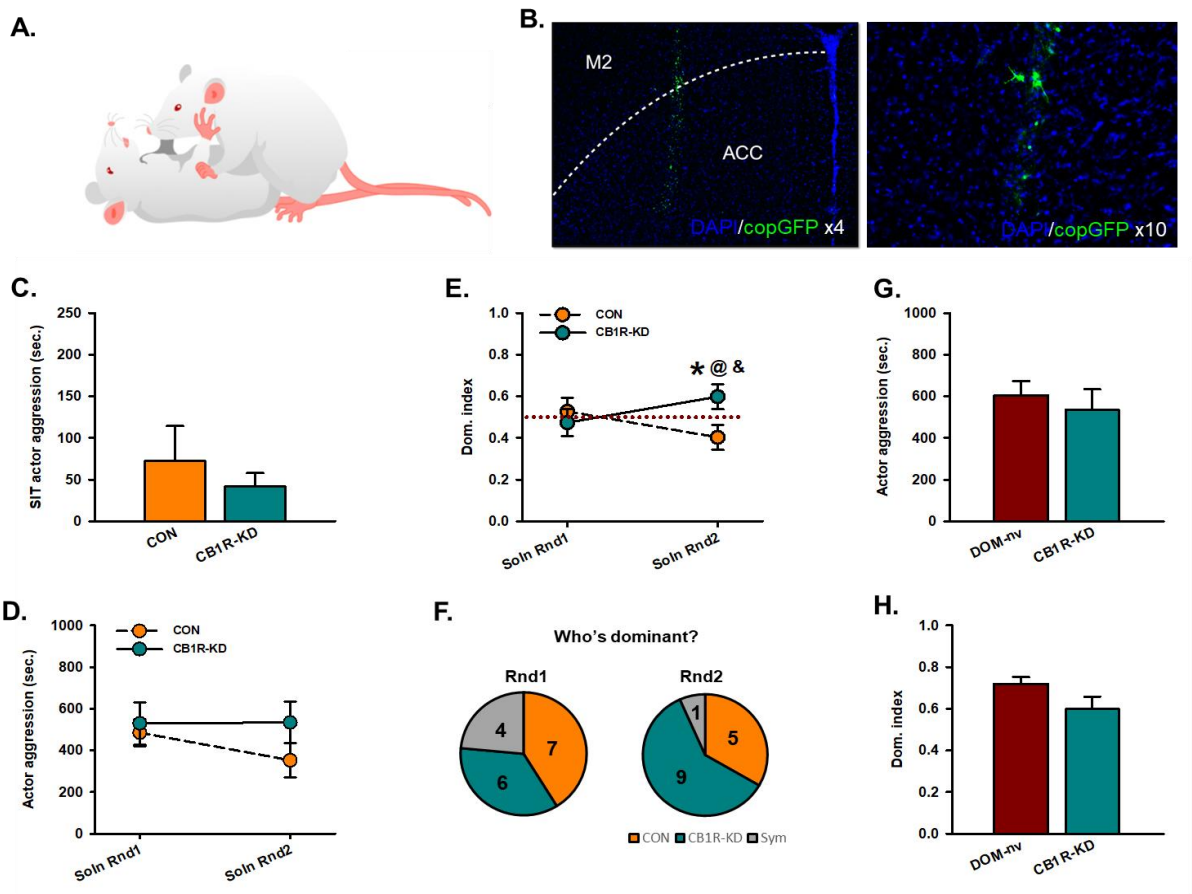


*Figure* 11. **(A)** Illustration of interacting rats that were previously infused with different viral vectors in the ACC. **(B)** Photographic example showcasing transfection of lentivirus particles with copGFP as reporter in the ACC used to standardize injection coordinates. **(C)** Actor aggression of CON (n=15) or CB1R-KD (n=15) rats observed in SIT. Actor aggression **(D)**, dominance index **(E)**, and dominance status **(F)** of CON or CB1R-KD rats two (Rnd1) and four weeks (Rnd2) after dyad assignment. **(G)** Comparison of aggression and **(H)** dominance index between DOM-nv (n=15) and CB1R-KD (n=15) rats. *p<0.05 CON vs. CB1R-KD. @p<0.05 CON SoIn Rnd1 vs. CON SoIn Rnd2. &p<0.05 CB1R-KD SoIn Rnd1 vs. CON SoIn Rnd2.

in their dominance score, surpassing CON rats (Fig. 5E). On the other hand, CON rats demonstrated a decreased dominance score by SoIn Rnd2 (Fig. 5E). When comparing the second round to the first, we observed a reduction in the count of symmetric dyads, with the important observation that most individuals identified as dominant (9/14) were CB1R-KD subjects (Fig. 5F). Taken together, this data indicates that CB1R-KD rats established dominance over CON rats. The actor aggression exhibited by CB1R-KD rats and their dominance scores were on par with the levels seen in naïve dominant rats (DOM-nv), the latter also evaluated during SoIn Rnd2.

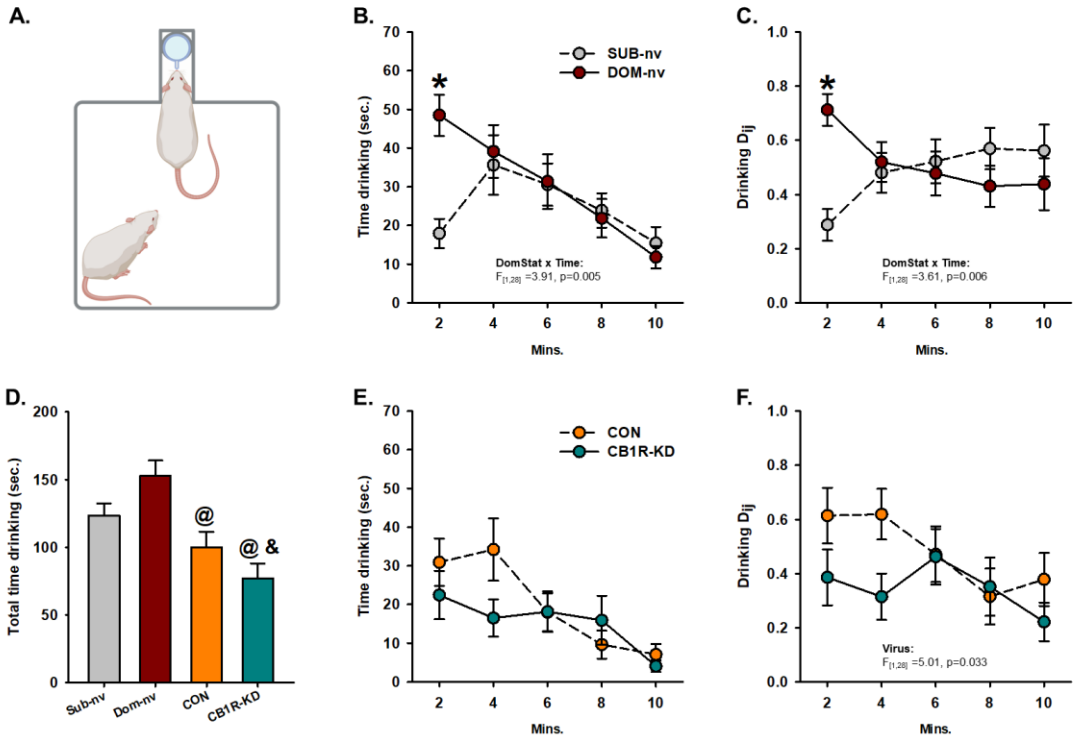


*Figure* 12. **(A)** Illustration of two rats in the RCT apparatus. **(B)** The duration of drinking and **(C)** drinking Dij by DOM-nv (n=15) and SUB-nv (n=15) rats throughout the duration of RCT. *p<0.05 SUB-nv vs. DOM-nv. **(D)** Total time spent drinking by naïve rats and rats infused with a viral vector. @p<0.05 vs. DOM-nv, &p<0.05 vs. SUB-nv. **(E)** Temporal pattern of drinking behavior in CON (n=15) and CB1R-KD (n=15) rats throughout RCT and **(F)** drinking Dij. *p<0.05 CON vs. CB1R-KD. The time-series data was binned and displayed in two-minute intervals.

*V.3.2. ACC CB1R-KD does not affect competitiveness for a vital resource.* The association between dominance status and exclusive access to essential resources is well-established[255,270]. Therefore, we conducted RCT in which dyads subjected to water restriction were placed in an arena where only one animal could access water at a time (Fig. 7A). To have a reference point, RCT was first carried out with rats that were not subjected to stereotaxic surgery four weeks after dyad formation. Social dominance was evaluated using an identical protocol to that used for rats receiving viral vector infusions, and their dominance status was used for classification as either naïve subordinate (SUB-nv) or naïve dominant (DOM-nv).

DOM-nv individuals spent significantly more time drinking than SUB-nv in the first two minutes of the test (Fig. 7B). For the remaining duration, the time spent drinking between DOM-nv and SUB-nv overlapped. Furthermore, a consistent decrease in the time allocated to drinking was witnessed in both groups over the course of the test, suggesting the onset of satiety. To evaluate dominion over the waterspout relative to dyad membership, $D_{ij}$ (i.e. dyadic dominance index) was calculated. Asymmetry was only observed in the first 2 minutes of the test, replicating what was seen in the previous measurement (Fig. 7C). Lastly, the total accumulated time drinking did not differ between DOM-nv and SUB-nv (Fig. 7D). These measurements confirm that dominant individuals have privileged access to water.

No statistical difference was reported in the time spent drinking between CB1R-KD and CON rats (Fig. 7E). Nonetheless, it does seem that CON rats tend to spend more time drinking at the four-minute mark. Though variability of data is high, statistical analysis of drinking $D_{ij}$ did report an effect by virus, indicating that CON rats had more access to the water than CB1R-KD rats (Fig. 7F). Comparison of the cumulative drinking time among all groups indicated that CB1R-KD rats spent less time drinking than both DOM-nv and SUB-nv rats, while CON rats drank less only when compared to DOM-nv (Fig. 7D).

*V.3.2. ACC CB1R-KD does not affect anxiety.* Anxiety has been proposed to play a mediating role between social dominance and drug reward[57,58,60], therefore we used EPM to assess anxiety-like behavior. Our observations did not reveal any variations in the exploration of open or enclosed arms, as well as locomotion, between the CON and CB1R-KD groups (Fig. 8B). Similar results were obtained in a follow-up assessment conducted two weeks later (Fig. 8C), where no differences were detected between groups for all measurements previously mentioned.

*V.3.3. ACC CB1R-KD increases drug reward.* Lastly, we used ACEA-CPP (Fig. 8D) to evaluate the effects of CB1R-KD on drug reward. We previously showed that dominant rats were more sensitive to the rewarding effects of d-amphetamine[32] and ACEA (Ostos-Valverde, unpublished). We also showed that dominant rats has a lower endogenous expression of CB1R receptor in the mPFC[32]. Accordingly, we tested if CB1R-KD in the ACC could also affect drug-reward to ACEA. Our observations revealed that CB1R-KD rats were the sole group to exhibit a conditioned preference for the context associated with ACEA delivery, as evidenced by the comparison of preference

indices between the preconditioning and test sessions (Fig. 8E). In addition, CB1R-KD rats exhibited a greater preference for the context associated with ACEA during the test session compared to CON (Fig. 8E).

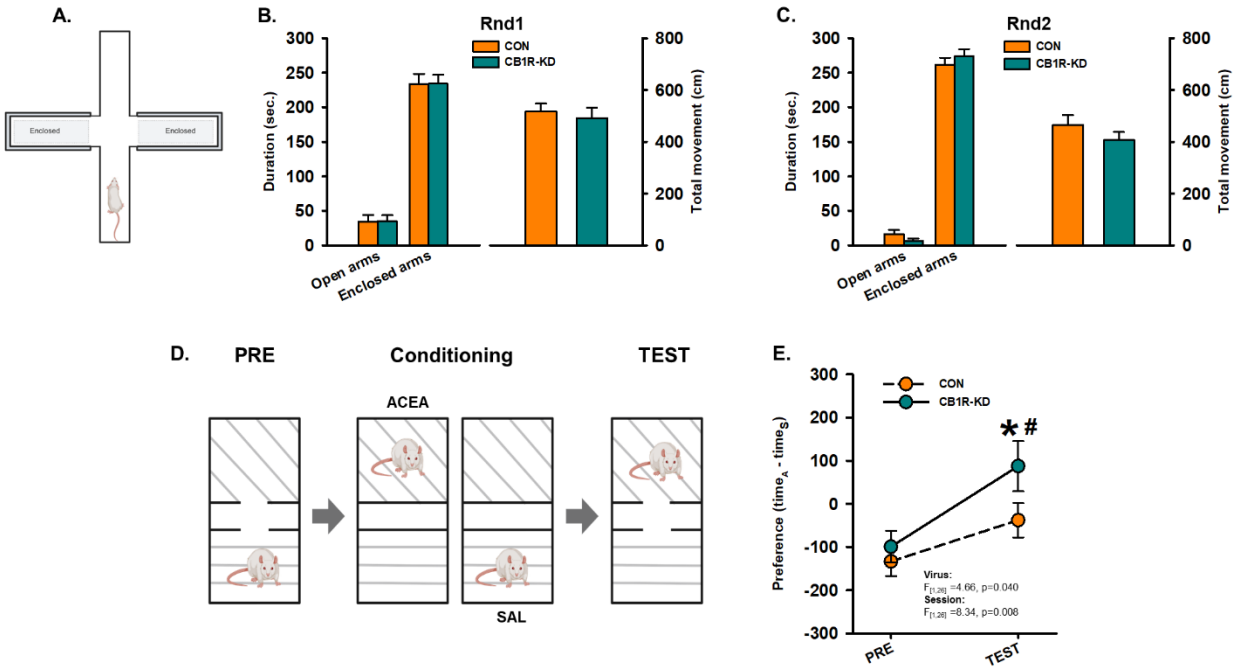


*Figure* 13. **(A)** Illustration of a rat exploring the open arm of EPM. **(B)** Left, results from Rnd1 demonstrating exploration of open and enclosed arms of EPM, comparing CON (n=15) and CB1R-KD (n=15) rats. Right, total distance traveled within the maze. **(C)** Findings obtained during the second evaluation using the EPM, conducted two weeks after the initial assessment with the same subjects. **(D)** Illustration of ACEA-CPP protocol sectioned into three phases: pre-conditioning (PRE), conditioning, and test. For full description of the protocol, please see Methods. **(E)** Preference for the context associated with ACEA delivery (10 µg/kg) in PRE and TEST comparing CON (n=14) and CB1R-KD (n=14) rats. Preference was calculated by subtracting time spent in the context associated with ACEA minus the time spent in the context associated with saline (sec.). *p<0.05 CON vs. CB1R-KD. #p<0.05 CB1R-KD PRE vs. CB1R-KD TEST.

