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Orbitofrontal cortex encoding delayed gratification in chronic pain

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Orbitofrontal Cortex encoding of reward delayed gratification in chronic pain

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Abbreviations

ANOVA	Analysis of variance
AP	Anterio-posterior
BLA	Basolateral amygdala
CFA	Complete Freund's Adjuvant
D1R	Dopamine D1 receptors
D2R	Dopamine D2 receptors
DA	Dopamine
DG	Delayed gratification
DGt	Delayed gratification task
DV	Dorso-ventral
FSV	Fast-scan cyclic voltammetry
HPLC	High precision liquid chromatography
IP	Intraperitoneal
KS	One-sample Kolmogorov-smirnov
KS2	Two-sample Kolmogorov-smirnov
KW	Kruskal-Wallis
LO	Lateral orbital
ML	Medio-lateral
MO	Medial orbital
NAc	Nucleus accumbens
OFC	Orbitofrontal cortex
PFC	Prefrontal cortex
SD	Standard Deviation
VTA	Ventral tegmental area

Sumário

A dor crónica causa alterações estruturais e funcionais em várias regiões do cérebro, entre os quais o córtex orbitofrontal (OFC), caracterizadas por uma redução da substância cinzenta e uma diminuição da transmissão dopaminérgica. Dado que a dopamina, é fundamental para funcionamento do OFC, este declínio compromete o seu desempenho. O OFC desempenha um papel crucial no processamento de recompensas, particularmente na monitorização e atualização do valor dos efeitos /resultados esperados. Para além disso, está também associado à regulação de comportamentos impulsivos, através da promoção de escolhas por recompensas mais vantajosas e tardias. Dessa forma, as alterações provocadas pela dor crónica no OFC contribuem para a mudança observada em indivíduos com essa condição, por uma maior preferência por escolhas de gratificação instantânea. No entanto, na literatura atual surgem papéis contraditórios para o OFC na gratificação atrasada, o que destaca a necessidade por mais investigação. Para abordar este problema, este trabalho foi projetado em duas fases. A primeira visou validar se, sob condições de dor inflamatória crónica, há de facto uma maior preferência por recompensas instantâneas numa tarefa de gratificação atrasada (DGt). A segunda fase, explorou o papel do OFC na DGt e como a modulação farmacológica dos recetores de dopamina D2 (D2r) afetou o desempenho na DGt, ambos sob dor inflamatória crónica. A DGt consistiu em 2 alavancas retráteis com diferentes contingências de recompensa: a “alavanca instantânea”, que entregava 1 “pellet” se pressionada 5 segundos após exposição, e a “alavanca atrasada”, que entregava 3 “pellets” se pressionada 10 segundos após exposição. Para induzir o modelo de dor inflamatória crónica foram injetados 50 µL de Adjuvante de Freund Completo (CFA) no lado dorsal da pata traseira. Na segunda parte do projeto, antes da indução da dor inflamatória, os ratos foram submetidos a lesões unilaterais do OFC usando ácido quinolínico (0.4 µl, 0.18 M). O desempenho comportamental foi avaliado 1, 2 e 3 semanas após a injeção de CFA. Cada sessão de teste compreendeu 3 tratamentos: veículo (NaCl 0.9% w/v, IP), raclopride (antagonista D2r, 0.05 mg/kg, IP), e quimpirol (agonista D2/3r, 0.05 mg/kg, IP). As respostas nociceptivas foram avaliadas usando filamentos de von Frey. Os resultados revelaram que os dois grupos experimentais preferiram recompensas instantâneas. No entanto, foram observadas diferenças nos ensaios não recompensados, visto que o grupo CFA exibiu uma maior preferência pela alavanca instantânea. Esta semelhança não foi observada após as lesões no OFC, uma vez os animais sem dor mostraram uma maior

preferência por ensaios recompensados de forma atrasada e ensaios não recompensados de forma instantânea. Por outro lado, o grupo CFA com lesão mostrou desempenhos semelhantes ao grupo CFA sem lesão. Relativamente à modulação dos D2r, a administração sistêmica de quimpirol aumentou o número de trocas entre alavancas, enquanto que o raclopride levou a uma diminuição. Estes resultados sugerem que lesões unilaterais no OFC podem comprometer a capacidade de atualizar os valores das recompensas, prejudicando a aprendizagem associativa, sem aumentar a impulsividade nestes animais. No grupo CFA, foram observados desempenhos semelhantes, provavelmente porque a dor crônica induz défices semelhantes aos causados por lesões no OFC. Por último, o quimpirol aumentou o número de trocas e o raclopride levou a uma diminuição, devido a alterações na sensibilidade à receção de recompensa, sem afetar os índices de preferência, possivelmente devido a um efeito sistémico ao invés de localizado.

Palavras-chave: Córtex orbitofrontal, gratificação atrasada, impulsividade, dopamina, dor inflamatória crônica, ácido quinolínico, quimpirole, raclopride

Abstract

Chronic pain induces structural and functional changes in several brain regions, including the orbitofrontal cortex (OFC), characterized by reduced gray matter and decreased dopaminergic transmission. Since dopamine is crucial for the OFC's optimal functioning, this decline impairs its performance. The OFC plays a key role in reward processing, particularly in monitoring and updating the value of expected outcomes. Additionally, it has been associated with regulation of impulsive behaviors by promoting the pursuit of more advantageous and delayed rewards. Thus, the changes caused by chronic pain likely contribute to the observed shift towards instant gratification over delayed in individuals with chronic pain. However, current evidence indicates contradictory roles for the OFC in delayed gratification, highlighting the need for further investigation. To address this problem, this study was designed in two phases. The first, aimed to validate whether, under conditions of chronic inflammatory pain, there is indeed a greater preference for instant rewards in a delayed gratification task (DGt). The second, explored the role of OFC in executing the DGt and how pharmacological modulation of dopamine D2 receptors (D2r) affected performance in DGt, both under chronic inflammatory pain. The DGt consisted of 2 retractable levers with different reward delivery contingencies: the "instant lever", which delivered 1 food-pellet if pressed 5 sec after exposure, and the "delayed lever", which delivered 3 food-pellets if pressed 10 sec after exposure. To induce the chronic inflammatory pain model, 50 μ L of Complete Freund's Adjuvant (CFA) were injected into the dorsal side of the hindpaw. In the second part of the project, prior to induction of inflammatory pain, rats underwent to unilateral lesions of the OFC using quinolinic acid (0.4 μ L, 0.18 M). Behavior performance was assessed 1, 2, and 3 weeks post-CFA injection. Each probe session comprises 3 treatments: vehicle (NaCl 0.9% w/v, IP), raclopride (antagonist D2r, 0.05 mg/kg, IP), and quinpirole (agonist (D2/3r, 0.05 mg/kg, IP). Mechanical noxious stimulation was assessed using von Frey filaments. The results revealed that both experimental groups preferred instant rewards. However, the difference between groups emerged during non-rewarded trials, as CFA group exhibited a higher preference for the instant lever. This similarity in rewarded trials was not observed after OFC lesions, as animals without pain showed a higher preference for delayed rewarded trials and instant non-rewarded trials. Conversely, CFA group showed similar results to their non-lesioned counterpart. Regarding D2r modulation, systemic administration of quinpirole increased lever-shifting, while raclopride decreased

it. These results suggest that unilateral OFC lesions may impair the ability to update reward values, compromising associative learning, without increasing impulsivity. This impairment was not observed in CFA group, likely because chronic pain induces deficits similar to those caused by OFC lesions. Furthermore, quinpirole increased shifting behavior, while raclopride had the opposite effect, possible due to alterations in reward sensitivity, without affecting overall preference indices, likely due as result of a systemic rather than a localized effect.

Keywords: orbitofrontal cortex, delayed gratification, impulsivity, dopamine, chronic inflammatory pain, quinolinic acid, quinpirole, raclopride.

I. Introduction

I.1 Time–Reward Associations

In our everyday lives, we often face decisions that involve a trade-off between two different dimensions: time and magnitude. In simpler terms, sometimes we must choose between an instant reward and another that is temporally distant but more beneficial [1, 2]

Healthy individuals generally maintain a balance between these types of choices (instant and delayed). However, some people consistently favor instant choices, a tendency linked to impulsive traits and associated with various pathologies such as obesity, substance abuse, and gambling [3, 4]. The preference for immediate rewards can be explained by a phenomenon known as temporal discounting, where the value of choice or reward is decreased as time passes. For example, addicted individuals show increased temporal discounting rates compared to healthy individuals, which is consistent with their inability to pursue later rewards [1].