# Chapter VI: General discussion

| Chapter highlights | |
|---|---|
| | CB1R is a link |
| | Translational relevance |
| | Wisdom of the body |

### VI.1. Summary of key results

Co-occurrence of traits associated with social dominance and drug consumption in humans led to the idea that there might be neurophysiological connection between these seemingly disparate behavioral patterns[4,53], offering a fascinating avenue for exploring the underlying mechanisms of reward and social status. It is worth noting being dominant and the consumption of drugs of abuse are both rewarding experiences[164], thus dispelling their apparent distance. At a critical junction, CB1R-mediated signaling assumes a central role in the modulation of both aggressive behavior and the pursuit of rewards[34].

The first study established the groundwork by demonstrating that dominant male rats, when compared to subordinates, had a lower reward-threshold to AMPH in CPP. Dominant rats required a lesser dose to engage in drug-seeking behavior, seen as a preference for the drug-paired compartment. Protein levels for various ECS components were measured in samples from the mPFC and NAc, where only CB1R differed between groups. Dominant rats showed a lower endogenous CB1R level in both regions of interest. Moreover, a robust negative correlation was found between the dominance score and CB1R expression, signifying a gradient relation between the variables. Nevertheless, associations were insufficient to infer a causal relation. Rigor demanded more evidence to ascertain such claims.

In the second study, the spotlight was set on the ACC, a region of the mPFC implicated in effort-based decision making that involves the procurement of rewards and overpowering of opponents[56,185,186]. To test if CB1R expression was a common link between social dominance and drug-reward, we sought out to artificially lower its expression. A long-term (several weeks) silencing of CB1R expression was needed, thus eliminating techniques involving the onsite infusion of small interference RNA (siRNA). Thus, a commercially available alternative was chosen, which involved the transduction via a viral vector of a plasmid coding for a shRNA. Levels of aggression observed in CB1R-KD rats did not differ from naïve dominant rats, suggesting that dominance was not attained through an aberrant form of aggression. Also, like naïve dominant rats, CB1R-KD rats demonstrated preference for the context associated with ACEA delivery (10 µg/kg), whereas rats infused with the control virus did not show a preference. However, not all aspects of naïve dominants were replicated, namely, CB1R-KD rats did not have privileged access to a vital resource.

### VI.2. CB1R in social dominance and resource competition

Dominance relationships involve submitting to a powerful individual that possesses the ability to inflict costs [68,92]. Thus, the formation of dominance relationships are driven by a cost-benefit analysis, where participants learn to interact with each other[174,277]. The assessment of outcomes in social encounters is acquired through trial-and-error, with victories strengthening competitive tendencies (i.e. winner effect) and losses leading to the gradual fading of competitive behavior (i.e. looser effect) [278]. From an ethological perspective, the spontaneous and unrestrained interaction between animals is informative of how they perceive and relate to one another[63]. As

such, we studied the patterns of cooccurring aggressive and submissive behaviors to elucidate the emergence of dominance relationships.

While the literature on the eCBS and aggression is extensive[34], the present study is the first to analyze the relationship between the mammalian eCBS and dominance status. Our data revealed that CB1R has a differential expression between dominants and subordinates in the mPFC and NAc, while no differences are observed in CB2R, FAAH1, and DAGla. Furthermore, a robust negative linear relationship between CB1R and the dominance score in both brain structures is observed, where higher expression of the receptor is associated with lower success in agonistic encounters.

In an ethological sense, social dominance is not a characteristic of two strangers interacting for the first time in a neutral arena (i.e., SIT), given that social relationships develop as conspecifics become acquainted with each other[63,88]. Nonetheless, heighted aggression towards an stranger rodent is a form of abnormal aggression that is characteristic of stressed rodents[279,280] or ACC hypofunction[281]. In the second study, our analysis of the initial encounter revealed that actor aggression levels did not exhibit significant variance between animals infused with different viral vectors, downplaying the importance of CB1R signaling in expression of aggression/submission between two strangers. The ECS has been implicated in a wide range of non-aggressive social behaviors[282], thus it is possible that other social behaviors, like those geared towards social exploration, might have been affected. After a cohabitation period of two weeks after dyad assignment (SoIn Rnd1), dominance index of CON and CB1R-KD rats was indistinguishable even though aggression/submission was present. The reason for this is twofold, 1) dominant individuals were almost equally distributed between groups and 2) a considerable number of dyads were symmetrical (i.e., absence of dominance relationship). Dominance of CB1R-KD is apparent four weeks after the first meeting (SoIn Rnd2), where the dominance index is higher in CB1R-KD rats and most dominant subjects belong to the CB1R-KD.

Neurons of the ACC has been shown to compute decision costs[182], which are particularly important guiding action selection. Chemogenetic inhibition of pyramidal neurons in the ACC was observed to decrease the number of reinforced responses to qualitatively-preferred / high-effort option[283]. Chemogenetic activation of excitatory pyramidal neurons of the ACC increases latency to attack and number of attacks in mice with high propensity to aggression[281]. This study also reported that the activation of the ACC also lead to a reduced the activity of subcortical structures that contributive to aggressive arousal and the probability of an attack, namely, BLA, LH, and VMH[281]. In another study, chemogenetic inhibition of pyramidal neurons in the ACC and PL (dmPFC) decreased displacement of cage-mates in the tube test, which involves effortful pushing an opponent out of a tube which can be contingent with a reward[185]. Rankings based on a round-robin tournament involving all pairings within a social group have demonstrated a robust correlation with rankings derived from spontaneous aggressive interactions, along with several other tests that assess social dominance[56]. In addition, when dmPFC pyramidal neurons were

optogenetically activated in subordinate mice, their performance in the tube test improved, and the resulting rise in their social rank endured for several days after the optical stimulation[185]. Taken together, glutamatergic output from the ACC is contingent with the inhibition of high-effort behavior, which manifests in both social and non-social decisions.

What, then, might be the function of CB1R signaling within the ACC, explaining the outcomes we have presented regarding the acquisition of dominance status? Microinjections of ACEA delivered into the ACC of rats were observed to reduce the choice for a high-reward/ high-effort option[228]. When there was no impediment, rats consistently selected the high-reward option, regardless of CB1R activation. Complementary to our results, intra-mPFC (including the ACC) infusion of an AAV designed to overexpress CB1R into the mPFC of mice resulted in the emergence of a behavioral pattern reminiscent of a subordinate animal, including a decrease in contact initiation, increase in active voidance, and increase in anogenital exploration[284]. Taken together, it seems that potentiating CB1R activity in the ACC elicits avoidance of decision costs, leading to the adoption of non-confrontational strategies in social contexts. Therefore, it seems logical to infer that gene silencing of CB1R would have the opposite effect, likely mitigating the costs associated with losing. Given that the actor aggression and dominance index of CB1R-KD rats was dissimilar from naïve dominant rats, it is suggested that the attainment of social dominance is unlikely to be attributed to aberrant levels of aggression. Thus, it seems possible that dominance attainment favored by CB1R-KD is not best explained by a victory through overwhelming force, but rather could understood as a resistance to submit, akin to a victory in a war of attrition[247]. In this scenario, CB1R-KD rats ultimately become dominant due to hampered ability to factor in costs associated with fighting, a capacity that remains intact in their counterparts.

Competition for vital resources is another scenario where dominance manifests[129,255,270,285]. Herein, we have shown clear evidence that rats identified as dominant based on patterns of aggressive/submissive interactions with conspecifics also have a privileged access to water when it is most needed, namely, following a period of scarcity. Nevertheless, CB1R-KD did not behave like DOM-nv since they were not able monopolize the access to the waterspout. One feasible explanation is that the viral treatment may have affected water consumption directly, as demonstrated by substantial reduction in total drinking time in CB1R-KD rats in comparison to both SUB-nv and DOM-nv. Another explanation is that CB1R-KD does not simulate the entirety of social dominance. While raw drinking durations across the test showed no discernible differences between virus-treated groups, a main factor effect of virus type was evident in the $D_{ij}$ score. Although not as compelling as the findings in DOM-nv, this result suggests that it was CON, not CB1R-KD rats, who exhibited greater resource control. Why would CB1R-KD be able to divorce two closely intertwined behavioral patterns? SoIn and RCT both provide information about the outcomes of pairwise competition, however, the time scales for evaluation are widely different. SoIn entails sampling from a cumulative twelve hours of video footage over six days for

each dyad, whereas RCT is a brief, single-session assessment lasting only ten minutes. Hence, the presumed alteration in cost-benefit analysis by CB1R-KD might favor the attainment of social dominance over the long run, but not give an advantage in punctual competitions.

### VI.3. Social dominance, drug reward, and CB1R

In the first study, naïve dominant male rats were observed to prefer the drug-paired compartment with the low and intermediate doses of AMPH, suggesting that the threshold to engage in reward-seeking behavior is lower in dominant rats. This finding is in tune with previous reports that showed a stronger preference for the context associated with cocaine delivery in dominant mice[121]. Furthermore, recent unpublished data (Ostos-Valverde) from our research team indicates that dominant rats exhibit a reduced reward threshold when exposed to ACEA. This pattern mirrors observations made with AMPH, implying a potential generalization of these effects to both psychostimulants and cannabinoids. Regardless of dominant status, we observed that the highest dose of AMPH did not evoke conditioned preference, which can be attributed to the adverse effects of psychostimulants at this dose[286]. Furthermore, our results are consonant with human studies and therefore retain translational value. In people, high social status has been reported to promote approach-related tendencies to rewards[110] and personality traits associated with dominance have been found to predict substance use and abuse[53].

The pharmacological blockade of CB1R signaling in the mPFC of rodents has been shown to increase the rewarding effects of cocaine[224] and morphine[225]. Furthermore, lower availability of CB1R in the ACC was associated the self-reported hedonic sensation of amphetamine in humans[226]. In the second study, we focused on studying the rewarding effects of a selective CB1R agonist, ACEA. Assessing cannabinoid reward has long been acknowledged as a challenging endeavor, partly due to the widespread and abundant expression of CB1R throughout the central nervous system, as well as the multitude of localized signaling functions in which CB1R is involved. Δ9-THC induces conditioned place aversion in subordinate mice at the higher of the two doses but has no effect on dominants at either dose[122]. Notably, conditioned preference for the drug-paired compartment was not achieved in either group with Δ9-THC at doses tested. This compound is notorious for its offsite effects; therefore, we opted for a highly selective CB1R agonist instead. Nonetheless, we observed a conditioned preference for the context associated with ACEA delivery exclusively in CB1R-KD rats. Using positron emission tomography, a downregulation of CB1R in the ACC was observed in chronic cannabis consumers[287]. From these results, authors argued that changes in CB1R levels could be a mechanism promoting cannabis dependence. Collectively, our results align with prior research suggesting that the reduction of CB1R signaling boosts drug-reward.

### VI.4. Anxiety as possible driver of drug reward for dominants

Subordination stress has been hypothesized to be a mediating variable between dominance status and drug consumption, particularly alcohol[44,45,58,60,288]. Anxiety-like behavior is oftentimes an indicator of subjects that suffer social chronic stress[279], however, we did not find differences in anxiety-like behavior between dominants and subordinates measured by open arms exploration. Since rats are nocturnal foragers and photophobic[289], we evaluated EPM exploration under two lighting conditions to test if there was a differential reactivity to contextual stressors[243]. Not surprisingly, subjects under HIWL explored the open arms less and moved less compared to LIRL, regardless of dominance status. Consequently, it seems unlikely that anxiety nor the reactivity to stressors are involved in the differential sensitivity to the rewarding effects AMPH.

Available evidence suggests mPFC-CB1R plays a role in regulating anxiety-related behaviors[201]. It has been suggested that upregulation of CB1R in the mPFC by the exposure to chronic stressors underlies anxiety, as suggested by animal studies and postmortem analysis in patients that suffered mood disorders[201]. However, in a rodent study where a viral vector was used to overexpress CB1R in the mPFC did not lead to an anxious profile in EPM[284]. In another study, CB1R expression in the mPFC has been shown to positively correlate with open arm exploration in EPM[246]. Herein, CB1R-KD in the ACC does not seem to affect anxiety-like behavior as measured by EPM. Due to these inconsistencies, it is possible that CB1R involvement in anxiety-related behaviors within the mPFC is region-specific and calls for more granular research to elucidate the precise mechanisms governing these effects.

### VI.5. Disclosure of section contents

A portion of text in the current chapter has been published in Migliaro et al. (2022) and Migliaro et al. (2023), in which Martin Migliaro wrote and revised.

# Chapter VII: General conclusions

| Chapter highlights | |
|---|---|
| | CB1R is a link |
| | Translational relevance |
| | Wisdom of the body |

### VII.1. CB1R is a link between dominance status and drug-reward

This thesis amalgamates two investigations aimed at understanding vulnerability to drug abuse. Parting from the epidemiological observation that aggressiveness and drug use are co-occurring phenomena, and through the adjusting lens of ethology, CB1R was identified as a potential link between the two behavioral tendencies. Having the evidence in hand, we can now more confidently assert that CB1R signaling in the ACC plays a role in an underlying mechanism for both social dominance and drug reward. It's conceivable that CB1R-KD is reshaping the processes underlying general decision-making, ultimately leading to a bias towards social confrontation and approach-related tendencies. Moreover, both domineering and the sensation evoked by substances of abuse are positive valence experiences. This commonality between dominance and drugs is not shared with resource competition, where behavior is motivated by the drive to quench a negative sensation (i.e., thirst) possibly explaining why CB1R-KD failed to replicate all behavioral aspects of naïve dominants.

Research begets research. CB1R is primarily found in the terminals of GABAergic interneurons in the ACC, although a small fraction is observed in glutamatergic afferents[228].It is tempting to attribute the behavioral consequences observed as GABA-dependent mechanism, but the evidence presented herein is not sufficient. The technique used to measure endogenous CB1R content in naïve rats was not informative about the type of cell where differential expression occurred. Even more, the gene silencing virus was not engineered to be selective to cell type, thus opening the possibility of a non-neural mediator (e.g., astrocytes or microglia). As alternative or even cooccurring mechanism can be attributed to offsite effect. Since CB1R is predominantly presynaptic, it is reasonable to infer that gene silencing of CB1R in pyramidal neurons should have a reduced count of CB1R in their terminals at the target structure. Supporting evidence comes from results showing that conditional knockout of CB1R in terminals of mPFC pyramidal neurons boosts optic self-stimulation of these terminals in the NAc[144]. Said differently, CB1R activity in terminals of the mPFC in the NAc has a limiting function on reward-seeking. Thus, by eliminating CB1R, inputs from the mPFC that promote reward seeking are disinhibited. This leads us to the next point. In the first study, dominant male rats were observed to have a lower expression of CB1R in NAc. It is not entirely out of the realm of possibility that this lower CB1R count could be occurring in mPFC terminals. However, answers beget time. As such, granular clarity of mechanistic intricacies will likely grace us further down the road.

### VII.2. Translational value for psychiatry

The discoveries elucidated in this thesis hold translational significance, especially within the context of male adolescents. This demographic warrants special attention due to its heightened propensity for overt, physical aggression relative to their female counterparts and a higher likelihood of involvement in substance use[290–292]. Adolescence is characterized by a tendency towards risk-taking, a curiosity for novelty, and impulsivity[293]. Various elements of decision-making, including intertemporal choice, prospective evaluation, and the incorporation of positive and negative feedback, have yet to match with the patterns seen in typical adults[294]. Concurrently, this phase in life holds significant importance in the late development of the PFC,

a process that involves synaptic pruning to fine-tune the intricately woven prefrontal networks[295]. Thus, it has been argued that the imbalance between heightened sensation-seeking and reward sensitivity, coupled with incomplete PFC development, can lead to impulsive or risky choices[294,295].

Without fail, brain CB1R expression serves as a consistent factor in elucidating the disparities in reward-based decision-making between male adolescents and adults. In a previous work from our laboratory, adolescent rats when compared to adults demonstrated elevated seeking behavior of natural rewards[296] and are more sensitive to the rewarding effects of cocaine, where a smaller dose is needed to engage drug-seeking[297]. Moreover, adolescents demonstrate lower expression of CB1R in both PFC and NAc[296]. These results agree with previously published report that showed lower binding to CB1R in the frontal cortex and striatum in adolescent rats compared to adult rats[298]. These findings bear resemblance to the observations in dominant adult rats. When compared to subordinates, dominants also exhibit elevated seeking of natural rewards[54] and (as demonstrated in the first study within this dissertation) reduced CB1R expression in both the mPFC and NAc[32]. Taken with the evidence showing that in vivo gene silencing of CB1R in the ACC facilitates approach behavior, it is appropriate to low expression of CB1R in the mPFC promotes reward-seeking behavior. Henceforth, by accentuating the role CB1R in the mPFC and NAc in drug-seeking behavior, we are provided with a potential biomarker for addiction vulnerability.

### VII.3. On the wisdom of the body

> *"For unto every one that hath shall be given, and he shall have abundance: but from him that hath not shall be taken away even that which he hath."*
>
> *-Matthew 25:29, KJV*

The embodiment of ideas and concepts, which implies the manifestation of the abstract in the flesh, has long captivated inquisitive minds. Exemplar is the notion of a species-level wisdom contained within the body, as proposed by Arthur Schopenhauer[299] and Friedrich Nietzsche[300]. Wisdom in this context is understood as an impulse to act according to principles of proliferation and survival. In the *Descent of Man* (1871), Charles Darwin exposes the idea that the obeisance to authority motivated by moral sentiments is an evolved strategy of the human species[301]. Admitting to non-originality, the current thesis was an effort to elucidate the embodiment of power. Proverbial understanding holds that those that hold power are changed and in a pessimistic interpretation, corrupted. Herein, it has been shown and discussed that the behavior and the brains of animals of different social strata are different. Moreover, meddling with brain circuits can favor domineering behavior. What is the evolutionary meaning of this?

Konrad Lorenz introduces *On Aggression* (1974) with vivid visual imagery of his personal observations snorkeling in coral reefs off the coast of Florida[30]. These bustling metropolises

beneath the sea are vibrant and mesmerizing underwater tapestries of biodiversity, which reveal stories to the trained eyes. The out-of-place primate noted that aggression was most evident between conspecifics, rather than between species. Particularly notorious were the most colorful fish, which darted towards others bearing the same flag. Additionally, aggression was notably pronounced in species that coexisted in proximity, constrained by the spatial confines of the reef. It is therefore inferred that aggression is a strategy to safeguard resources and the rival that will have matching needs is another member of species. Hence, there are incentive to being aggressive: kill, incapacitate, or expel a rival eliminates any dispute. Pack hunters, like wolves, have the inherent arsenal to annihilate one another. An engagement between armed killers can be very costly, but the pressure to compete is ever present. Something has got to give.

Dominance relationships emerged as an evolutionary stable solution, replicated across taxa over millions of years. However, the name given to this type of social relationship can be misleading, as it disregards the intricate bidirectional dynamics at play. In zero-sum competitions, credit all too often is given to the victor. Can't we realize that to win someone else has to make the effort to lose? As with any other relationship, social dominance is a coordinated effort. Dominant and subordinate are molded from a process of mutual learning, with the outcome of a fight influencing subsequent encounters. Winning is rewarding: an invigorating experience that boosts self-appraisal and provides motivation to continue striving in the pursuit of victories. On the other hand, constant losing reduces self-appraisal, making individuals more likely to submit.

The goal of this dominance game is to acquire status[94], a intersubjective quality with serious consequences in health and reproductive fitness[106]. Those at the top will have secure access to resources and mates, ensuring that their genetic lineage will pass on to the next generation. The adaptive function for individuals is clear: when resources are limited, it is beneficial to minimize the costs of intragroup fighting and organize groups into hierarchies. As the Invisible Hand is one of the forces guiding value of goods in the market, the winner/loser effect guides individuals in competitive contexts to their status. When diverting attention to those at the bottom, the implication of the winner-loser becomes ominous: losers surrender their value as individuals and their genetic lineage for the betterment of the species. Nonetheless, Nature prefers diversity and does not play only one game. For example, tactical deception in cuttlefish[302] and coalition formation in chimpanzees[303] and humans[91] are stable strategies developed to circumvent the grip of dominance. As a heuristic to ward off erroneous and exaggerated social imperatives extrapolated to human societies (e.g. social Darwinism), it is best to acknowledge that complex social species play several games simultaneously and it is best to assume that no single evolutionary stable strategy is determinant on how fitness is calculated for each species.

If dominance is constituted by learned patterns of behavior, what then are the changes within the brain that account for these behavioral adaptations? We know that winning competitive interactions adjusts how future motivated behavior of the individual is engaged[72,111]. Thus, ascending the social hierarchy strongly influences reward-seeking behavior by enhancing the perceived value of rewards and minimizing the perception of potential losses[92,112]. This effect is especially pronounced when individuals with high social status feel secure in their positions[113].

Dominance is characterized by an asymmetry in social power, which implies power *over* others and freedom *from* others[304]. In this privileged position, consequences of anti-social and self-interested behaviors are attenuated, thus tipping cost-benefit scale towards the actuation of one's whims. Consistently, a position of high power is understood as disinhibitory force over behavior that fosters a propensity for approach-related tendencies to rewards and displays of aggression[84,110,111]. As such, it is of no surprise that RMC changes in response to status attainment[111]. For example, the dopamine type-2 (D2) receptor was upregulated in NAc of monkeys that became dominant[62]. Additionally, reduced D2 expression has been documented in the NAc of dominant rats, accompanied by decreased dopamine (DA) levels and heightened expression of the dopamine transporter (DAT)[120].

The ECS plays a pivotal role in reward processing[305,306] and this thesis presents compelling evidence of a common neurophysiological mechanism in the mPFC driven by CB1R signaling that explains both dominance and a lower reward-threshold. Given that the spontaneous expression of CB1R was compared between dominant and subordinates after the formation of social relationships, it is yet unclear if the difference precedes or is a consequence of dominance attainment. Social factors have been shown to regulate gene expression[307], hence it is possible that social dominance exerts an epigenetic regulation of CB1R expression. It is hypothesized that the diminished expression of CB1R in dominant rats stems from downregulation following the attainment of status. If this conjecture holds true, then a downregulation mPFC-CB1R in subjects that become dominant could be considered a mechanism underlying the winner-loser effect, thereby explaining the propensity of dominant individuals to partake in behaviors motivated by rewards (i.e. drug-seeking and aggression).

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# References

1. Volkow, N. D., Michaelides, M. & Baler, R. The neuroscience of drug reward and addiction. *Physiol. Rev.* **99**, 2115–2140 (2019).

2. Anthony, J. C., Warner, L. A. & Kessler, R. C. Comparative Epidemiology of Dependence on Tobacco, Alcohol, Controlled Substances, and Inhalants: Basic Findings From the National Comorbidity Survey. *Exp. Clin. Psychopharmacol.* **2**, 244–268 (1994).

3. Koob, G. F. & Volkow, N. D. Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry* **3**, 760–773 (2016).

4. Iacono, W. G., Malone, S. M. & McGue, M. Behavioral disinhibition and the development of early-onset addiction: Common and specific influences. *Annu. Rev. Clin. Psychol.* **4**, 325–348 (2008).

5. Ernst, M. *et al.* Behavioral Predictors of Substance-Use Initiation in Adolescents With and Without Attention-Deficit/Hyperactivity Disorder. *Pediatrics* **117**, 2030–2039 (2006).

6. De Wit, H. Impulsivity as a determinant and consequence of drug use: A review of underlying processes. *Addict. Biol.* **14**, 22–31 (2009).

7. Lischinsky, J. E. & Lin, D. Neural mechanisms of aggression across species. *Nat. Neurosci.* **23**, 1317–1328 (2020).

8. Tomlinson, M. F., Brown, M. & Hoaken, P. N. S. Recreational drug use and human aggressive behavior: A comprehensive review since 2003. *Aggress. Violent Behav.* **27**, 9–29 (2016).

9. Krueger, R. F., Markon, K. E., Patrick, C. J. & Iacono, W. G. Externalizing psychopathology in adulthood: A dimensional-spectrum conceptualization and its implications for DSM-V. *J. Abnorm. Psychol.* **114**, 537–550 (2005).

10. Mathias, C. W. *et al.* Aggression as a Predictor of Early Substance Use Initiation Among Youth With Family Histories of Substance Use Disorders. *Addict. Disord. Their Treat.* **14**, 230–240 (2015).

11. Sijtsema, J. J., Garofalo, C., Jansen, K. & Klimstra, T. A. Disengaging from Evil: Longitudinal Associations Between the Dark Triad, Moral Disengagement, and Antisocial Behavior in Adolescence. *J. Abnorm. Child Psychol.* **47**, 1351–1365 (2019).

12. Stenason, L. & Vernon, P. A. The Dark Triad, reinforcement sensitivity and substance use. *Pers. Individ. Dif.* **94**, 59–63 (2016).

13. Fernández, D. *et al.* Testing the properties of the triarchic model of psychopathy in a community sample: Self-reported trait aggression and drug consumption associations. *Scand. J. Psychol.* **60**, 377–385 (2019).

14. Creswell, K. G., Wright, A. G. C., Flory, J. D., Skrzynski, C. J. & Manuck, S. B. Multidimensional assessment of impulsivity-related measures in relation to externalizing behaviors. *Psychol. Med.* **49**, 1678–1690 (2019).

15. Snowden, R. J., Smith, C. & Gray, N. S. Risk taking and the triarchic model of psychopathy. *J. Clin. Exp. Neuropsychol.* **39**, 988–1001 (2017).

16. Patrick, C. J., Fowles, D. C. & Krueger, R. F. Triarchic conceptualization of psychopathy: Developmental origins of disinhibition, boldness,

and meanness. *Dev. Psychopathol.* **21**, 913–938 (2009).

17. Dalley, J. W. & Robbins, T. W. Fractionating impulsivity: Neuropsychiatric implications. *Nat. Rev. Neurosci.* **18**, 158–171 (2017).

18. Khadka, S. *et al.* Genetic association of impulsivity in young adults: A multivariate study. *Transl. Psychiatry* **4**, (2014).

19. Knutson, K. M. *et al.* Areas of Brain Damage Underlying Increased Reports of Behavioral Disinhibition. *J. Neuropsychiatry Clin. Neurosci.* **27**, 193–198 (2015).

20. Glenn, A. L. & Yang, Y. The potential role of the striatum in antisocial behavior and psychopathy. *Biol. Psychiatry* **72**, 817–822 (2012).

21. Kim, S. H., Yoon, H. S., Kim, H. & Hamann, S. Individual differences in sensitivity to reward and punishment and neural activity during reward and avoidance learning. *Soc. Cogn. Affect. Neurosci.* **10**, 1219–1227 (2014).

22. Buschman, T. J., Miller, E. K. & Miller, E. K. Goal-direction and top-down control. (2014).

23. Heilig, M., Epstein, D. H., Nader, M. A. & Shaham, Y. Time to connect: Bringing social context into addiction neuroscience. *Nat. Rev. Neurosci.* **17**, 592–599 (2016).

24. Shemesh, Y. & Chen, A. A paradigm shift in translational psychiatry through rodent neuroethology. *Mol. Psychiatry* (2023). doi:10.1038/s41380-022-01913-z

25. Belzung, C. Innovative drugs to treat depression: Did animal models fail to be predictive or did clinical trials fail to detect effects. *Neuropsychopharmacology* **39**, 1041–1051 (2014).

26. Haller, J., Aliczki, M. & Gyimesine Pelczer, K. Classical and novel approaches to the preclinical testing of anxiolytics: A critical evaluation. *Neurosci. Biobehav. Rev.* **37**, 2318–2330 (2013).

27. Peters, S. M., Pothuizen, H. H. J. & Spruijt, B. M. Ethological concepts enhance the translational value of animal models. *Eur. J. Pharmacol.* **759**, 42–50 (2015).

28. Venniro, M., Banks, M. L., Heilig, M., Epstein, D. H. & Shaham, Y. Improving translation of animal models of addiction and relapse by reverse translation. *Nat. Rev. Neurosci.* **21**, 625–643 (2020).

29. Bailoo, J. D. *et al.* Effects of weaning age and housing conditions on phenotypic differences in mice. *Sci. Rep.* **10**, 1–13 (2020).