Regarding time-reward associations, there are two paradigms that, while seemingly similar, are distinct: delayed discounting and delayed gratification (DG). Delayed discounting is a cognitive process in which the subjective value of a reward decreases as the delay to its receipt increases. In contrast, DG is a behavioral process where an individual is presented with two choices and opts for the delayed reward, waiting for its delivery [2, 5].

One of the pioneering studies on DG was conducted in the 1970s by Walter Mischel and his colleagues [6]. This research, famously known as the “Marshmallow Test”, involved children aged 4 to 5. Each child was tested individually, seated in a chair with a marshmallow placed on a table in front of them. The researchers instructed the children that they would leave the room and that if the marshmallow was still uneaten upon their return, the child would receive an additional marshmallow (thus, offering a choice between one marshmallow “now” or two later). The findings revealed distinct behaviors among the children: some resisted the immediate temptation and patiently waited for the researchers to return, while others ate the marshmallow almost immediately after the researchers left. The study also included variations, such as altering the duration of the researchers’ absence and initially not showing the marshmallow to the children.

These variations showed that when the reward was not visible, the more impulsive infants, performed better [7, 8]. However, the intriguing aspect of this research emerged in follow-up studies conducted over the following 40 years. These studies found that the children who chose the delayed reward exhibited positive life outcomes. They achieved higher academic performance, demonstrated lower rates of obesity and substance abuse, possessed stronger social skills, and displayed better coping mechanisms for stress. Conversely, children with greater impulsivity showed less favorable outcomes in these areas [9-11].

This long-term work highlighted the critical role that the ability to delay gratification plays in our lives, leading to the question of what differences exist between these two groups of children/adults.

I.1.1 Delayed gratification tasks in rodents

Humans have been shown to be valuable for DG studies, as demonstrated by the “Marshmallow test”. However, the conclusions from such studies are often limited [2, 5, 8]. Consequently, there is a need to use other models, such as rodents, which allow for more invasive techniques and robust conclusions [12, 13]. Currently, several behavioral tasks in rodents effectively evaluate the DG paradigm, including the intertemporal choice task, waiting zone/reward zone task, time-based diminishing return task, and effort-based delay task.

In the intertemporal choice task [5, 14], rodents must choose between two levers associated with rewards of different magnitudes. One lever provides a small reward after a fixed short delay, while the other offers a larger reward after a fixed longer delay. Distinct auditory cues for each delay are used to guide the animal’s behavior. Pressing a lever before the associated delay results in no reward. A significant advantage of this task is its simplicity and the clear measurement it provides between the preference for an instant smaller reward and a delayed larger reward.

The waiting zone/reward zone task [3] assesses an animal’s ability to wait for a reward that increases in value the longer it waits. The animal is placed in a chamber, and the duration it spends there determines the size of its reward. While this task effectively evaluates the animal’s self-control to obtain larger rewards, it is less straightforward than

the intertemporal choice task due to the lack of clear distinction between instant and delayed rewards.

The time-based diminishing returns task [15] uses two levers: one with a fixed delay (FD) and the other (PD) with a progressively increasing delay with each press. The FD lever is set higher than the progressive lever, making the optimal strategy to start with the progressive option. A light cue signals after each press, followed by reward delivery. Importantly, pressing the FD lever resets the PD timer to zero. This task, while more complex and challenging for the animal to learn, is a good measure of self-control. The reset feature tests the animal's ability to wait for the FD reward to reduce the delay for the PD reward.

Lastly, the effort-based delay task [16] is conducted in a T-maze, assesses both delayed gratification and an animal's willingness to exert effort. One arm offers a larger reward after navigating obstacles and waiting for a delay, while the other provides a smaller with a simpler path. While valuable for measuring both self-control and motivation, this task introduces additional complexity in interpreting results solely for delayed gratification.

Overall, these tasks are excellent for measuring delayed gratification as their correct execution requires self-control and the consideration of the best choice. Among these tasks, the intertemporal choice task is the most intuitive. It effectively captures the inhibitory control and sustained decision-making required to choose the larger and delayed reward over the instant one.

I.2 Orbitofrontal Cortex: Localization and Architecture

For the last two decades, there has been a considerable increase in the number of studies conducted on the orbitofrontal cortex (OFC), demonstrating a growing interest in this area and its impact on our lives [17-19]. In rodents, this brain region is located on the dorsal side of the rostral rhinal sulcus. In contrast, in primates, it resides in the prefrontal cortex (PFC), specifically occupying a ventral position [20-22]. Regarding the cellular structure, the rodent OFC is comprised of 70-80% excitatory glutamatergic pyramidal neurons and 20-30% inhibitory GABAergic interneurons, further organized into five distinct layers [23, 24]. The most superficial layer (layer I) contains dendrites from pyramidal neurons in deeper layers and groups of interneurons. Layer II is primarily

composed of small excitatory pyramidal neurons and inhibitory interneurons. Layer III houses medium-sized pyramidal neurons and interneurons. Lastly, layers V and VI include large pyramidal neurons and interneurons [23-25].

The OFC of rodents can be subdivided into four distinct subregions with different functionalities: medial orbital (MO), ventrolateral orbital (VLO), lateral orbital (LO), and dorsolateral orbital (DLO) areas [17]. However, there is a key distinction between the primate and rodent OFC beyond the smaller size of latter, is that the rodent OFC is composed exclusively of agranular zones, lacking a distinct layer IV [22, 24, 26]. This fundamental difference raises questions about the existence of true homology between the rodent and primate OFC. Despite this evident distinction, similarities have been identified between the OFC of the two species, particularly in terms of location and connectivity [17, 26]. These similarities are crucial for translating findings from preclinical rodent studies to the human OFC, leading to a deeper understanding of the OFC's role in time-reward associations and impulsive behaviors.

I.2.1 OFC and its role on delayed gratification

The OFC plays a critical role in reward processing [18, 21]. This region integrates information from sensory cortices and from other brain areas including the basolateral amygdala (BLA), thalamus, hypothalamus, and insula [17, 20]. Additionally, it sends information to the nucleus accumbens (NAc), ventral tegmental area (VTA), PFC, periaqueductal gray (PAG), and exhibits reciprocal interconnections with the amygdala and thalamus [17, 20, 27]. Due to its connections, it is not surprising that the core function of the OFC is to encode the value of outcomes by integrating sensory and emotional information, making it crucial in both association learning and in intertemporal choices [21, 28, 29].

Since Damásio and Bechara's work, it has been recognized a link between OFC and impulsivity, as lesions in this area resulted in a preference for immediate rewards [30, 31]. However, subsequent research has yielded inconsistent findings regarding this premise. Some studies reported a shift towards instant rewards following OFC lesion, while others observed a preference for delayed rewards [28, 32, 33]. These contradictory findings suggested functional heterogeneity within the OFC [12]. In this regard, it has

been shown that lesions in the MO subregion decreases impulsive behavior, while lesions in the LO subregion develops an opposite effect [32, 33]. Together these findings suggest that the MO and LO subregions may contribute to distinct circuits underlying delayed gratification. In fact, it has been shown the LO participates in a circuit that mediates cognitive control to pursue long-term goals, whereas the MO is mainly involved in immediate goals processing [28, 33].

In terms of the OFC interplay with other brain areas, it has been shown that the OFC cooperates with the NAc and BLA to regulate impulsive behavior [12, 20, 34]. These brain regions are densely interconnected, facilitating their function [34]. Studies on NAc and BLA lesions but intact OFC showed a shift towards instant rewards, supporting the premise that these brain regions contribute to the pursuit of delayed and more beneficial rewards [34]. Within this network, the OFC is responsible for monitoring and updating the value of expected outcomes. Adding to this, the BLA, through OFC input, holds the value of the outcome and the NAc coordinates the appropriate behavioral responses taking into consideration the value of the outcome [12, 34]. In essence, this collaborative network of the OFC, NAc, and BLA regulates impulsive behavior through the pursuit of more advantageous and delayed rewards.

I.3 Chronic Pain and its impact on delayed gratification

Chronic pain is a complex and debilitating condition that affects millions of people worldwide [35]. Unlike acute pain, which serves a protective role, chronic pain has no biological value and significantly reduces the quality of life of affected individuals [36]. Essentially, chronic pain results from a malfunction in the peripheral nociceptors, which are responsible for reporting injuries [37]. This system becomes sensitized, lowering its activation threshold and increasing its firing rate [37, 38]. This peripheral sensitization impacts the central nervous system, leading to its sensitization and causing functional and structural reorganization of the brain [38]. These changes alter pain processing and ultimately contribute to the perpetuation of pain [37]. Since the brain allocates the majority of its resources to continuous pain processing, it leaves limited capacity for cognitive functions, thereby compromising them [39]. It is well known that

several cognitive functions are impaired, including decision-making and reward processing [40, 41].