30. Lorenz, K. *On Agression*. *A University Paperback* (1974).

31. Tinbergen, N. On aims and methods of Ethology. *Z. Tierpsychol.* **20**, 410–433 (1963).

32. Migliaro, M. *et al.* Dominance status is associated with a variation in cannabinoid receptor 1 expression and amphetamine reward. *Pharmacol. Biochem. Behav.* **221**, (2022).

33. Migliaro, M. Effects on Behavior by Different Exposure Durations of Predator Scent Stress. *Acta Investig. Psicológica* **10**, 17–26 (2020).

34. Migliaro, M., Ruiz-Contreras, A. E., Herrera-Solís, A., Méndez-Díaz, M. & Prospéro-García, O. E. Endocannabinoid system and aggression across animal species. *Neurosci. Biobehav. Rev.* **153**, 105375 (2023).

35. Brüne, M. On aims and methods of psychiatry - A reminiscence of 50 years of Tinbergen's famous questions about the biology of behavior. *BMC Psychiatry* **14**, 1–8 (2014).

36. Tinbergen, N. Ethology and stress diseases. *Science (80-. ).* **185**, 20–27 (1974).

37. Darwin, C. *On the origin of the species*. (Race Point Publishing, 2017).

38. Luo, G. *et al.* Rapid oxygenation of Earth's atmosphere 2.33 billion years ago. *Sci. Adv.* **2**, 1–10 (2016).

39. Knoll, A. H. & Nowak, M. A. The timetable of evolution. *Sci. Adv.* **3**, 1–14 (2017).

40. Sharov, A. A. Genome increase as a clock for the origin and evolution of life. *Biol. Direct* **1**, 1–10 (2006).

41. Haugan, T. Social anxiety in modern societies from an evolutionary perspective. *Discov. Psychol.* **3**, (2023).

42. Nesse, R. M. Evolutionary psychiatry: foundations, progress and challenges. *World Psychiatry* **22**, 177–202 (2023).

43. Miller, A. H. & Raison, C. L. The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* **16**, 22–34 (2016).

44. Lende, D. H. & Smith, E. O. Evolution meets biopsychosociality: An analysis of addictive

behavior. *Addiction* **97**, 447–458 (2002).

45. Prospéro-García, O. E., Ruiz-Contreras, A. E., Morelos, J., Herrera-Solis, A. & Mendez-Díaz, M. Fragility of reward vs antifragility of defense brain systems in drug dependence. *Soc. Neurosci.* **16**, 145–152 (2021).

46. Kelley, A. E. & Berridge, K. C. The Neuroscience of Natural Rewards: Relevance to Addictive Drugs. *J. Neurosci.* **22**, 3306–3311 (2002).

47. Durrant, R., Adamson, S., Todd, F. & Sellman, D. Drug use and addiction: evolutionary perspective. *Aust. N. Z. J. Psychiatry* **43**, 1049–1056 (2009).

48. Dellazizzo, L. *et al.* Association between the use of cannabis and physical violence in youths: A meta-analytical investigation. *Am. J. Psychiatry* **177**, 619–626 (2020).

49. Hoaken, P. N. S. & Stewart, S. H. Drugs of abuse and the elicitation of human aggressive behavior. *Addict. Behav.* **28**, 1533–1554 (2003).

50. Ostrowsky, M. K. Does marijuana use lead to aggression and violent behavior? *J. Drug Educ.* **41**, 369–389 (2011).

51. Brook, J. S. & Newcomb, M. D. Childhood aggression and unconventionality: Impact on later academic achievement, drug use, and workforce involvement. *J. Genet. Psychol.* **156**, 393–410 (1995).

52. Giancola, P. R., Godlaski, A. J. & Parrott, D. J. 'So I can't blame the booze?': Dispositional aggressivity negates the moderating effects of expectancies on alcohol-related aggression. *J. Stud. Alcohol* **66**, 815–824 (2005).

53. Tarter, R. E. *et al.* Social Dominance Mediates the Association of Testosterone and Neurobehavioral Disinhibition With Risk for Substance Use Disorder. *Psychol. Addict. Behav.* **21**, 462–468 (2007).

54. Davis, J. F., Krause, E. G., Melhorn, S. J., Sakai, R. R. & Benoit, S. C. Dominant rats are natural risk takers and display increased motivation for food reward. *Neuroscience* **162**, 23–30 (2009).

55. Kunkel, T. & Wang, H. Socially dominant mice in C57BL6 background show increased social motivation. *Behav. Brain Res.* **336**, 173–176 (2018).

56. Wang, F. *et al.* Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science (80-. ).* **334**, 693–697 (2011).

57. Wolffgramm, J. & Heyne, A. From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat. *Behav. Brain Res.* **70**, 77–94 (1995).

58. Wolffgramm, J. An ethopharmacological approach to the development of drug addiction. *Neurosci. Biobehav. Rev.* **15**, 515–519 (1991).

59. Blanchard, D. C. & Blanchard, R. J. Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neurosci. Biobehav. Rev.* **14**, 455–462 (1990).

60. Blanchard, R. J., Hori, K., Tom, P. & Blanchard, D. C. Social structure and ethanol consumption in the laboratory rat. *Pharmacol. Biochem. Behav.* **28**, 437–442 (1987).

61. Nader, M. A. *et al.* Social dominance in female monkeys: Dopamine receptor function and cocaine reinforcement. *Biological Psychiatry* **72**, 414–421 (2012).

62. Morgan, D. *et al.* Social dominance in monkeys: Dopamine D2 receptors and cocaine self-administration. *Nat. Neurosci.* **5**, 169–174 (2002).

63. Whitehead, H. Analyzing Animal Societies: Quantitative Methods for Vertebrate Social Analysis. *Univ. Chicago Press* 332 (2008). doi:10.1017/CBO9781107415324.004

64. Krause, J. & Ruxton, G. D. *Living in groups*. (Oxford University Press, 2002).

65. Markham, A. C. & Gesquiere, L. R. Costs and benefits of group living in primates: An energetic perspective. *Philos. Trans. R. Soc. B Biol. Sci.* **372**, (2017).

66. Chapman, C. A. & Valenta, K. Costs and benefits of group living are neither simple nor linear. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 14751–14752 (2015).

67. Dunbar, R. I. M. The social brain hypothesis. *Evol. Anthropol.* **6**, 178–190 (1998).

68. Van Vugt, M. & Smith, J. E. A Dual Model of Leadership and Hierarchy: Evolutionary Synthesis. *Trends Cogn. Sci.* **23**, 952–967 (2019).

69. Tibbetts, E. A., Pardo-Sanchez, J. & Weise, C. The establishment and maintenance of dominance hierarchies. *Philos. Trans. R. Soc. B Biol. Sci.* **377**, (2022).

70. Thomas, A. L., Davis, S. M. & Dierick, H. A. Of Fighting Flies, Mice, and Men: Are Some of the Molecular and Neuronal Mechanisms of

Aggression Universal in the Animal Kingdom? *PLoS Genet.* **11**, 1–14 (2015).

71. Grether, G. F. *et al.* The evolutionary consequences of interspecific aggression. *Ann. N. Y. Acad. Sci.* **1289**, 48–68 (2013).

72. Price, J., Sloman, L., Gardner, R., Gilbert, P. & Rohde, P. The social competition hypothesis of depression. *Br. J. Psychiatry* **164**, 309–315 (1994).

73. Rutte, C., Taborsky, M. & Brinkhof, M. W. G. What sets the odds of winning and losing? *Trends Ecol. Evol.* **21**, 16–21 (2006).

74. Hsu, Y. & Wolf, L. L. The winner and loser effect: Integrating multiple experiences. *Anim. Behav.* **57**, 903–910 (1999).

75. Dugatkin, L. A. & Druen, M. The social implications of winner and loser effects. *Proc. R. Soc. B Biol. Sci.* **271**, 488–489 (2004).

76. Rappaport, B. I. *et al.* Peer victimization and dysfunctional reward processing: ERP and behavioral responses to social and monetary rewards. *Front. Behav. Neurosci.* **13**, 1–11 (2019).

77. Ethridge, P., Sandre, A., Dirks, M. A. & Weinberg, A. Past-year relational victimization is associated with a blunted neural response to rewards in emerging adults. *Soc. Cogn. Affect. Neurosci.* **13**, 1259–1267 (2018).

78. Kureta, Y. & Watanabe, S. Influence of social dominance on self-stimulation behavior in male golden hamsters. *Physiol. Behav.* **59**, 621–624 (1996).

79. Maxim, P. E. Self-stimulation of a hypothalamic site in response to tension or fear. *Physiol. Behav.* **18**, 197–201 (1977).

80. Goffman, E. *Stigma: Notes on the Management of Spoiled Identity*. *Penguin Books* (1963). doi:10.2307/2575995

81. Becker, H. S. *Outsiders: Studies In The Sociology Of Deviance*. (The Free Press, 1966).

82. Zimbardo, P. *The Lucifer Effect: Understanding How Good People Turn Evil*. (Ramdom House, 2007).

83. Chen Zeng, T., Cheng, J. T. & Henrich, J. Dominance in humans. *Philos. Trans. R. Soc. B Biol. Sci.* **377**, (2022).

84. Fast, N. J. & Chen, S. When the boss feels inadequate: Power, incompetence, and aggression. *Psychol. Sci.* **20**, 1406–1413 (2009).

85. Cheng, J. T., Tracy, J. L., Foulsham, T., Kingstone, A. & Henrich, J. Two ways to the top: Evidence that dominance and prestige are distinct yet viable avenues to social rank and influence. *J. Pers. Soc. Psychol.* **104**, 103–125 (2013).

86. Henrich, J. & Gil-White, F. J. The evolution of prestige: freely conferred deference as a mechanism for enhancing the benefits of cultural transmission. *Evol. Hum. Behav.* **22**, 165–196 (2001).

87. Watanabe, N. & Yamamoto, M. Neural mechanisms of social dominance. *Front. Neurosci.* **9**, (2015).

88. Hinde, R. A. Interactions, Relationships and Social Structure. *Man* **11**, 1 (1976).

89. Rivenbark, J. *et al.* Adolescents' perceptions of family social status correlate with health and life chances: A twin difference longitudinal cohort study. *Proc. Natl. Acad. Sci.* 201820845 (2020). doi:10.1073/pnas.1820845116

90. Roberts, A., Palermo, R. & Visser, T. A. W. Effects of dominance and prestige based social status on competition for attentional resources. *Sci. Rep.* **9**, 1–11 (2019).

91. Cheng, J. T. Dominance, prestige, and the role of leveling in human social hierarchy and equality. *Curr. Opin. Psychol.* **33**, 238–244 (2020).

92. Anderson, C. & Berdahl, J. L. The experience of power: Examining the effects of power on approach and inhibition tendencies. *J. Pers. Soc. Psychol.* **83**, 1362–1377 (2002).

93. von Rueden, C., Gurven, M. & Kaplan, H. Why do men seek status? Fitness payoffs to dominance and prestige. *Proc. R. Soc. B Biol. Sci.* **278**, 2223–2232 (2011).

94. Van Vugt, M. & Smith, J. E. A Dual Model of Leadership and Hierarchy: Evolutionary Synthesis. *Trends Cogn. Sci.* **23**, 952–967 (2019).

95. Smith, D. G. A 15-year study of the association between dominance rank and reproductive success of male rhesus macaques. *Primates* **34**, 471–480 (1993).

96. Francis, R. C. On the Relationship between Aggression and Social Dominance. *Ethology* **78**, 223–237 (1988).

97. Drews, C. The Concept and Definition of Dominance in Animal Behaviour. *Behaviour* **125**, 283–313 (1993).

98. Grant, E. C. & Mackintosh, J. H. A Comparison of the Social Postures of Some Common Laboratory Rodents. *Behaviour* **21**, 246–259 (1963).

99. Reddon, A. R., Ruberto, T. & Reader, S. M. Submission signals in animal groups. *Behaviour* (2021). doi:10.1163/1568539X-bja10125

100. Miczek, K. A. & de Boer, S. F. *Aggressive, Defensive, and Submissive Behavior*. (Oxford University Press, 2005).

101. Chase, I. D. & Seitz, K. *Self-structuring properties of dominance hierarchies. A new perspective*. *Advances in Genetics* **75**, (Elsevier Inc., 2011).

102. Drummond, H. Dominance in Vertebrate Broods and Litters. *Q. Rev. Biol.* **81**, 3–32 (2006).

103. Shizuka, D. & McDonald, D. B. A social network perspective on measurements of dominance hierarchies. *Anim. Behav.* **83**, 925–934 (2012).

104. Varholick, J. A. *et al.* Social dominance hierarchy type and rank contribute to phenotypic variation within cages of laboratory mice. *Sci. Rep.* **9**, 1–11 (2019).

105. Fournier, M. A. Dimensions of human hierarchy as determinants of health and happiness. *Curr. Opin. Psychol.* **33**, 110–114 (2020).

106. Sapolsky, R. M. The Influence of Social Hierarchy on Primate Health. *Science (80-. ).* **308**, 648–652 (2005).

107. Sherman, G. D. & Mehta, P. H. Stress, cortisol, and social hierarchy. *Curr. Opin. Psychol.* **33**, 227–232 (2020).

108. Wallace, K. J. *et al.* Social ascent changes cognition, behaviour and physiology in a highly social cichlid fish. *Philos. Trans. R. Soc. B Biol. Sci.* **377**, (2022).

109. Anderson, C. & Galinsky, A. D. Power, optimism, and risk-taking. *Eur. J. Soc. Psychol.* **36**, 511–536 (2006).

110. Cho, M. & Keltner, D. Power, approach, and inhibition: empirical advances of a theory. *Curr. Opin. Psychol.* **33**, 196–200 (2020).

111. Ghosal, S., Sandi, C. & van der Kooij, M. A. Neuropharmacology of the mesolimbic system and associated circuits on social hierarchies. *Neuropharmacology* 0–1 (2019). doi:10.1016/j.neuropharm.2019.01.013

112. Fast, N. J., Sivanathan, N., Mayer, N. D. & Galinsky, A. D. Power and overconfident decision-making. *Organ. Behav. Hum. Decis. Process.* **117**, 249–260 (2012).

113. Maner, J. K., Gailliot, M. T., Butz, D. A. & Peruche, B. M. Power, Risk, and the Status Quo: Does Power Promote Riskier or More Conservative Decision Making? *Personal. Soc. Psychol. Bull.* **33**, 451–462 (2007).

114. Kunstman, J. W. & Maner, J. K. Sexual Overperception: Power, Mating Motives, and Biases in Social Judgment. *J. Pers. Soc. Psychol.* **100**, 282–294 (2011).

115. Mazur, A. & Booth, A. Testosterone and dominance in men. *Behav. Brain Sci.* **21**, 353–397 (1998).

116. Williamson, C. M., Lee, W., Romeo, R. D. & Curley, J. P. Social context-dependent relationships between mouse dominance rank and plasma hormone levels. *Physiol. Behav.* **171**, 110–119 (2017).

117. Vogel, M., Rees, C. E., McCuddy, T. & Carson, D. C. The Highs That Bind: School Context, Social Status and Marijuana Use. *J. Youth Adolesc.* **44**, 1153–1164 (2015).

118. Moody, J., Brynildsen, W. D., Osgood, D. W., Feinberg, M. E. & Gest, S. Popularity trajectories and substance use in early adolescence. *Soc. Networks* **33**, 101–112 (2011).

119. Ghosal, S., Sandi, C. & van der Kooij, M. A. Neuropharmacology of the mesolimbic system and associated circuits on social hierarchies. *Neuropharmacology* 0–1 (2019). doi:10.1016/j.neuropharm.2019.01.013

120. Jupp, B. *et al.* Social dominance in rats: Effects on cocaine self-administration, novelty reactivity and dopamine receptor binding and content in the striatum. *Psychopharmacology (Berl).* **233**, 579–589 (2016).

121. Yanovich, C., Kirby, M. L., Michaelevski, I., Yadid, G. & Pinhasov, A. Social rank-associated stress vulnerability predisposes individuals to cocaine attraction. *Sci. Rep.* **8**, 1–9 (2018).

122. Kardash, T. *et al.* Link between personality and response to THC exposure. *Behav. Brain Res.* **379**, 112361 (2020).

123. Katsidoni, V., Kastellakis, A. & Panagis, G. Biphasic effects of Δ9-tetrahydrocannabinol on brain stimulation reward and motor activity. *Int. J. Neuropsychopharmacol.* **16**, 2273–2284 (2013).

124. Gould, R. W., Czoty, P. W., Porrino, L. J. & Nader, M. A. Social Status in Monkeys: Effects of Social

Confrontation on Brain Function and Cocaine Self-Administration. *Neuropsychopharmacology* **42**, 1093–1102 (2017).

125. Dilleen, R. *et al.* High anxiety is a predisposing endophenotype for loss of control over cocaine, but not heroin, self-administration in rats. *Psychopharmacology (Berl).* **222**, 89–97 (2012).

126. Pelloux, Y., Costentin, J. & Duterte-Boucher, D. Anxiety increases the place conditioning induced by cocaine in rats. *Behav. Brain Res.* **197**, 311–316 (2009).

127. Blanchard, C., Sakai, R. R., McEwen, B., Weiss, S. M. & Blanchard, R. J. Subordination stress: Behavioral, brain, and neuroendocrine correlates. *Behav. Brain Res.* **58**, 113–121 (1993).

128. Dijk, C., van Emmerik, A. A. P. & Grasman, R. P. P. P. Social anxiety is related to dominance but not to affiliation as perceived by self and others: A real-life investigation into the psychobiological perspective on social anxiety. *Pers. Individ. Dif.* **124**, 66–70 (2018).

129. Varholick, J. A., Bailoo, J. D., Jenkins, A., Voelkl, B. & Würbel, H. A Systematic Review and Meta-Analysis of the Relationship Between Social Dominance Status and Common Behavioral Phenotypes in Male Laboratory Mice. *Front. Behav. Neurosci.* **14**, 1–13 (2021).

130. Kondrakiewicz, K., Kostecki, M., Szadzińska, W. & Knapska, E. Ecological validity of social interaction tests in rats and mice. *Genes, Brain Behav.* **18**, 1–14 (2019).

131. Bechara, A. *et al.* A Neurobehavioral Approach to Addiction: Implications for the Opioid Epidemic and the Psychology of Addiction. *Psychol. Sci. Public Interes.* **20**, 96–127 (2019).

132. Adamson, S., Todd, F., Sellman, D. & Durrant, R. Drug use and addiction: Evolutionary perspective. *Aust. N. Z. J. Psychiatry* **43**, 1049–1056 (2009).

133. Lewis, R. G., Florio, E., Punzo, D. & Borrelli, E. The Brain's Reward System in Health and Disease. *Adv. Exp. Med. Biol.* **1344**, 57–69 (2021).

134. Salamone, J. D. & Correa, M. The Mysterious Motivational Functions of Mesolimbic Dopamine. *Neuron* **76**, 470–485 (2012).

135. Volkow, N. D. & Morales, M. The Brain on Drugs: From Reward to Addiction. *Cell* **162**, 712–725 (2015).

136. Wenzel, J. M. *et al.* Phasic Dopamine Signals in the Nucleus Accumbens that Cause Active Avoidance Require Endocannabinoid Mobilization in the Midbrain. *Curr. Biol.* **28**, 1392-1404.e5 (2018).

137. Covey, D. P. & Yocky, A. G. Endocannabinoid Modulation of Nucleus Accumbens Microcircuitry and Terminal Dopamine Release. *Front. Synaptic Neurosci.* **13**, 1–18 (2021).

138. Kupchik, Y. M. *et al.* Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nat. Neurosci.* **18**, 1230–1232 (2015).

139. Soares-Cunha, C. *et al.* Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion. *Mol. Psychiatry* **25**, 3241–3255 (2020).

140. Salamone, J. D., Correa, M., Nunes, E. J., Randall, P. A. & Pardo, M. THE BEHAVIORAL PHARMACOLOGY OF EFFORT-RELATED CHOICE BEHAVIOR: DOPAMINE, ADENOSINE AND BEYOND. *J. Exp. Anal. Behav.* **97**, 125–146 (2012).

141. Berridge, K. C. & Robinson, T. E. What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* **28**, 309–369 (1998).

142. Berridge, K. C. Evolving concepts of emotion and motivation. *Front. Psychol.* **9**, 1–20 (2018).

143. Britt, J. P. *et al.* Synaptic and Behavioral Profile of Multiple Glutamatergic Inputs to the Nucleus Accumbens. *Neuron* **76**, 790–803 (2012).

144. Mateo, Y. *et al.* Endocannabinoid Actions on Cortical Terminals Orchestrate Local Modulation of Dopamine Release in the Nucleus Accumbens. *Neuron* **96**, 1112-1126.e5 (2017).

145. Lafferty, C. K., Yang, A. K., Mendoza, J. A. & Britt, J. P. Nucleus Accumbens Cell Type- and Input-Specific Suppression of Unproductive Reward Seeking. *Cell Rep.* **30**, 3729-3742.e3 (2020).

146. Stuber, G. D. *et al.* Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* **475**, 377–380 (2011).

147. Shen, C. J. *et al.* Cannabinoid CB1 receptors in the amygdalar cholecystokinin glutamatergic afferents to nucleus accumbens modulate depressive-like behavior. *Nat. Med.* **25**, 337–349 (2019).

148. Pastor, V. & Medina, J. H. Medial prefrontal cortical control of reward- and aversion-based behavioral output: Bottom-up modulation. *Eur. J. Neurosci.* **53**, 3039–3062 (2021).

149. Schaeffer, D. J. *et al.* Divergence of rodent and primate medial frontal cortex functional connectivity. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 21681–21689 (2020).

150. Laubach, M., Amarante, L. M., Swanson, K. & White, S. R. What, If Anything, Is Rodent Prefrontal Cortex? *eneuro* **5**, ENEURO.0315-18.2018 (2018).

151. Peters, J., Kalivas, P. W. & Quirk, G. J. Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn. Mem.* **16**, 279–288 (2009).

152. Voorn, P., Vanderschuren, L. J. M. J., Groenewegen, H. J., Robbins, T. W. & Pennartz, C. M. A. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci.* **27**, 468–474 (2004).

153. James, M. H., McGlinchey, E. M., Vattikonda, A., Mahler, S. V. & Aston-Jones, G. Cued Reinstatement of Cocaine but Not Sucrose Seeking Is Dependent on Dopamine Signaling in Prelimbic Cortex and Is Associated with Recruitment of Prelimbic Neurons That Project to Contralateral Nucleus Accumbens Core. *Int. J. Neuropsychopharmacol.* **21**, 89–94 (2018).

154. Cameron, C. M., Murugan, M., Choi, J. Y., Engel, E. A. & Witten, I. B. Increased Cocaine Motivation Is Associated with Degraded Spatial and Temporal Representations in IL-NAc Neurons. *Neuron* **103**, 80-91.e7 (2019).

155. Nett, K. E. *et al.* Infralimbic Projections to the Nucleus Accumbens Shell and Amygdala Regulate the Encoding of Cocaine Extinction Learning. *J. Neurosci.* **43**, 1348–1359 (2023).

156. Flanigan, M. E. & Kash, T. L. Coordination of social behaviors by the bed nucleus of the stria terminalis. *Eur. J. Neurosci.* **55**, 2404–2420 (2022).

157. Haller, J. The role of central and medial amygdala in normal and abnormal aggression: A review of classical approaches. *Neurosci. Biobehav. Rev.* **85**, 34–43 (2018).

158. Lo, L. *et al.* Connectional architecture of a mouse hypothalamic circuit node controlling social behavior. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 7503–7512 (2019).

159. Lipp, H. P. & Hunsperger, R. W. Threat, Attack and Flight Elicited by Electrical Stimulation of the Ventromedial Hypothalamus of the Marmoset Monkey Callithrix jacchus. *Brain. Behav. Evol.* **15**, 260–275 (1978).

160. Falkner, A. L., Grosenick, L., Davidson, T. J., Deisseroth, K. & Lin, D. Hypothalamic control of male aggression-seeking behavior. *Nat. Neurosci.* **19**, 596–604 (2016).

161. Stagkourakis, S. *et al.* A neural network for intermale aggression to establish social hierarchy. *Nat. Neurosci.* **21**, 834–842 (2018).

162. Chang, C. H. & Gean, P. W. The Ventral Hippocampus Controls Stress-Provoked Impulsive Aggression through the Ventromedial Hypothalamus in Post-Weaning Social Isolation Mice. *Cell Rep.* **28**, 1195-1205.e3 (2019).

163. Cameron, A. A., Khan, I. A., Westlund, K. N. & Willis, W. D. The efferent projections of the periaqueductal gray in the rat: A Phaseolus vulgaris-leucoagglutinin study. II. Descending projections. *J. Comp. Neurol.* **351**, 585–601 (1995).

164. Golden, S. A., Jin, M. & Shaham, Y. Animal models of (or for) aggression reward, addiction, and relapse: Behavior and circuits. *J. Neurosci.* **39**, 3996–4008 (2019).

165. Goodwin, N. L., Nilsson, S. R. O. & Golden, S. A. Rage Against the Machine: Advancing the study of aggression ethology via machine learning. *Psychopharmacology (Berl).* **237**, 2569–2588 (2020).

166. Golden, S. A. *et al.* Persistent conditioned place preference to aggression experience in adult male sexually-experienced CD-1 mice. *Genes, Brain Behav.* **16**, 44–55 (2017).

167. Van Erp, A. M. M. & Miczek, K. A. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *J. Neurosci.* **20**, 9320–9325 (2000).

168. Becker, E. A. & Marler, C. A. Postcontest blockade of dopamine receptors inhibits development of the winner effect in the California Mouse (Peromyscus californicus). *Behav. Neurosci.* **129**, 205–213 (2015).

169. Lozano-Montes, L. *et al.* Latency to reward predicts social dominance in rats: A causal role for the dopaminergic mesolimbic system. *Front. Behav. Neurosci.* **13**, 1–15 (2019).

170. Dong, H. W. & Swanson, L. W. Projections from Bed Nuclei of the Stria Terminalis, Posterior Division: Implications for Cerebral Hemisphere Regulation of Defensive and Reproductive Behaviors. *J. Comp. Neurol.* **471**, 396–433 (2004).

171. Molero-Chamizo, A., Martín Riquel, R., Moriana, J.

A., Nitsche, M. A. & Rivera-Urbina, G. N. Bilateral Prefrontal Cortex Anodal tDCS Effects on Self-reported Aggressiveness in Imprisoned Violent Offenders. *Neuroscience* **397**, 31–40 (2019).

172. Choy, O., Raine, A. & Hamilton, R. H. Stimulation of the prefrontal cortex reduces intentions to commit aggression: A randomized, double-blind, placebo-controlled, stratified, parallel-group trial. *J. Neurosci.* **38**, 6505–6512 (2018).

173. Takahashi, A., Nagayasu, K., Nishitani, N., Kaneko, S. & Koide, T. Control of Intermale Aggression by Medial Prefrontal Cortex Activation in the Mouse. *PLoS One* **9**, e94657 (2014).

174. Hillman, K. L. Cost-benefit analysis: The first real rule of fight club? *Front. Neurosci.* **7**, 1–10 (2013).

175. Padilla-Coreano, N. *et al.* Cortical ensembles orchestrate social competition through hypothalamic outputs. *Nature* **603**, 667–671 (2022).

176. Gangopadhyay, P., Chawla, M., Dal Monte, O. & Chang, S. W. C. Prefrontal–amygdala circuits in social decision-making. *Nat. Neurosci.* **24**, 5–18 (2021).

177. Mateo, Y. *et al.* Endocannabinoid Actions on Cortical Terminals Orchestrate Local Modulation of Dopamine Release in the Nucleus Accumbens. *Neuron* **96**, 1112-1126.e5 (2017).

178. Caballero, J. P., Scarpa, G. B., Remage-Healey, L. & Moorman, D. E. Differential effects of dorsal and ventral medial prefrontal cortex inactivation during natural reward seeking, extinction, and cue-induced reinstatement. *eNeuro* **6**, (2019).

179. Franklin, T. B. *et al.* Prefrontal cortical control of a brainstem social behavior circuit. *Nat. Neurosci.* **20**, 260–270 (2017).

180. Chudasama, Y. *et al.* The role of the anterior cingulate cortex in choices based on reward value and reward contingency. *Cereb. Cortex* **23**, 2884–2898 (2013).

181. Monosov, I. E., Haber, S. N., Leuthardt, E. C. & Jezzini, A. Anterior Cingulate Cortex and the Control of Dynamic Behavior in Primates. *Curr. Biol.* **30**, R1442–R1454 (2020).

182. Hillman, K. L. & Bilkey, D. K. Neural encoding of competitive effort in the anterior cingulate cortex. *Nat. Neurosci.* **15**, 1290–1297 (2012).