Recent studies have aimed to understand how chronic pain affects decision-making in conditions involving trade-offs between time and magnitude [42, 43]. It has been observed that subjects with chronic pain tend to alter the outcome value of intertemporal choices, showing a preference for more immediate rewards over delayed ones, particularly (but not exclusively) in decisions related to pain relief and management [20, 42]. They adopt an impulsive behavior, undervaluing the potential benefits of long-term treatments. This leads to ineffective therapeutic adherence and poor medication management, including the misuse of opioids [43]. In this regard, opioid misuse is a significant issue in chronic pain management, with approximately 10% of chronic pain patients reporting opioid misuse, thereby increasing their risk of addiction and overdose [42, 44]. Therefore, it is crucial to gain a deeper understanding of the underlying processes in intertemporal choices in chronic pain to improve pain management and the overall well-being of individuals.

I.3.1 Persistent inflammatory pain model

Rodents are extensively used in pain research compared to other animal models, and significantly contribute to advancing our understanding of chronic pain [45, 46]. One origin of chronic pain can be persistent inflammation, being one of the most prevalent types of chronic pain [47, 48]. This has led to the development of various models to study its underlying mechanisms in greater depth, including Complete Freund's Adjuvant (CFA), carrageenan, and formalin [49].

Among these models, CFA is the most widely used, representing over half of published studies on inflammatory pain [46]. A key advantage of CFA over other inflammatory models is its ability to induce longer-lasting effects, making it particularly valuable for chronic pain studies. This is due to an extended period of hypersensitivity caused by the CFA [46, 49]. Additionally, compared to models of different pain etiologies, CFA is significantly simpler to induce. Its non-invasive nature minimizes complications and enhances the reliability of results [46]. A limitation of CFA model is its clinical relevance [46]. While CFA is a mineral oil containing a suspension of inactivated Mycobacteria wall, accidental exposure and subcutaneous accumulation of

dead bacteria is not a leading cause of chronic inflammatory pain in humans [46, 49, 50]. Despite this limitation, the CFA model is a robust choice. This model is well-characterized and studies have shown it replicates the cognitive deficits observed in human chronic pain patients [40, 43, 51]. For all these reasons, CFA serves as an excellent model for investigating the mechanisms underlying chronic inflammatory pain.

I.4 Role of dopaminergic modulation in OFC in delayed gratification

Dopamine plays a well-established role in supporting OFC's function [52-54]. Most of the dopaminergic input to the OFC originates from the VTA via the mesocortical pathway [52, 55, 56]. In the OFC, dopamine activity is regulated through phasic and tonic transmission, with these two modes of action working together to maintain dopamine release homeostasis [57, 58]. Phasic activity results from the depolarization of VTA dopaminergic neurons, leading to the opening of voltage-dependent calcium channels and subsequent dopamine release [59, 60]. This phasic dopamine release is associated to responses to environmental stimuli and unexpected rewards, playing a critical role in learning reinforcement [56]. On the other hand, tonic activity occurs intrinsically, where glutamatergic activity at dopaminergic terminals triggers dopamine release [60]. This helps maintaining the steady-state level of extracellular dopamine and stabilizing learning patterns [57, 59].

The OFC expresses both D1-like and D2-like dopamine receptor families [54, 61]. These receptors are in the dendritic arborizations of pyramidal neurons and in the cell bodies of GABAergic interneurons [57, 62]. D1-like receptors are more abundantly expressed [63] and are exclusively postsynaptically, whereas D2-like receptors are found both presynaptically and postsynaptically [54, 64]. Given the functional heterogeneity of the OFC, the distribution of these receptors varies. The MO exhibits a higher density of dopaminergic receptors compared to LO [55, 61]. In the LO, D1 receptors are more prevalent than D2 receptors [61]. Additionally, D1 receptors are distributed across all layers, whereas D2 receptors are primarily located in layer V [57, 65].

Dopamine plays a pivotal role in reward learning and goal-directed behavior [14, 56, 66]. It mediates the prediction error signal, the difference between received and predicted reward [67, 68]. This signal is triggered by unexpected rewards, enabling the

association between the cue and the reward [69, 70]. When a reward-predicting cue is encountered, it prompts the release of dopamine. Higher dopaminergic release corresponds to rewards of greater magnitude, while a decrease in dopamine release occurs if no reward or a lesser-than-predicted reward is received [67]. In addition to magnitude, dopamine release is also associated with the delay of reward [71]. Delayed rewards elicit a diminished dopamine response compared to an immediate one [14, 71]. However, larger delayed rewards cue triggers a strong dopamine response than smaller immediate, reflecting the best available option. The OFC contributes to the calculation of reward prediction errors by signaling information about expected outcomes [72]. When an unexpected reward is detected by a release of dopamine, the OFC uses this information to update the value of the option [52, 72]. This interaction is supported by a study that found that disrupting the connection between the OFC and VTA impairs the OFC from assigning value to new and unexpected rewards, preventing the animal from recognizing reward-cues and choosing the best option [73]. Therefore, the interplay between dopamine from the VTA and the OFC is crucial for assigning value to rewards, which is essential for making correct judgments of cost-benefits options.

The characteristic impulsive behavior and consequent preference for immediate rewards is linked to deficient dopaminergic signaling in OFC [74, 75]. This is supported by studies that demonstrated that dopamine depletion in rat OFC led to a decreased preference for larger and delayed rewards [75]. This may be because reduced dopaminergic transmission affects the OFC's ability to assign and update the value of rewards, leading to incorrect time-reward associations. While dopamine's relevance is clear, it is crucial to determine which receptors are involved. D1-like and D2-like receptors have distinct functions and thus, may play different roles in these cognitive processes [66, 76]. Research indicates that D2 receptors are more relevant in regulating impulsive behaviors, while the role of D1 receptors is less clear [53, 77, 78]. This is demonstrated by studies on rodents that found that injections into OFC of a D2 receptor antagonist resulted in a reduced preference for delayed rewards compared to vehicle animals, whereas D2 receptor agonists shifted the preference towards delayed rewards [79]. Another work found that blocking D2 receptors but not D1 receptors resulted in decreased preference for delayed rewards [80]. However, other studies were less conclusive, showing similar performances when D1 and D2 receptors were blocked with antagonists or when agonists of both receptors were injected into the OFC [53, 74]. This highlights the importance of considering the functional heterogeneity of the OFC, as

different areas are involved in distinct circuits for immediate and delayed rewards preference [28, 33]. Therefore, the injection site of dopamine receptors modulators can significantly impact results. Additionally, the dosage of the neuromodulators is critical. Dopamine's performance on cognitive functions, such as time-reward association follows an inverted U-shaped curve, emphasizing its dependence on the level of dopaminergic transmission [53, 81]. Thus, both reduced and excessive activation of dopaminergic receptors can impair performance. This impairment can be directly caused by abnormal activation of dopamine receptors or indirectly by the influence that one type of receptor has on the other. The D1 and D2 receptors work synergistically, and abrupt changes in one can disrupt the balance of the other [78, 81]. Moreover, this synergy is seen in tasks demanding behavioral flexibility and efficient decision-making. D2 receptors help the system determine the new course of action, while D1 receptors facilitate the stabilization of this new strategy [76]. This suggest that the balance between the two receptors is necessary for optimal decision-making.

Current evidence suggests a critical role for dopamine signaling through D2 receptors in regulating impulsive behaviors. However, inconsistencies in research highlight the need for further experimentation. These inconsistencies likely arise from the functional heterogeneity within OFC and the interplay between D1 and D2 receptors. In essence, impaired D2 dopaminergic transmission appears to disrupt the balance between cost and benefits evaluation, biasing decision-making towards immediate and lesser valuable rewards.

I.5 Chronic pain impact on OFC and dopaminergic modulation

Chronic pain induces significant structural and functional reorganization in the OFC [82]. This remodeling, according to rodent and human studies, includes a reduction in gray matter, diminished dopaminergic transmission and an increase in GABAergic activity [20, 83-85]. These changes impair OFC functionality, leading to cognitive deficits similar to those observed in cases of OFC lesions [86]. Pais-Vieira and colleagues [40], demonstrated this by implanting multielectrodes in the OFC and assessing the animal's performance in a risk uncertainty task at two distinct periods: before and after the induction of inflammatory pain. Before the induction of pain, OFC neurons distinguished the value of each lever associated with rewards of different frequencies and

magnitudes. However, in animals experiencing pain, only a small percentage of OFC neurons exhibited altered firing patterns in response to different reward receipt. Specifically, some neurons could not differentiate between choosing the different levers, resulting ultimately in a risky- prone behavior. This study demonstrated that the onset of inflammatory pain disrupts OFC's ability of encoding the value of rewards. This raises the possibility that the delayed gratification impairments observed in chronic pain may also be due to OFC's inability to encode reward value.

Other works on OFC and chronic pain are not focused on cognitive functions, but rather on modulating the nociceptive response, through dopamine. Dopamine, in addition to supporting cognitive functions, is crucial in modulating pain [60, 87]. This modulation is mediated by distinct dopamine receptors, D1r and D2r, that have opposing effects [82, 87]. While D1r contributes to the development and maintenance of nociception, D2r have an antinociceptive effect [60]. This functional dichotomy is supported by studies that have shown that microinjections of quinpirole, a D2r agonist, into the VLO alleviate pain, and this effect is reversed by using a D2r antagonist (raclopride), but not by blocking D1r [82, 88]. Further evidence of the antinociceptive role of D2r comes from research indicating that quinpirole microinjections alleviate allodynia, while microinjections of raclopride maintain it [60, 65]. Additionally, bilateral lesions in the VTA, an area that is a source of dopaminergic innervation to many brain regions, including OFC, exacerbate pain syndromes [89].

In the persistence of pain syndromes there is a disruption of dopaminergic transmission that leads to a hypodopaminergic state [87, 90, 91]. This reduction in dopamine levels has also been observed in OFC [83]. In this study, [83] HPLC (high precision liquid chromatography) was performed to measure tonic levels of dopamine and DOPAC (3,4-Dihydroxyphenylacetic acid) in animals with inflammatory pain at three different time points (5, 21, and 56 days) in areas including the OFC [83]. The study found that tonic levels of dopamine and DOPAC were lower on the 56th compared to the 5th day, with the lowest levels observed on the 21st day [83]. This demonstrated that chronic pain is indeed accompanied by reduced dopaminergic levels in OFC.

The effects of chronic pain extend beyond tonic dopamine transmission, also disrupting phasic dopamine release [92, 93]. This was showed by Taylor A. Gee and colleagues using fast-scan cyclic voltammetry (FSCV) [92]. Unlike HPLC, FSCV does not have temporal limitations, allowing for a clear correlation between behavior and phasic dopaminergic activity [94, 95]. They found that in animals with chronic pain, the

reduction in tonic levels resulted in an increase in phasic dopamine activity in comparison to baseline levels [92]. This may occur due to decreased D2 receptor (D2r) activity, as D2r antagonism increases phasic dopamine release. This disrupted ratio of low tonic/high phasic dopamine release, due to D2r hypoactivity, is thought to be responsible for increased impulsive behaviors and cognitive impairments [77, 92].

The role of dopamine in chronic pain, whether through the modulation of nociceptive responses or understanding of cognitive function, has been of big interest [53, 60, 96, 97]. This has resulted in the adoption of techniques in rodent studies, such as HPLC and FSCV, which have been mentioned earlier. There is an emerging technique with higher spatiotemporal resolution named dLight [98]. This technique relies on the engineering of a fluorescent protein fused with the dopamine receptor [94, 95]. When endogenous dopamine binds to the receptor, the conformational change increases fluorescence [94, 98]. Using this genetically encoded dopamine sensor allows for the real-time measurement of dopamine dynamics in relation to a given behavior, enabling the detection of both tonic and phasic dopamine release [95, 98]. Although there are currently few studies using dLight in chronic pain, it is a promising and interesting technique that is likely to gain significant relevance in dopamine studies in the context of chronic pain.

The hypodopaminergic state under chronic pain syndromes is thought to occur due to neuroplastic changes (that accompany chronic pain), causing an upregulation of GABAergic neurons in the VTA, that contribute to an inhibitory tone of dopamine, and consequently a decrease of dopaminergic transmission to areas such as OFC [56, 99-101]. Dopamine is essential for the optimal function of the OFC, so this reduction impairs its performance [53, 66]. This chain of events may underlie the impulsive behaviors and deficits in delayed gratification present in chronic pain syndromes.

I.6 AIMS

Given the inconsistencies and limited research on the interplay between orbitofrontal cortex (OFC) and D2 dopamine receptors (D2r) in delayed gratification tasks (DGt) performed by animals under chronic pain), this study aimed to elucidate the role of the OFC and dopaminergic modulation of D2r in these tasks. The study was conducted in two stages. Initially, I investigated whether chronic inflammation pain increases the preference for immediate rewards during a DGt. Then, I explored the role of OFC and D2r modulation on DGt performance with the goal of reversing these impairments.

Specifically, this work aimed to answer the following questions:

1. How does chronic inflammatory pain affect performance in DGt?
2. What is the role of the OFC in executing the DGt, and how does the systemic pharmacological blockade or activation of D2r influence performance under chronic inflammatory pain?

II. Materials and Methods

II.1 Animal model

This study included 16 male rats of CD strain weighing between 275 and 365 grams. The animals were maintained under a 12-hour light/dark cycle (normal cycle), alongside a temperature of 20 ± 2 °C and relative humidity between 45-60%. All handling sessions and arena habituation occurred at the same time of the day, during the light cycle. Furthermore, throughout the experiments, the rats experienced a controlled food deprivation, restricting their intake to 90-95% of their *ad libitum* feeding body weight to enhance their motivation to participate in the behavioral sessions. This study followed the guidelines set by the European Union directive (2010/63/CE) and the research and ethical standards of the International Association for the Study of Pain [102] .

II.2 Behavioral paradigm

II.2.1 Apparatus

The behavioral sessions were conducted in an arena measuring 45x45x40, with opaque and dark walls (**Figure II.1**). The arena was equipped with 2 retractable levers (A and B), positioned on the same wall and at same height. Between the retractable levers and at the same distance was a feeder cup, where chocolate-flavored pellets were supplied (TestDiet -5TUT, 45mg sucrose, Richmond, Indiana, USA). At a height of 90 cm, a camera was positioned to record the behavioral sessions. The maze's position and light intensity remained consistent throughout the experiments.

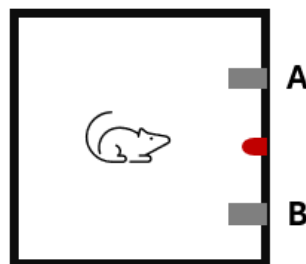


Figure II.1- Schematic representation of the apparatus used on the Delayed Gratification task.

II.2.2 Delayed gratification task

The experimental setup comprised two phases: a learning curve (**Figure II.2**) and probe sessions (**Figure II.3**). The learning curve consisted of 10 sessions, each with 50 trials, performed daily. In this phase, trials began with the exposure of the 2 retractable levers, accompanied by a sound cue (frequency = 1 kHz, duration = 1 sec). At 5 seconds of trial, a sound cue (frequency = 0.2 kHz, duration = 1 sec) is activated. To be rewarded, animals were required to press either lever after the 5 seconds of trial. Therefore, they had a 15-second window to choose and press either lever A or lever B, both resulting in an equal reward: 1 pellet. An incomplete trial (or omission) was considered if the animal did not press any lever within 20 seconds. In these situations, the trial was concluded accompanied by a sound (frequency = 0.2 kHz, duration = 0.2 sec). Trials were separated by 10 seconds inter-trial interval.