183. Báez-Mendoza, R., Vázquez, Y., Mastrobattista, E. P. & Williams, Z. M. Neuronal Circuits for Social Decision-Making and Their Clinical Implications. *Front. Neurosci.* **15**, 1–21 (2021).

184. De Wit, S., Kosaki, Y., Balleine, B. W. & Dickinson, A. Dorsomedial prefrontal cortex resolves response conflict in rats. *J. Neurosci.* **26**, 5224–5229 (2006).

185. Zhou, T. *et al.* History of winning remodels thalamo-PFC circuit to reinforce social dominance. *Science (80-. ).* **357**, 162–168 (2017).

186. Kingsbury, L. *et al.* Correlated Neural Activity and Encoding of Behavior across Brains of Socially Interacting Animals. *Cell* **178**, 429-446.e16 (2019).

187. Lu, H. C. & MacKie, K. An introduction to the endogenous cannabinoid system. *Biological Psychiatry* **79**, (2016).

188. Alger, B. E. & Kim, J. Supply and demand for endocannabinoids. *Trends Neurosci.* **34**, 304–315 (2011).

189. Cristino, L., Bisogno, T. & Di Marzo, V. Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nat. Rev. Neurol.* (2020). doi:10.1038/s41582-019-0284-z

190. Cravatt, B. F. *et al.* Chemical characterization of a Family of Brain Lipids That Induce Sleep. *Science (80-. ).* **268**, 1506–1509 (1995).

191. Berdyshev, E. V, Boichot, E. & Lagente, V. Anandamide--a new look on fatty acid ethanolamides. *J. Lipid Mediat. Cell Signal.* **15**, 49–67 (1996).

192. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).

193. Di Marzo, V. & Maccarrone, M. FAAH and anandamide: is 2-AG really the odd one out? *Trends Pharmacol. Sci.* **29**, 229–233 (2008).

194. McKinney, M. K. & Cravatt, B. E. Structure and function of fatty acid amide hydrolase. *Annu. Rev. Biochem.* **74**, 411–432 (2005).

195. Sugiura, T., Kishimoto, S., Oka, S. & Gokoh, M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog. Lipid Res.* **45**, 405–446 (2006).

196. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).

197. Farrell, E. K. & Merkler, D. J. Biosynthesis,

degradation and pharmacological importance of the fatty acid amides. *Drug Discov. Today* **13**, 558–568 (2008).

198. Pertwee, R. G. *et al.* International Union of Basic and Clinical Pharmacology . LXXIX . Cannabinoid Receptors and Their Ligands : Beyond CB 1 and CB 2. *Pharmacol. Rev.* **62**, 588–631 (2010).

199. Winters, B. L. & Vaughan, C. W. Mechanisms of endocannabinoid control of synaptic plasticity. *Neuropharmacology* **197**, 108736 (2021).

200. Piomelli, D. The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* **4**, 873–884 (2003).

201. McLaughlin, R. J., Hill, M. N. & Gorzalka, B. B. A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. *Neurosci. Biobehav. Rev.* **42**, 116–131 (2014).

202. Martinez Ramirez, C. E. *et al.* Endocannabinoid signaling in the central nervous system. *Glia* **71**, 5–35 (2023).

203. Atwood, B. K. & MacKie, K. CB2: A cannabinoid receptor with an identity crisis. *Br. J. Pharmacol.* **160**, 467–479 (2010).

204. Zhang, H. Y. *et al.* Repeated cocaine administration upregulates CB2 receptor expression in striatal medium-spiny neurons that express dopamine D1 receptors in mice. *Acta Pharmacol. Sin.* **43**, 876–888 (2022).

205. Morgan, N. H., Stanford, I. M. & Woodhall, G. L. Functional CB2 type cannabinoid receptors at CNS synapses. *Neuropharmacology* **57**, 356–368 (2009).

206. Sánchez-Zavaleta, R. *et al.* Presynaptic cannabinoid CB2 receptors modulate [ 3 H]-Glutamate release at subthalamo-nigral terminals of the rat. *Synapse* **72**, e22061 (2018).

207. Reekie, T. A., Scott, M. P. & Kassiou, M. The evolving science of phytocannabinoids. *Nat. Rev. Chem.* **2**, (2018).

208. Howlett, A. C. *et al.* Cannabinoid physiology and pharmacology: 30 Years of progress. *Neuropharmacology* **47**, 345–358 (2004).

209. Gaston, T. E. & Friedman, D. Pharmacology of cannabinoids in the treatment of epilepsy. *Epilepsy Behav.* **70**, 313–318 (2017).

210. Tham, M. *et al.* Allosteric and orthosteric pharmacology of cannabidiol and cannabidiol-dimethylheptyl at the type 1 and type 2 cannabinoid receptors. *Br. J. Pharmacol.* **176**, 1455–1469 (2019).

211. Peng, J. *et al.* A narrative review of molecular mechanism and therapeutic effect of cannabidiol (CBD). *Basic Clin. Pharmacol. Toxicol.* **130**, 439–456 (2022).

212. Bisogno, T. *et al.* Molecular targets for cannabidiol and its synthetic analogues: Effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br. J. Pharmacol.* **134**, 845–852 (2001).

213. de Almeida, D. L. & Devi, L. A. Diversity of molecular targets and signaling pathways for CBD. *Pharmacol. Res. Perspect.* **8**, 1–10 (2020).

214. Tripathi, R. K. P. A perspective review on fatty acid amide hydrolase (FAAH) inhibitors as potential therapeutic agents. *Eur. J. Med. Chem.* **188**, 111953 (2020).

215. Gil-Ordóñez, A., Martín-Fontecha, M., Ortega-Gutiérrez, S. & López-Rodríguez, M. L. Monoacylglycerol lipase (MAGL) as a promising therapeutic target. *Biochem. Pharmacol.* **157**, 18–32 (2018).

216. Ramiro-Fuentes, S., Ortiz, O., Moratalla, R. & Fernandez-Espejo, E. Intra-accumbens rimonabant is rewarding but induces aversion to cocaine in cocaine-treated rats, as does in vivo accumbal cannabinoid CB1 receptor silencing: Critical role for glutamate receptors. *Neuroscience* **167**, 205–215 (2010).

217. Rodriguez, J. S., Boctor, S. Y., Flores, L. C., Phelix, C. F. & Martinez, J. L. Local pretreatment with the cannabinoid CB1 receptor antagonist AM251 attenuates methamphetamine intra-accumbens self-administration. *Neuroscience Letters* **489**, 187–191 (2011).

218. Oleson, E. B. & Cheer, J. F. A brain on cannabinoids: The role of dopamine release in reward seeking. *Cold Spring Harb. Perspect. Med.* **2**, 1–14 (2012).

219. Kleijn, J. *et al.* Effects of amphetamine on dopamine release in the rat nucleus accumbens shell region depend on cannabinoid CB1 receptor activation. *Neurochemistry International* **60**, 791–798 (2012).

220. Li, X. *et al.* Dissecting the role of CB1 and CB2 receptors in cannabinoid reward versus aversion using transgenic CB1- and CB2-knockout mice. *Eur. Neuropsychopharmacol.* **43**, 38–51 (2021).

221. Mitra, S. *et al.* A role for the endocannabinoid enzymes monoacylglycerol and diacylglycerol lipases in cue-induced cocaine craving following prolonged abstinence. *Addict. Biol.* **26**, 1–13 (2021).

222. Deroche, M. A., Lassalle, O., Castell, L., Valjent, E. & Manzoni, O. J. Cell-type- And endocannabinoid-specific synapse connectivity in the adult nucleus accumbens core. *J. Neurosci.* **40**, 1028–1041 (2020).

223. Wright, W. J., Schlüter, O. M. & Dong, Y. A Feedforward Inhibitory Circuit Mediated by CB1-Expressing Fast-Spiking Interneurons in the Nucleus Accumbens. *Neuropsychopharmacology* **42**, 1146–1156 (2017).

224. Hu, S. S. J., Liu, Y. W. & Yu, L. Medial prefrontal cannabinoid CB1 receptors modulate consolidation and extinction of cocaine-associated memory in mice. *Psychopharmacology (Berl).* **232**, 1803–1815 (2015).

225. Ahmad, T., Lauzon, N. M., de Jaeger, X. & Laviolette, S. R. Cannabinoid transmission in the prelimbic cortex bidirectionally controls opiate reward and aversion signaling through dissociable kappa versus μ-opiate receptor dependent mechanisms. *J. Neurosci.* **33**, 15642–15651 (2013).

226. Ceccarini, J., Koole, M. & Van Laere, K. Cannabinoid receptor availability modulates the magnitude of dopamine release in vivo in the human reward system: A preliminary multitracer positron emission tomography study. *Addict. Biol.* **27**, 1–11 (2022).

227. Monosov, I. E. Anterior cingulate is a source of valence-specific information about value and uncertainty. *Nat. Commun.* **8**, 1–11 (2017).

228. Khani, A. *et al.* Activation of cannabinoid system in anterior cingulate cortex and orbitofrontal cortex modulates cost-benefit decision making. *Psychopharmacology (Berl).* **232**, 2097–2112 (2015).

229. Fatahi, Z. *et al.* Exogenous Cannabinoids Impair Effort-Related Decision-Making via Affecting Neural Synchronization between the Anterior Cingulate Cortex and Nucleus Accumbens. *Brain Sci.* **13**, (2023).

230. Abel, E. L. The relationship between cannabis and violence: A review. *Psychol. Bull.* **84**, 193–211 (1977).

231. Abel, E. L. Cannabis and aggression in animals. *Behav. Biol.* **14**, 1–20 (1975).

232. Kolla, N. J. & Mishra, A. The endocannabinoid system, aggression, and the violence of synthetic cannabinoid use, borderline personality disorder, antisocial personality disorder, and other psychiatric disorders. *Front. Behav. Neurosci.* **12**, 1–8 (2018).

233. Sorkhou, M., Johnstone, S., Kivlichan, A. E., Castle, D. J. & George, T. P. Does cannabis use predict aggressive or violent behavior in psychiatric populations? A systematic review. *Am. J. Drug Alcohol Abuse* **48**, 631–643 (2022).

234. Salzman, C., Van Der Kolk, B. A. & Shader, R. I. Marijuana and hostility in a small group setting. *Am. J. Psychiatry* **133**, 1029–1033 (1976).

235. De Sousa Fernandes Perna, E. B., Theunissen, E. L., Kuypers, K. P. C., Toennes, S. W. & Ramaekers, J. G. Subjective aggression during alcohol and cannabis intoxication before and after aggression exposure. *Psychopharmacology (Berl).* **233**, 3331–3340 (2016).

236. Rodriguez-Arias, M. *et al.* CB1 cannabinoid receptor-mediated aggressive behavior. *Neuropharmacology* **75**, 172–180 (2013).

237. Chang, C. H., Liu, Y. C., Sun, C. Y., Su, C. L. & Gean, P. W. Regulation of stress-provoked aggressive behavior using endocannabinoids. *Neurobiol. Stress* **15**, 100337 (2021).

238. Hartmann, A. *et al.* Cannabidiol attenuates aggressive behavior induced by social isolation in mice: Involvement of 5-HT1A and CB1 receptors. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **94**, 109637 (2019).

239. Aliczki, M., Varga, Z. K., Balogh, Z. & Haller, J. Involvement of 2-arachidonoylglycerol signaling in social challenge responding of male CD1 mice. *Psychopharmacology (Berl).* **232**, 2157–2167 (2015).

240. Chambers, A. P. *et al.* A neutral CB1 receptor antagonist reduces weight gain in rat. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **293**, 2185–2193 (2007).

241. Ryberg, E. *et al.* The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharmacol.* **152**, 1092–1101 (2007).

242. Martin, M., Ledent, C., Parmentier, M., Maldonado, R. & Valverde, O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl).* **159**, 379–387 (2002).

243. Haller, J., Varga, B., Ledent, C., Barna, I. & Freund, T. F. Context-dependent effects of CB1

cannabinoid gene disruption on anxiety-like and social behaviour in mice. *Eur. J. Neurosci.* **19**, 1906–1912 (2004).

244. Rodríguez-Arias, M. *et al.* Role of CB2 receptors in social and aggressive behavior in male mice. *Psychopharmacology (Berl).* **232**, 3019–3031 (2015).

245. Amancio-Belmont, O., Becerril Meléndez, A. L., Ruiz-Contreras, A. E., Méndez-Díaz, M. & Prospéro-García, O. Maternal separation plus social isolation during adolescence reprogram brain dopamine and endocannabinoid systems and facilitate alcohol intake in rats. *Brain Res. Bull.* **164**, 21–28 (2020).

246. Amancio-Belmont, O., Becerril Meléndez, A. L., Ruiz-Contreras, A. E., Méndez-Díaz, M. & Prospéro-García, O. Opposed cannabinoid 1 receptor (CB1R) expression in the prefrontal cortex vs. nucleus accumbens is associated with alcohol consumption in male rats. *Brain Research* **1725**, (2019).

247. Kennedy, A. The what, how, and why of naturalistic behavior. *Curr. Opin. Neurobiol.* **74**, 102549 (2022).

248. Miczek, K. A. & Barry, H. Δ9-Tetrahydrocannabinol and Aggressive Behavior in Rats. *Behav. Biol.* **11**, 261–267 (1974).

249. Sieber, B. Influence of hashish extract on the social behaviour of encountering male baboons (Papio c. anubis). *Pharmacol. Biochem. Behav.* **17**, 209–216 (1982).

250. Ely, D. L., Henry, J. P. & Jarosz, C. J. Effects of marihuana (Δ9-THC) on behavior patterns and social roles in colonies of CBA Mice. *Behav. Biol.* **13**, 263–276 (1975).

251. Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates*. (Elsevier Inc., 2007).

252. Cordero, M. I. & Sandi, C. Stress amplifies memory for social hierarchy. *Front. Neurosci.* **1**, 175–184 (2007).

253. Gentsch, C., Lichtsteiner, M. & Feer, H. Competition for sucrose-pellets in triads of male Wistar rats: the individuals' performances are differing but stable. *Behav. Brain Res.* **27**, 37–44 (1988).

254. Jones, C. E. & Monfils, M. H. Dominance status predicts social fear transmission in laboratory rats. *Anim. Cogn.* **19**, 1051–1069 (2016).

255. Murlanova, K., Kirby, M., Libergod, L., Pletnikov, M. & Pinhasov, A. Multidimensional nature of dominant behavior: Insights from behavioral neuroscience. *Neurosci. Biobehav. Rev.* **132**, 603–620 (2022).