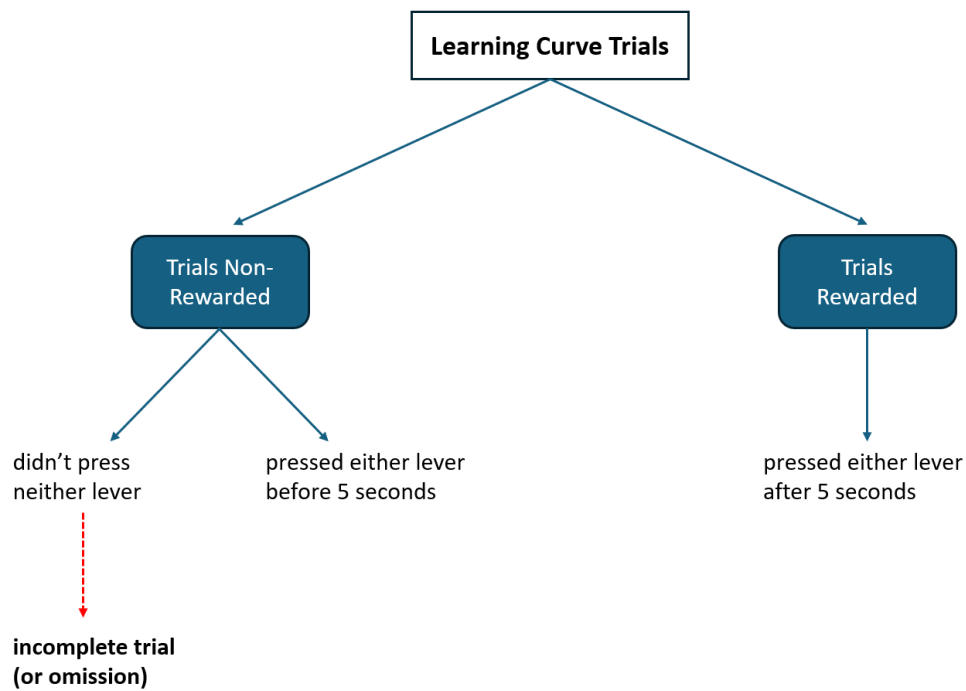


Figure II.2- Representation of Learning Curve Trials.

Only animals that presented, on average, less than 10% of incomplete trials in the last 3 days of learning curve advanced to the probe sessions. Each probe session was

composed by 100 trials. Moreover, in this phase, the two levers yielded different outcomes: the "instant lever," functioned similarly to those in the learning phase. On the other hand, the "delayed lever" required a 10-second wait before pressing but rewarded with triple amount of pellets. Both 5 second and 10 second mark were accompanied with a sound cue (frequency = 0.2 kHz, duration = 1 sec). The lever assignment (instant or delayed) was reversed every block of 50 trials, and each session had a distinct pattern to prevent bias. After each session, the Von Frey Filaments test was performed to assess mechanical noxious responses (see details below). The OpenControl software [103] was utilized for the hardware configuration of the task and behavioral data recording, while the Bonsai software [104] for video recording of the behavioral sessions and navigation vector of the animal position.

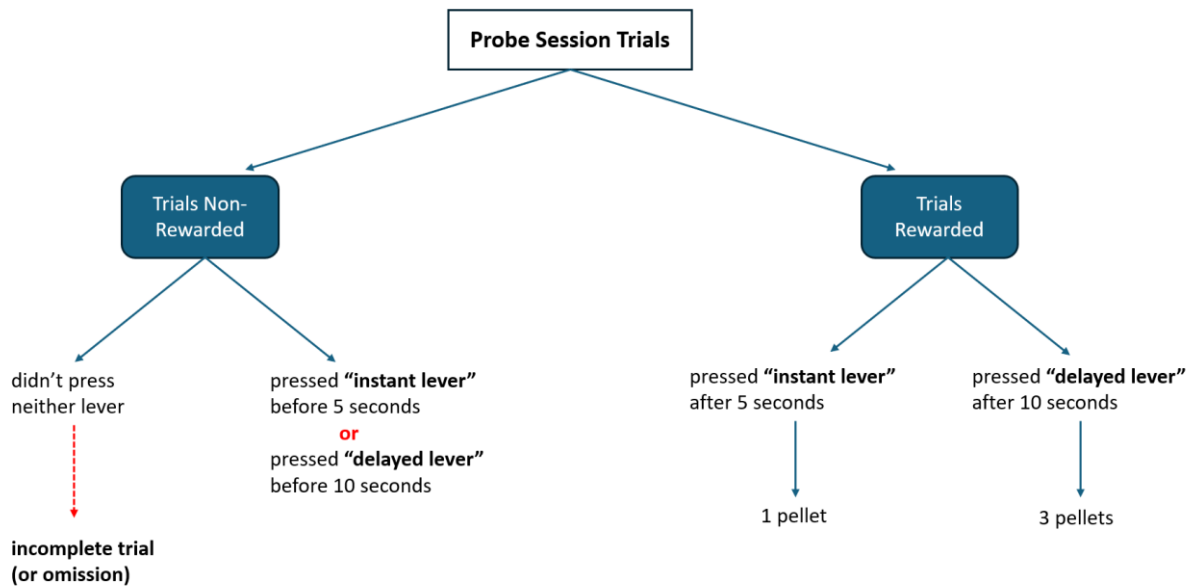


Figure II.3- Representation of Probe Sessions Trials.

II.3 Orbitofrontal cortex lesion

To study the role of OFC in the performance of the “delayed gratification task”, quinolinic acid was unilaterally injected into this brain area with random allocation: half animals were injected on the right hemisphere and the other half on the left hemisphere. To accomplish this, rats were anesthetized with a mixture of ketamine (75mg/kg; IMALGENE 1000) and medetomidine (0.5mg/kg; DOMITOR), injected

intraperitoneally (IP). The level of unconsciousness was monitored through hindpaw withdrawal and tail-pinch reflexes. If the surgery lasted more than 2 hours, extra ketamine (1/3 of initial dose) was given. During the surgery, body temperature was maintained at 37°C using a homeothermic blanket, and it was applied an ophthalmic ointment to avoid eyes drying. Once, the animals were securely positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with ears bars, their head fur was shaved. A skin incision was made, and the muscle tissue was displaced to expose the skull. After identifying bregma, the following stereotaxic coordinates of OFC from “Paxinos and Watson, Atlas for rats” [105] were used: +3.5 mm anteroposterior, ± 2.4 mm mediolateral and -4.5 mm dorsoventral. Then 0.4 μ L quinolinic acid (prepared from 0.18 M and diluted in water) [106] was administered at a rate of 0.1 μ L/min with a microliter syringe (Model 7002 Knurled Hub 2 μ L HAMILTON). The syringe remained in the place, stabilized, for additional 10 minutes to ensure the optimal absorption and diffusion of quinolinic acid in the targeted area. After this step the syringe was slowly removed. Then, the skin was sewed with non-absorbable surgical suture and disinfected with Betadine. The anesthesia was reversed with subcutaneous (SC) administration of Atipamezol (5mg/kg; ANTIDORM). Moreover, for analgesic purposes Ketoprofen (5mg/kg, SC; ROMEFEN 10%) and for antibiotic therapy Enrofloxacin (5mg/kg, SC; BAYTRIL) were administered over the course of the following 7 days. During the 7-day recovery period, animals were individually housed with unrestricted access to water and food. Throughout that period, their health status and weight were consistently monitored until subsequent induction of inflammatory pain model.

II.4 Rodent model of inflammatory pain

To enlighten the impact of inflammatory pain in a delayed gratification task, rats were anesthetized with a mixture of ketamine (75mg/kg, IP; IMALGENE 1000) and medetomidine (0.5mg/kg, IP; DOMITOR) and injected with 50 μ L of Complete Freund's Adjuvant (CFA; CAS: 9007-81-2; Sigma-Aldrich) into the dorsal side of the hindpaw to induce persistent inflammatory pain. For control purposes, the sham-treated group received an injection of the same volume of saline solution (NaCl 0.9% w/v). The CFA and saline injections were contralateral to the lesion in the OFC.

II.4.1 Evaluation of mechanical responses

The evaluation of sensitivity to noxious stimuli was conducted through the Von Frey filaments test [107]. The behavioral assessment occurred in a confined chamber with a metal mesh floor. Prior to test, each rat underwent a 15 minutes of acclimation period. Subsequently, Von Frey filaments were applied delicately perpendicular to the plantar surface of the paw. Each application lasted a maximum of 10 seconds or until a positive response occurred. A positive response was considered if the animal exhibited paw withdrawal, paw flinching or paw licking. The series of monofilaments involved decreasing forces to identify the filament eliciting at least 5 out of 10 positive responses, utilizing the up/down Dixon method. Two additional series of 10 stimulations were performed with 1 minute interval between sessions, using the same filament. The Von Frey test was performed at the end of each session, during the probe sessions phase.