256. Pohorecky, L. A. Acute novel stressors modify ethanol intake of psychosocially stressed rats. *Pharmacol. Biochem. Behav.* **95**, 390–400 (2010).

257. Todd, W. D. *et al.* A hypothalamic circuit for the circadian control of aggression. *Nat. Neurosci.* **21**, 717–724 (2018).

258. de Vries, H., Stevens, J. M. G. & Vervaecke, H. Measuring and testing the steepness of dominance hierarchies. *Anim. Behav.* **71**, 585–592 (2006).

259. Funkhouser, J. A., Mayhew, J. A. & Mulcahy, J. B. Social network and dominance hierarchy analyses at Chimpanzee Sanctuary Northwest. *PLoS One* **13**, 1–19 (2018).

260. Balasubramaniam, K. N. *et al.* Consistency of dominance rank order: A comparison of David's scores with I&SI and Bayesian methods in macaques. *Am. J. Primatol.* **75**, 959–971 (2013).

261. Bayly, K. L., Evans, C. S. & Taylor, A. Measuring social structure: A comparison of eight dominance indices. *Behav. Processes* **73**, 1–12 (2006).

262. Forkosh, O. *et al.* Identity domains capture individual differences from across the behavioral repertoire. *Nat. Neurosci.* **22**, 2023–2028 (2019).

263. Gammell, M. P., de Vries, H., Jennings, D. J., Carlin, C. M. & Hayden, T. J. David's score: A more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Anim. Behav.* **66**, 601–605 (2003).

264. David, H. A. Ranking from Unbalanced Paired-Comparison Data. *Biometrika* **74**, 432 (1987).

265. Lee, W. *et al.* Social status in mouse social hierarchies is associated with variation in oxytocin and vasopressin 1a receptor densities. *Horm. Behav.* **114**, (2019).

266. So, N., Franks, B., Lim, S. & Curley, J. P. A social network approach reveals associations between mouse social dominance and brain gene expression. *PLoS One* **10**, 1–27 (2015).

267. Neumann, C., Mcdonald, D. B. & Shizuka, D. Dominance ranks , dominance ratings and linear hierarchies : a critique. *Anim. Behav.* **144**, e1–e16 (2018).

268. Williamson, C. M., Lee, W. & Curley, J. P.

Temporal dynamics of social hierarchy formation and maintenance in male mice. *Anim. Behav.* **115**, 259–272 (2016).

269. Shizuka, D. & McDonald, D. B. The network motif architecture of dominance hierarchies. *J. R. Soc. Interface* **12**, (2015).

270. Wang, F., Kessels, H. W. & Hu, H. The mouse that roared: Neural mechanisms of social hierarchy. *Trends Neurosci.* **37**, 674–682 (2014).

271. Mathis, A. *et al.* DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281–1289 (2018).

272. Sturman, O. *et al.* Deep learning-based behavioral analysis reaches human accuracy and is capable of outperforming commercial solutions. *Neuropsychopharmacology* **45**, 1942–1952 (2020).

273. Nilsson, S. R. *et al.* Simple Behavioral Analysis (SimBA) – an open source toolkit for computer classification of complex social behaviors in experimental animals. **02**, 1–29 (2020).

274. McKendrick, G. & Graziane, N. M. Drug-Induced Conditioned Place Preference and Its Practical Use in Substance Use Disorder Research. *Front. Behav. Neurosci.* **14**, 1–15 (2020).

275. Amancio-Belmont, O. *et al.* Chloramphenicol decreases CB1 receptor expression in the nucleus accumbens and prefrontal cortex and prevents amphetamine-induced conditioned place preference in rats. *Pharmacol. Biochem. Behav.* **159**, 1–5 (2017).

276. Sánchez-Zavaleta, R. *et al.* GPR55 activation prevents amphetamine-induced conditioned place preference and decrease the amphetamine-stimulated inflammatory response in the ventral hippocampus in male rats. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **120**, 110636 (2022).

277. Dehnen, T. *et al.* Costs dictate strategic investment in dominance interactions. *Philos. Trans. R. Soc. B Biol. Sci.* **377**, (2022).

278. Fawcett, T. W. & Johnstone, R. A. Learning your own strength: Winner and loser effects should change with age and experience. *Proc. R. Soc. B Biol. Sci.* **277**, 1427–1434 (2010).

279. Sandi, C. & Haller, J. Stress and the social brain: Behavioural effects and neurobiological mechanisms. *Nat. Rev. Neurosci.* **16**, 290–304 (2015).

280. Takahashi, A. The role of social isolation stress in escalated aggression in rodent models. *Neurosci. Res.* (2022). doi:10.1016/j.neures.2022.07.009

281. van Heukelum, S. *et al.* A central role for anterior cingulate cortex in the control of pathological aggression. *Curr. Biol.* **31**, 2321-2333.e5 (2021).

282. Ahmed, M., Best, L. M., Pereira, C. F., Boileau, I. & Kloiber, S. Effects of endocannabinoid system modulation on social behaviour: A systematic review of animal studies. *Neurosci. Biobehav. Rev.* **138**, 104680 (2022).

283. Hart, E. E., Blair, G. J., O'Dell, T. J., Blair, H. T. & Izquierdo, A. Chemogenetic modulation and single-photon calcium imaging in anterior cingulate cortex reveal a mechanism for effort-based decisions. *J. Neurosci.* **40**, 5628–5643 (2020).

284. Klugmann, M., Goepfrich, A., Friemel, C. M. & Schneider, M. AAV-mediated overexpression of the CB1 receptor in the mPFC of adult rats alters cognitive flexibility, social behavior, and emotional reactivity. *Front. Behav. Neurosci.* **5**, 1–10 (2011).

285. Fulenwider, H. D., Caruso, M. A. & Ryabinin, A. E. Manifestations of domination: Assessments of social dominance in rodents. *Genes, Brain Behav.* **21**, 1–15 (2022).

286. Graham, J. & Coghill, D. Adverse effects of pharmacotherapies for attention-deficit hyperactivity disorder: Epidemiology, prevention and management. *CNS Drugs* **22**, 213–237 (2008).

287. Hirvonen, J. *et al.* Reversible and regionally selective downregulation of brain cannabinoid CB 1 receptors in chronic daily cannabis smokers. *Mol. Psychiatry* **17**, 642–649 (2012).

288. Blanchard, C. D. *et al.* Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. *Psychoneuroendocrinology* **20**, 117–134 (1995).

289. Whishaw, I. Q. & Kolb, B. *The Behavior of the Laboratory Rat*. (Oxford University Press, 2004). doi:10.1093/acprof:oso/9780195162851.001.0001

290. Doran, N., Luczak, S. E., Bekman, N., Koutsenok, I. & Brown, S. A. Adolescent Substance Use and Aggression: A Review. *Crim. Justice Behav.* **39**, 748–769 (2012).

291. Espelage, D. L., Low, S., Rao, M. A., Hong, J. S. & Little, T. D. Family violence, bullying, fighting, and substance use among adolescents: A longitudinal

mediational model. *J. Res. Adolesc.* **24**, 337–349 (2014).

292. Bukstein, O. G. Aggression, Violence, and Substance Abuse in Adolescents. *Child Adolesc. Psychiatr. Clin. N. Am.* **5**, 93–109 (1996).

293. Spear, L. P. The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* **24**, 417–463 (2000).

294. Hartley, C. A. & Somerville, L. H. The neuroscience of adolescent decision-making. *Curr. Opin. Behav. Sci.* **5**, 108–115 (2015).

295. Kolk, S. M. & Rakic, P. Development of prefrontal cortex. *Neuropsychopharmacology* **47**, 41–57 (2022).

296. Amancio-Belmont, O., Romano-López, A., Ruiz-Contreras, A. E., Méndez-Díaz, M. & Prospéro-García, O. From adolescent to elder rats: Motivation for palatable food and cannabinoids receptors. *Dev. Neurobiol.* **77**, 917–927 (2017).

297. Badanich, K. A., Adler, K. J. & Kirstein, C. L. Adolescents differ from adults in cocaine conditioned place preference and cocaine-induced dopamine in the nucleus accumbens septi. *Eur. J. Pharmacol.* **550**, 95–106 (2006).

298. Verdurand, M. *et al.* Comparison of Cannabinoid CB 1 Receptor Binding in Adolescent and Adult Rats: A Positron Emission Tomography Study Using [ 18 F]MK-9470 . *Int. J. Mol. Imaging* **2011**, 1–11 (2011).

299. Arthur Schopenhauer. Dolores del mundo. in *El amor, las mujeres y la muerte* (9786079014704, 2012).

300. Nietzsche, F. W. *Also sprach Zarathustra: Ein Buch für alle und keinen*. (C. G. Naumann, 1907).

301. Hodgson, G. M. The enduring relevance of Darwin's theory of morality. *Bioscience* **63**, 513–514 (2013).

302. Brown, C., Garwood, M. P. & Williamson, J. E. It pays to cheat: Tactical deception in a cephalopod social signalling system. *Biol. Lett.* **8**, 729–732 (2012).

303. Enigk, D. K., Thompson, M. E., Machanda, Z. P., Wrangham, R. W. & Muller, M. N. Competitive ability determines coalition participation and partner selection during maturation in wild male chimpanzees (Pan troglodytes schweinfurthii). *Behav. Ecol. Sociobiol.* **74**, 89 (2020).

304. Lammers, J., Stoker, J. I. & Stapel, D. A. Differentiating social and personal power: Opposite effects on stereotyping, but parallel effects on behavioral approach tendencies. *Psychol. Sci.* **20**, 1543–1549 (2009).

305. Sagheddu, C., Muntoni, A. L., Pistis, M. & Melis, M. *Endocannabinoid Signaling in Motivation, Reward, and Addiction: Influences on Mesocorticolimbic Dopamine Function*. *International Review of Neurobiology* **125**, (Elsevier Inc., 2015).

306. Parsons, L. H. & Hurd, Y. L. Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neurosci.* **16**, 579–594 (2015).

307. Guerrero, T. P., Fickel, J., Benhaiem, S. & Weyrich, A. Epigenomics and gene regulation in mammalian social systems. *Curr. Zool.* **66**, 307–319 (2020).

# Appendix 1: Cover of article 1

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## Dominance status is associated with a variation in cannabinoid receptor 1 expression and amphetamine reward

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ABSTRACT

The rewarding effects of psychostimulants appear to be distinct between dominant and subordinate individuals. In turn, the endocannabinoid system is an important modulator of drug reward in the nucleus accumbens and medial prefrontal cortex, however the connection with social dominance is yet to be established. Male rats were classified as dominant or subordinate on the basis of their spontaneous agonistic interactions and drug reward was assessed by means of conditioned place preference with amphetamine (AMPH). In addition, the expression of CB1R, CB2R, FAAH1, and DAGLa was quantified from accumbal and cortical tissue samples. Our findings demonstrate that dominant rats required a lesser dose of AMPH to acquire a preference for the drug-associated compartment, thereby suggesting a higher sensitivity to the rewarding effects of AMPH. Furthermore, dominants exhibited a lower expression of CB1R in the medial prefrontal cortex and nucleus accumbens. This study illustrates how CBR1 expression could differentiate the behavioral phenotypes associated to social dominance.

### 1. Introduction

Psychostimulants act upon the neuronal substrates that code for reward and their use can induce highly gratifying experiences that increase the probability of their future consumption (Koob and Volkow, 2016). Drug reward is a crucial component of substance abuse disorder and furthering our understanding of the factors that modulate reward is a fundamental step towards more effective treatments (Volkow et al., 2019). Across species, the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC) are crucial brain structures mediating reward to natural stimuli and drugs of abuse (Sabatinelli et al., 2007; Volkow et al., 2019).

Participation in social relationships shapes an individual's brain and behavior (Falk and Bassett, 2017; Hari et al., 2015; Kingsbury et al., 2019). Across the animal kingdom, individuals partake in dominance relationships that have serious consequences beyond the social domain; including on health, emotional wellbeing, cognition, and motivated behavior (Fournier, 2020; Sapolsky, 2005; Sherman and Mehta, 2020; Wallace et al., 2022). Social dominance is a learned mode of interaction between conspecifics (Drummond, 2006) and emerges both in the wild and in captivity (Chase and Seitz, 2011). Dominance relationships are established in acquainted animals and manifest as an asymmetry of

*Abbreviations:* Dom, dominant; Sub, subordinate; eCBS, endocannabinoid system; CB1R, cannabinoid 1 receptor; CB2R, cannabinoid 2 receptor; FAAH1, fatty acid amide hydrolase 1; DAGLa, diacylglycerol lipase alpha; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; AG, aggressive grooming; DP, dominance posture; MDS, Modified David's Score; AMPH, d-amphetamine; A-CPP, d-amphetamine conditioned place preference; PRE, preconditioning session; TEST, test session; EPM, elevated plus maze; LIRL, low-intensity red lighting; HIWL, high-intensity white lighting.

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*Figure 14*

# Appendix 2: Acknowledgement of article 1



previous reports that showed a stronger cocaine attraction measured with CPP in dominant mice (Yanovich et al., 2018). Regardless of dominant status, we observed that the highest dose of AMPH did not evoke conditioned preference, which can be attributed to the adverse effects of psychostimulants at this dose (Graham and Coghill, 2008). Taken all together, it appears that the sensitivity to the rewarding effects of psychostimulants between male cynomolgus macaques and male rodents (rats and mice) is divergent, thus suggesting a species-dependent effect. Interestingly, our results are consonant with human studies and therefore retain translational value. In people, high social status has been reported to promote approach-related tendencies to rewards (Cho and Keltner, 2020) and personality traits associated with dominance have been found to predict substance use and abuse (Tarter et al., 2007).

Herein, we focused on male subjects, however, sex appears to be an important factor to understanding the relationship between dominance and drug reward. Social dominance is prevalent among female primates and rodents, albeit males engage in more overt forms of agonistic behavior (Chen Zeng et al., 2022; Varholick et al., 2021). Contrary to what has been reported in males, female dominant cynomolgus macaques self-administered cocaine at significantly higher rates than their subordinate counterparts (Nader et al., 2012). In the field of social dominance, males have received more attention and we consider that further study is needed to understand how sex contributes to the relationship between dominance and drug reward.