II.5 Dopamine D2 receptors Neuromodulators

To investigate the role of the dopaminergic system in the triad of inflammatory pain – OFC – delayed gratification, two dopaminergic neuromodulators: raclopride and quinpirole were used. Raclopride functions as an antagonist for dopamine D2 receptor, while quinpirole acts as an agonist for dopamine D2/D3 receptors. These neuromodulators were exclusively utilized in experiment 2 (II.6.2) during probe sessions. On probe sessions days, prior to animal testing, IP injections of 0.05 mg/kg of raclopride (prepared from 50 mg/ml and diluted in saline; CAS: 98185-20-7; Sigma-Aldrich) or quinpirole (prepared from 1 mg/ml and diluted in saline; CAS: 85798-08-9; Sigma-Aldrich) were administered. The dosage (0.05 mg/kg) of quinpirole and raclopride utilized were determined through previous studies, which confirmed that this dose did not impair the locomotion of the animals nor influence their pain responses [108, 109]. For control purposes, animals received an IP injection of the same volume of saline. After the injections, the animals spent 20 minutes in an open field before the beginning of behavioral testing

II.6 Experimental timeline

Both experiments started similarly: 8 animals (per experience) were habituated to the arena where behavioral tasks were conducted. After becoming comfortable with the arena, the rats had to learn to press levers and understand the association between “press lever-receive pellet”. After this period, the learning curve began, lasting for 10 days with a daily session of 50 trials. In the learning curve, as previously explained, both levers provided the same amount of pellet (1), but animals had to wait for 5 seconds before pressing either lever. If the animals did not press any lever for 20 seconds, the trial was considered incomplete. Only animals that demonstrated at least 90% completion of trials, (meaning only 10% omissions) were selected to continue the experiments. Taking this criterion into consideration none of the animals was discarded.

II.6.1 Experiment 1: effect of persistent inflammatory pain on delayed gratification task

In experiment 1 (**Figure II.4**), half of the animals were randomly injected with CFA to induce the inflammatory pain model, while the other half was injected with saline. After 1 week, probe sessions began (3 sessions corresponding to day 7,14,21 post injection, each consisting of 100 trials). During the probe session, 2 levers with different outcomes were introduced. An “instant lever” that provided one pellet if pressed after 5 seconds, and a “delayed lever” that yielded 3 pellets if pressed 10 seconds after the trial started. At the end of each probe session, measurements of mechanical response threshold were taken using Von Frey filaments test.

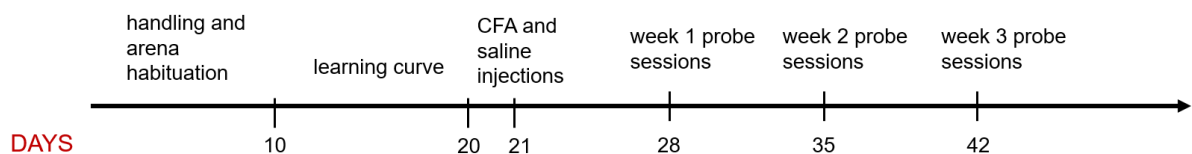


Figure II.4- Experience 1 Timeline.

II.6.2 Experiment 2: role of OFC and modulation of dopamine D2 receptors on delayed gratification task

In experience 2 (**Figure II.5**), after the learning curve, the animals underwent surgery to inject unilaterally quinolinic acid into the OFC (randomly, half animals were injected on the right hemisphere and the other half on the left hemisphere).

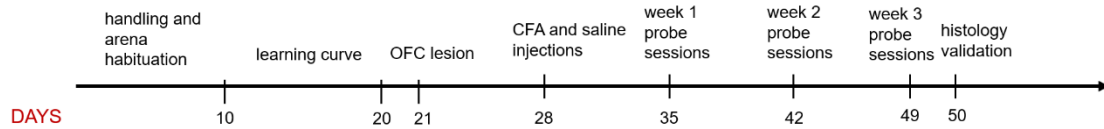


Figure II.5- Experiment 2 Timeline.

Seven days later, half of the animals were injected with CFA and the other half were injected with saline. One week later, probe sessions began, consisting of 2 consecutive days of sessions for 3 weeks. These sessions involved the administration of 3 different treatments: saline, raclopride and quinpirole. Saline treatment was always the first to be evaluated due the washout periods of both neuromodulators. Subsequently, animals were randomly injected with either raclopride or quinpirole. Depending on the neuromodulator assessed on the first day, the other was administered on the following day. Throughout the 3-week-period, the sequence of quinpirole and raclopride injections was varied to prevent any bias. Similar to experiment 1 (II.6.1), at the end of each behavioral session, the animal's sensory threshold was assessed using the Von Frey filament test. One day after the completion of all probe sessions, the animals were perfused for subsequent histological validation. The study concluded with the analysis of the obtained data.

Table II.1-Example of Probe Sessions plan. This is a plan of one of the animals tested. It is important to clarify that the evaluations of the saline/quinpirole or saline/raclopride treatments are not conducted consecutively. Between these assessments are behavioral sessions involving other animals.

	Day 1	Day 2
1 week after CFA/ Saline injections	Saline Quinpirole	Raclopride
2 weeks after CFA/ Saline injections	Saline Raclopride	Quinpirole
3 weeks after CFA/ Saline injections	Saline Quinpirole	Raclopride

II.7 Histological validation

To ascertain the extent of the OFC lesion, histological validation was conducted. Following the conclusion of the last probe session, the rats were anesthetized using pentobarbital sodium (200 mg/kg; IP). Subsequently, they were perfused with phosphate buffered saline (PBS 0.1 M) and then were fixed with paraformaldehyde (PFA 4% w/v). The brain was then extracted and put in a PFA solution (4% w/v) for 4 hours. Following that period, the brain underwent a sucrose gradient process: 10% for 24 hours, then 20% for 24 hours, ending at 30%, with sodium azide and stored at 4°C until further processing. The brain was sectioned into 60 µm coronal slices using a freezing microtome (LEICA CM1325, Leica Biosystems, Deer Park, IL, USA) equipped with a colling system (HUBER Ministat 240, Huber, Offenburg, Germany). These sections were rinsed with 0.1 M PBS and mounted onto gelatin-coated slides. Then, the sections were stained with thionine, following an adapted protocol (see annex I in supplementary data) and examined under a light microscope.

II.8 Data analysis

The performance of the animals in the DG task was assessed using various parameters. These included: completed trials (percentage of number of times the animal pressed either lever on total number of trials), omissions (percentage of omissions on total number of trials), instant rewarded trials (percentage of number of times the animal was rewarded by instant lever on total rewarded trials), delayed rewarded trials (percentage of number of times the animal was rewarded by delayed lever on total rewarded trials), non-rewarded delayed trials (percentage of number of times animal pressed delayed lever, being not rewarded on total number of non-rewarded trials), non-rewarded instant trials (percentage of number of times animal pressed instant lever, being not rewarded on total number of non-rewarded trials), total pellets consumed, index of preference (ratio of the difference between delayed trials and instant trials to the total number of completed trials, resulting in values ranging from -1 to +1, whether the animal always pressed the instant lever or delayed lever, respectively), shifting (number of shifts between levers) and variation (cumulative value of shifts between levers, whether instant lever equals -1 and delayed lever +1). Furthermore, temporal responses were also observed through latency average (mean of all latencies), latency rewarded trials (mean of rewarded trials latency), latency instant rewarded trials (mean of instant rewarded trials latency), latency delayed rewarded trials (mean of delayed rewarded trials latency) and latency no rewarded trials (mean of no rewarded trials latency). Lastly, motor responses, by measuring the total distance (pixel) and average speed (pixel/second). At the end of each session, an output file from Opencontrol was created containing information about each trial: whether the animal pressed a lever, which lever was pressed, whether it was rewarded, and the response latency (Annex II.A). Additionally, this file included performance data such as the total number of omissions, the number of instant rewarded trials, and the number of delayed rewarded trials. Latencies (Annexes III.A and III.B), variation and shifting (Annex III.C and III.D, respectively) were calculated using custom Python scripts (version 3.11.7; Python Software Foundation, Wilmington, DE, USA). Motor responses were obtained using both Python and MATLAB (MATLAB R2023b; MathWorks, Natick, MA, USA) scripts. After each session, Bonsai software created a text file containing the coordinates of the animal's position (Annex II.B), recorded at 15 coordinates per second (15 Hz). Once this file was generated, a Python script (Annex III.E.1) was used for pre-processing the data to improve readability before further

analysis. Then, a MATLAB script (Annex III.E.2) was used to calculate the total distance and average speed.

II.9 Statistics

To assess the normality of the data, the Shapiro-Wilk was conducted using GraphPad Prism (version 10.2.0; GraphPad Prism Software, Boston, USA). In the first experiment, to identify differences between experimental groups across the various parameters evaluated in the DGt, the Kruskal-Wallis (KW) test was used for the data that did not follow a normal distribution, followed by Dunn's post hoc test (if significant differences were observed). For the data following a normal distribution, a two-way ANOVA (F_1 : groups x F_2 : testing days /trial blocks) was used, followed by Sidak's post hoc test, if significant differences were found. To analyze the variation and the number of shifts throughout each session, the one-sample Kolmogorov-Smirnov test (using MatLab) was used to assess intragroup homogeneity within the session, and the two-sample Kolmogorov-Smirnov test (using MatLab) was used to examine differences in distribution between experimental groups. A similar strategy was applied to analyze both the impact of OFC lesions and the influence of dopaminergic modulation on the DGt. Results are mostly presented as Box and Whiskers plots based on the median and minimum/maximum values, except for learning curves, efficiency, variation, and shifting throughout each session, which are represented as mean $\pm$ standard deviation (SD). The significance level was set at 5% ($p=0.05$).

III. Results

III.1 Impact of persistent inflammatory pain on DGt

III.1.1 DGt Learning Curve

During the learning curve, the following parameters were evaluated: completed trials, rewarded trials, latency of rewarded trials and latency of non-rewarded trials (Figure III.1). Only animals that demonstrated an average of 90% completed trials in the last three sessions were selected to continue the experiment. As previously reported, no animals were discarded.

Completed trials rose from $93.375\% \pm$ on day 1 to $99.000\% \pm 0.655$ by day 10 (Figure III.1A). The percentage of rewarded trials showed a decrease from $37.250\% \pm 8.648$ on day 1 to $28.000\% \pm 12.421$ on the final day. Regarding response times (Figure III.1B), the latency of rewarded trials decreased slightly from 9.277 ± 2.438 sec on the first day to 8.546 ± 1.368 sec by the end of sessions, while the latency of non-rewarded trials increased from 2.439 ± 0.494 sec on day 1 to 2.792 ± 0.524 sec on day 10. Furthermore, performance in the early phase of learning curve (first 3 sessions) was compared to the late phase (last 3 sessions). No statistical differences were found between these phases for any of the parameters. Completed trials (t-test, $t=1.329$; $df=2$; $p=0.1576$), rewarded trials (t-test, $t=0.4675$; $df=2$; $p=0.3431$), latency of non-rewarded trials (t-test, $t=1.494$; $df=2$; $p=0.1369$) and latency of rewarded trials (t-test, $t=2.755$; $df=2$; $p=0.056$).

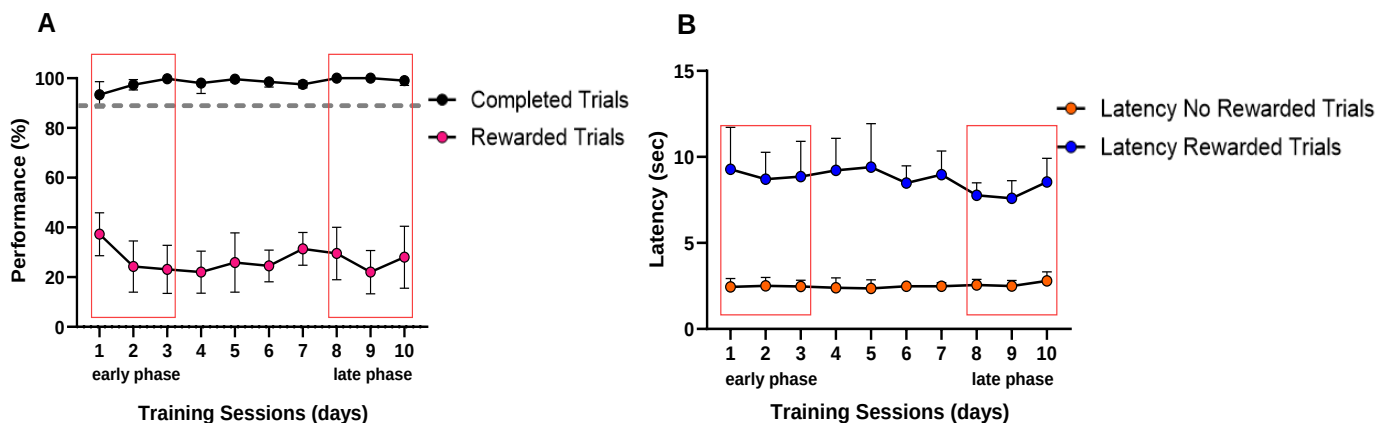


Figure III.1 – Learning curve of experiment 1. **A.** Over the 10-day learning curve, the percentage of completed trials consistently remained above 90%. Of these, only about 30% of the trials were rewarded. **B.** The latency of rewarded trials and latency of no rewarded trials showed a slight decrease and increase, respectively, indicating that the animals became more efficient, pressing the lever closer to 5 seconds. $n = 8$. Values are presented as mean $\pm$ SD.

III.1.2 CFA group showed higher mechanical sensitivity.

The results presented in **Figure III.2** shows that the animals in the CFA group exhibit a greater sensitivity to painful stimuli compared to Sham group at three time points. This is evidenced by a lower threshold to mechanical stimuli, measured in pressure (g/mm²). Statistically significant differences were observed between the two experimental groups using a two-way ANOVA test ($F_{(1,3)}=159.4$, $p<0.001$; $F_{1 \times F_2}$: $F_{(2,6)}=8.473$, $p=0.017$). A *post hoc* test revealed that the mechanical threshold in the CFA group is reduced compared to the Sham group on days 7, 14, and 21 (Sidak test, $p<0.001$). No statistically significant differences were found within the Sham and CFA groups across the three time points.

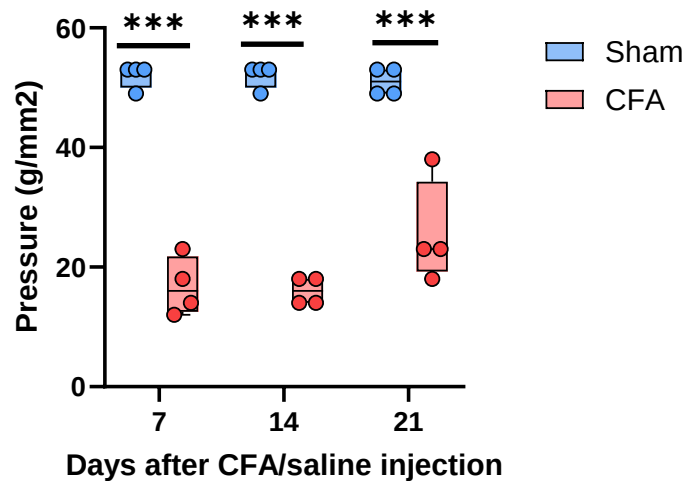


Figure III.2 – Mechanical sensitivity measured by the response to von frey filaments. Statistically significant reduction in the threshold required to elicit a response in the CFA group compared to the Sham group. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): $n=4$; Sham (blue): $n=4$. Comparisons of threshold between experimental groups were based on two-way ANOVA (group x sessions), followed by Sidak *post hoc* test. *** $p<0.001$.

III.1.3 CFA group exhibited differences compared to the Sham group in non-rewarded delayed trials.

Initially, to investigate potential differences between experimental groups, the performance of both was assessed by evaluating the percentage of completed trials and the percentage of rewarded trials (regardless, of whether the rewards were instant or delayed). Regarding completed trials (**Figure III.3 A**), CFA group exhibited similar performance in comparison to Sham group with no statistically significant differences found over the three sessions (Kruskal-Wallis test, $KW=3.290$, $p=0.655$). Similarly, no statistical differences were found between experimental groups over the three sessions on rewarded trials (**Figure III.3 B**, two-way ANOVA, $F_{(1,3)}=0.5029$, $p=0.5294$; $F_1 \times F_2$: $F_{(2,6)}=0.1407$, $p=0.8716$). Subsequently, it was examined whether, despite the absence of differences in percentage of the total rewarded trials, differences might exist in the percentage of trials rewarded only under delayed conditions (or instant), which was the primary objective of this experiment. However, no differences were observed between experimental groups and testing days (**Figure III.3 B**, $KW=2.187$, $p=0.8227$). Given the symmetrical nature of the data, there were also no differences in trials with instant rewards. However, differences were found regarding the percentage of only non-rewarded delayed trials (and only instant trials), **Figure III.3 D**, two-way ANOVA, $F_{(1,3)}=46.28$, $p=0.0065$; $F_1 \times F_2$: $F_{(2,6)}=0.1974$, $p=0.8260$. *Post hoc* analysis using Sidak test did not reveal any differences between the two experimental groups at any specific testing day. Although no differences were found between experimental groups in the percentage of trials rewarded only under delayed (or instant) conditions, differences were observed in non-rewarded delayed (and instant) trials.