The relationship between anxiety and psychostimulant reward may seem difficult to reconcile since these drugs have been reported to promote anxiety in both humans (Bystritsky et al., 1991) and rodents (Blanchard and Blanchard, 1999). Notwithstanding, it has been shown that rats displaying high anxiety on the EPM have a greater escalation of cocaine self-administration (Dilleen et al., 2012) and acquire cocaine CPP whereas low anxiety rats do not (Pelloux et al., 2009). On the other hand, subordination has been described as anxiogenic (Blanchard et al., 1993; Dijk et al., 2018), however, findings in rodents are not consistent (Varholick et al., 2021). Some groups report that subordinate individuals are the most anxious (Davis et al., 2009), while other groups have reported the opposite or no difference (Varholick et al., 2021). Housing conditions are likely to explain discrepancies between studies, since same-sex housing, unlimited food availability and a simplistic laboratory environment are known to reduce agonistic confrontations, potentially attenuating the anxiogenic effects of social conflict (Kondrakiewicz et al., 2019; Varholick et al., 2021). We did not find differences in anxiety-like behavior between dominants and subordinates measured by open arms exploration in EPM. Since rats are nocturnal foragers and photophobic in nature (Whishaw and Kolb, 2004), we evaluated EPM exploration under two lighting conditions to test if there was a differential reactivity to contextual stressors (Haller et al., 2004). Not surprisingly, subjects under HIWL explored the open arms less and moved less compared to LIRL, regardless of dominance status. Consequently, it seems unlikely that anxiety nor the reactivity to stressors are involved in the differential sensitivity to the rewarding effects AMPH.

While the literature on the eCBS and aggression is extensive (Kolla and Mishra, 2018; Miczek, 1978; Wei et al., 2017), the present study is the first to analyze the relationship between the mammalian eCBS and dominance status. Our data revealed that CB1R has a differential expression between dominants and subordinates in the mPFC and NAc, while no differences are observed in CB2R, FAAH1, and DAGla. Furthermore, a robust negative linear relationship between CB1R and the dominance score in both brain structures is observed, where higher expression of the receptor is associated with lower success in agonistic encounters. Social factors have been shown to regulate gene expression (Guerrero et al., 2020), hence it is possible that social dominance exerts an epigenetic regulation of CB1R expression. Supporting this hypothesis, a study in zebrafish found that whole-brain gene expression of CB1R is higher in subordinates (Orr et al., 2021).

It remains to be established if the differential expression of CB1R between dominants and subordinates directly contributes to the differences in drug reward. In rodents, projections from the prelimbic (PL) portion of the mPFC have been found to stimulate drug-seeking (Bravo-Rivera and Sotres-Bayon, 2020; Peters et al., 2009). Furthermore, infusions of a CB1R agonist into the PL inhibits drug reward, while infusions of an antagonist promotes it (Ahmad et al., 2013; Hu et al., 2015). In NAc, CB1R activity in cortical afferents that project to cholinergic interneurons downregulate the release of dopamine and reduce motivated behavior (Mateo et al., 2017). Taken together, we hypothesize that a low CB1R count in dominants implies a reduction in the number of binding sites for endocannabinoids, possibly making dominants more inclined to actuate drug-seeking.

The CB2R has been linked to agonistic interactions, where CB2R-defficient mice were found to be more aggressive than wild-type mice and isolation-induced aggression was attenuated by the pharmacological activation of the receptor (Rodriguez-Arias et al., 2015). Nevertheless, it is possible that CB2R contributes to establishment of dominance relationships without being affected by which status is attained.

## 5. Conclusion

The present study contributes to understanding why drugs of abuse affect members of a group differently. Previously, behavioral differences associated to dominance status have been attributed to the dopaminergic system (Ghosal et al., 2019). Our data indicates that CB1R could be an additional underlying mechanism that could explain why psychostimulant reward is distinct between dominant and subordinate male rats. Furthermore, CB1R receptor is known to modulate the dopaminergic system (Covey et al., 2017; Haj-Dahmane and Shen, 2011), thus painting the possibility of a multi-system crosstalk.

## Declaration of competing interest

The authors report there are no competing interests to declare.

## Data availability

Data will be made available on request.

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## References

Ahmad, T., Lauzon, N.M., de Jaeger, X., Laviolette, S.R., 2013. Cannabinoid transmission in the prelimbic cortex bidirectionally controls opiate reward and aversion signaling through dissociable kappa versus μ-opiate receptor dependent mechanisms. J. Neurosci. 33 (39), 15642–15651. https://doi.org/10.1523/JNEUROSCI.1686-13.2013.

Amancio-Belmont, O., Pérez-Vázquez, D., Ruiz-Contreras, A.E., Pérez de la Mora, M., Rueda-Orozco, P.E., Méndez-Díaz, M., Prospero-García, O.E., 2017. Chloramphenicol decreases CB1 receptor expression in the nucleus accumbens and prefrontal cortex and prevents amphetamine-induced conditioned place preference in rats. Pharmacol. Biochem. Behav. 159 (June), 1–5. https://doi.org/10.1016/j.pbb.2017.06.008.

Balasubramaniam, K.N., Berman, C.M., De Marco, A., Dittmar, K., Majolo, B., Ogawa, H., Thierry, B., De Vries, H., 2013. Consistency of dominance rank order: a comparison of David's scores with I&SI and Bayesian methods in macaques. Am. J. Primatol. 75 (9), 959–971. https://doi.org/10.1002/ajp.22160.

Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A.E., Ceresini, G., Poli, M.D., Parmigiani, S., 2001. Social status in mice: behavioral, endocrine and immune changes are context dependent. Physiol. Behav. 73 (3), 401–410. https://doi.org/10.1016/S0031-9384(01)00453-X.

# Appendix 3: Cover of article 2

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Review article

## Endocannabinoid system and aggression across animal species

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ABSTRACT

This narrative review article summarizes the current state of knowledge regarding the relationship between the endocannabinoid system (ECS) and aggression across multiple vertebrate species. Experimental evidence indicates that acute administration of phytocannabinoids, synthetic cannabinoids, and the pharmacological enhancement of endocannabinoid signaling decreases aggressive behavior in several animal models. However, research on the chronic effects of cannabinoids on animal aggression has yielded inconsistent findings, indicating a need for further investigation. Cannabinoid receptors, particularly cannabinoid receptor type 1, appear to be an important part of the endogenous mechanism involved in the dampening of aggressive behavior. Overall, this review underscores the importance of the ECS in regulating aggressive behavior and provides a foundation for future research in this area.

### 1. Introduction

Group-living is an evolutionary strategy readily expressed across the animal kingdom, from insects to humans (Krause and Ruxton, 2002). For these species, social interactions are crucial for an individual's survival and wellbeing, however, proximal living brings the problem of resource distribution between members(Chapman and Valenta, 2015; Markham and Gesquiere, 2017). Cooperative strategies, particularly among humans, have demonstrated to be essential in addressing this challenge and it has been hypothesized that the size of the neocortex in primates evolved primarily to support these complex social interactions (Dunbar, 1998; Van Vugt and Smith, 2019). On the other side, competitive strategies involving aggression are a ubiquitous across the animal kingdom and are another means to secure access to limited resources, involving the establishment of dominance hierarchies and territorial defense (Lorenz, 1974; Tibbetts et al., 2022; Van Vugt and Smith, 2019).

Aggression is defined as any hostile behavior directed towards a conspecific that has the goal of overpowering (Lischinsky and Lin, 2020). How aggression is expressed varies widely across taxa, but generally encompasses both threat displays and physical attacks (Grant and Mackintosh, 1963; Lischinsky and Lin, 2020). Human aggression is controversial due to the presence of both positive and negative associated aspects. On one side, aggression can serve as a means of self-defense, allowing individuals to protect themselves and others from harm. However, the dark side of aggression cannot be overlooked. Uncontrolled or excessive aggression is a public health problem that causes both physical and psychological harm, disrupts social cohesion, and raises legal and ethical concerns (Kolla and Mishra, 2018; Oram et al., 2022; Tomlinson et al., 2016; World Health Organization, 2014).

Animal behavior is underscored by the activity of neurobiological systems and entails a continuous information exchange between brain cells, involving both neurons and glia. The ECS is a widespread modulatory system that fine-tunes activity of several neuro/gliotransmitters, hormones, and cytokines involved in emotion, cognition, and social behavior (Ahmed et al., 2022; Gunduz-Cinar, 2021; Parsons and Hurd, 2015; Wei et al., 2017). Through the study of animal models, researchers can gain valuable insights into the neural mechanisms that drive aggressive behavior, which may help develop new treatments for preventing and treating excessive aggression in humans (Golden et al., 2019; Haller, 2017; Miczek et al., 2007).

The present review explores the relationship between the ECS and aggression across multiple vertebrate species, including fish, birds,

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# Appendix 4: Acknowledgement of article 2



phase, which revealed that the anti-aggressive effects of Δ9-THC persisted after repeated dosing. On the other hand, heightened aggression in adolescent monkeys is indicative of a sustained cumulative effect of chronic drug exposure and resembles what has been reported in humans. A longitudinal study in adolescents reported that an increase in cannabis use in a period of 5 years is associated with an increased risk of participating in fights (Norström and Rossow, 2014). A meta-analysis of the literature demonstrated a moderate association between cannabis use and physical aggression in adolescents and young adults independent of socioeconomic status and use of other substances (Dellazizzo et al., 2020). Increasing evidence indicates that adolescence represents a critical period during which cannabis use can have significant and lasting negative impacts on cortical areas associated with inhibitory control and emotional evaluation (Molla and Tseng, 2020; Sullivan et al., 2022; Wesley et al., 2016), which could possibly lead to an increased risk of aggressive behavior (Ostrowsky, 2011). While the monkey study suggests that heightened aggression is not directly associated with withdrawal, it does not discount the potential role of cannabis withdrawal syndrome in explaining increased aggression among certain chronic consumers (Tomlinson et al., 2016). Further pre-clinical research is necessary to fully understand the relationship aggression and chronic cannabis use.

### 3.5. Limitations and future directions

Despite the extensive research on role of the ECS in aggression, several limitations persist within the existing literature. Primarily, a significant proportion of studies have relied on pharmacological tools to manipulate the ECS's activity. While these interventions have provided valuable insights into the system's potential involvement in aggression, they have several inherent limitations. One key drawback of using pharmacological agents is the lack of specificity in targeting the ECS (see Introduction). These compounds often interact with multiple receptors and pathways beyond the ECS, leading to potential off-target effects. Consequently, interpreting the results solely based on pharmacological interventions may lead to inaccurate conclusions about the ECS's true role in aggression. Furthermore, most research has focused on understanding the consequences of manipulating the ECS rather than investigating endogenous neural mechanisms through which the ECS regulates aggression.

The development of novel methods for monitoring and manipulating the ECS promise to provide greater physiological relevance to findings (Covey and Yocky, 2021). Genetically encoded fluorescent sensor techniques (GEFST), such as gentically encoded calcium influx indicators, and the miniaturization of head-mounted fluorescent microscopes have allowed for cell-type specific monitoring of population activity during ongoing social interactions (Kingsbury et al., 2019; Nair et al., 2023). Employing this monitoring technique may shed light on the intricate pathways through which the ECS regulates aggression. On the other hand, G protein-coupled receptor (GPCR)-based sensors that convert ligand binding into a fluorescent signal have provided a rapid and precise detection of DA release in freely behaving mice (Sun et al., 2018). More recently, a genetically encoded eCBs sensor has been recently developed and implemented to probe eCBs release during a behavioral task in mice (Dong et al., 2022). On the other hand, advancements in optogenetic tools have enabled precise control of GPCRs and downstream effectors on a cell-type specific basis (Abreu and Levitz, 2022). Since cannabinoid receptors can express in multiple cell types in the same structure, the potential of cell-type optical manipulation of cannabinoid receptors opens up new and exciting avenues for dissecting their specific roles within complex microcircuits. Taken together, these tools for monitoring and manipulating eCB signaling are expected to significantly enhance our comprehension within physiologically relevant spatial and temporal scales.

Another significant limitation arises from traditional methods of quantifying aggression, which have relied on human observers to manually score complex social interactions. Among the issues inherent to manual annotation are observational bias and drift, long analysis times, and inter-rater reliability (Anderson and Perona, 2014). To overcome some of these limitations, researchers can leverage advancements in deep learning techniques, particularly pose estimation, to obtain fine-grained and quantifiable measurements of motor action without the need for human intervention (Lauer et al., 2022; Mathis and Mathis, 2020). Pose estimation involves using computer vision algorithms to track the positions and movements of key body points of one or multiple organisms from video data. Implementation of supervised classification algorithms using multi-animal pose estimation tracking has proven useful in automatizing the detection of aggressive behaviors between freely-interacting rodents (Nilsson et al., 2020; Segalin et al., 2021). Furthermore, simultaneously pairing of an automated analysis of behavior and a GEFST offers the opportunity to obtain a real-time correlate between behavior and a neural signaling mechanism (Nair et al., 2023), holding the potential to unlock new frontiers in our comprehension of the brain-behavior interface.

## 4. Conclusion

Animal models of aggression are an essential tool for understanding the neurobiological mechanisms underlying aggressive behavior. Accumulated evidence from the last six decades places the ECS as an important modulator of aggression. Herein, we have reported numerous studies conducted on various animal species, including rodents, non-human primates, birds, and fish. However, the findings are not always straightforward. The effects of ECS on aggression seem to be conditional on other variables (e.g., stress and dominance status) and can change with repeated administrations. Overwhelmingly, the acute activation of cannabinoid receptors or enhancement of eCB signaling reduces aggression in multiple animal models. Fewer studies have evaluated the long-term chronic administration and their results can diverge from what has been reported with acute administrations. An endogenous CB1R-mediated mechanism involved in the dampening of aggressive behavior seems probable; however, further research is needed to discern how CB1R influences brain circuits mediating aggression. Lastly, the inclusion of female subjects and implementations of novel technologies to monitor and manipulate the ECS are expected to be fruitful avenues in advancing our understanding into the neurobiology of aggression.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT-3.5 (OpenAI) to refine the language and improve the manuscript's overall quality. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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## References

Abel, E.L., 1975. Cannabis and aggression in animals. Behav. Biol. 14 (1), 1–20. https://doi.org/10.1016/S0091-6773(75)90266-7.
Abel, E.L., 1977. The relationship between cannabis and violence: a review. Psychol. Bull. 84 (2), 193–211. https://doi.org/10.1037/0033-2909.84.2.193.
Abreu, N., Levitz, J. (2022). Optogenetic Techniques for Probing G Protein-Coupled Receptor Signaling. https://doi.org/10.1007/978-1-0716-0755-8.