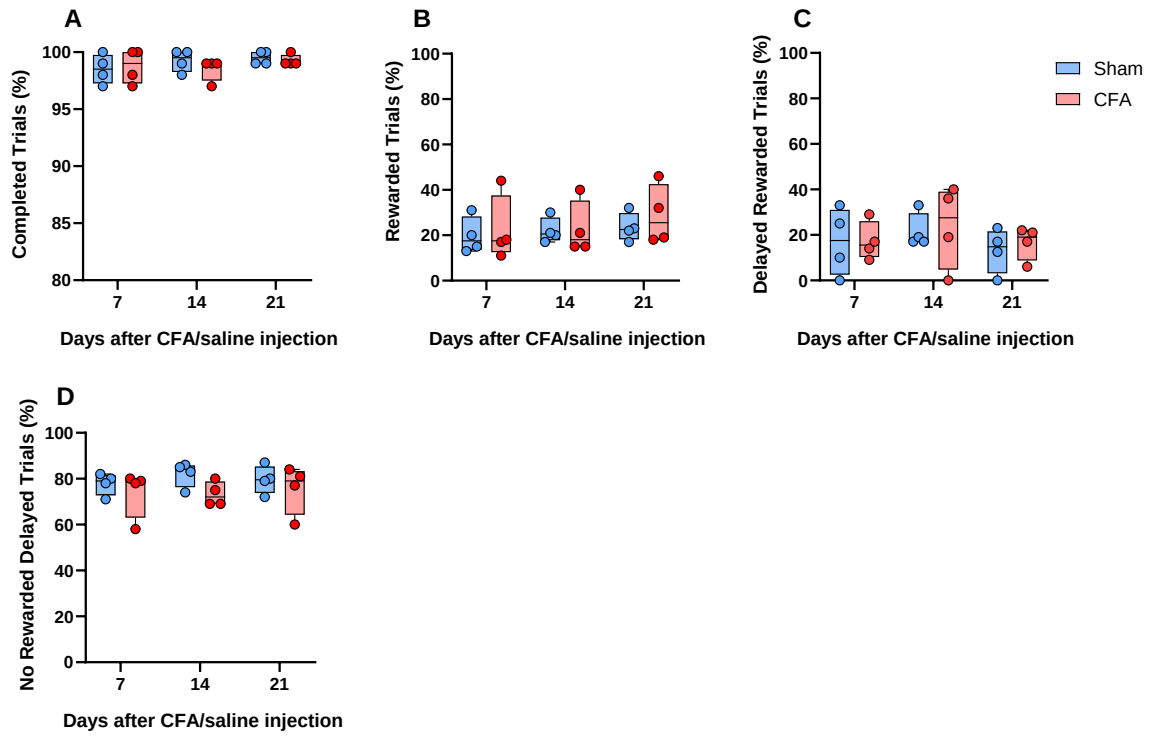


Figure III.3 – DGt performance assessed by completed trials, rewarded trials, delayed rewarded trials and delayed non-rewarded trials. A. Percentage of completed trials across the probe sessions. B. Percentage of all rewarded trials (instant + delayed) throughout the probe sessions. C. Percentage of only delayed rewarded trials on the three days of sessions. D. Percentage of non-rewarded delayed trials throughout the probe sessions. Comparisons between experimental groups and testing days were based on Kruskal-Wallis test (used to non-normal distributed data) and two-way ANOVA test (F1: group x F2: sessions), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): n = 4; Sham (blue): n = 4.

III.1.4 Pellet consumption is similar between experimental groups.

The number of pellets consumed was counted for both experimental groups during each of testing days (**Figure III.4**). No significant differences were found between the experimental groups across the three testing days: two-way ANOVA, $F_{(1,3)}=3.127$, $p=0.1752$, $F_{1 \times F_2}$: $F_{(2,6)}=0.1088$, $p=0.8987$. Therefore, the pellet consumption between experimental groups was similar.

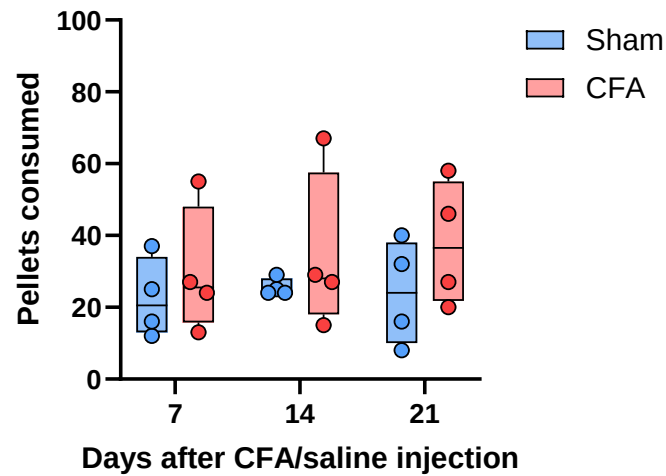


Figure III.4– Pellets consumed on DGt. Total number of pellets consumed between experimental groups across the three days of probe sessions. Comparisons between experimental groups throughout the testing days were based on two-way ANOVA (F1: group x F2: trials), followed by Sidak *post hoc*. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): n = 4; Sham (blue): n = 4.

III.1.5 On the first half of day 21 CFA group showed a preference to instant rewards when compared to Sham group.

Although no differences were found in delayed or instant rewarded trials, differences were observed in non-rewarded delayed or instant trials. As a result, preference indices between experimental groups were evaluated. Each session was divided into two halves as the on the 51st there is a shift of the rewards associated with each lever. The preference index was calculated for each block for each experimental group. In each of the 3 sessions (days 7, 14, and 21), it was assessed whether the preference index was statistically different across the session between the two groups. On day 7 (**Figure III.5 A**) and 14 (**Figure III.5 B**), no statistically significant differences were found between experimental groups across the trials (**day 7**: two-way ANOVA, $F_{(1,3)}=2.075$, $p=0.2454$, $F_{1 \times F_2}$: $F_{(1,3)}=0.0275$, $p=0.8788$; **day 14**: two-way ANOVA, $F_{(1,3)}=6.914$, $p=0.0784$, $F_{1 \times F_2}$: $F_{(1,3)}=0.0061$, $p=0.9424$). However, on **day 21** (**Figure III.5 C**), differences were seen between experimental groups (two-way ANOVA, $F_{(1,3)}=10.02$, $p=0.050$). *Post hoc* analysis, using Sidak test, revealed that the preference index of the CFA-group, on the first half of the session is reduced compared to Sham's ($p=0.011$). This means that, in the first half of the session of testing day 21, the CFA group showed a preference for instant rewards, that after 50th trial, shifted to delayed rewards.

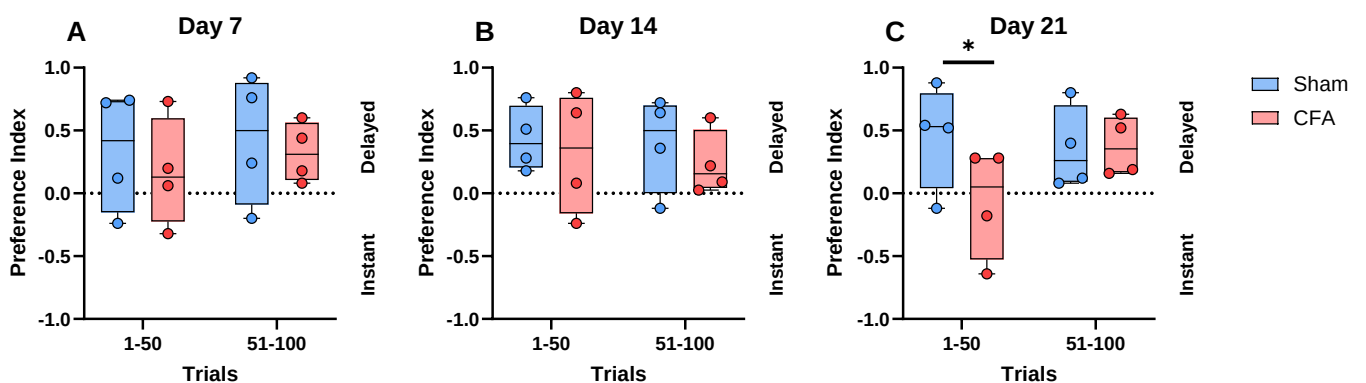


Figure III.5 – Preference index across days 7,14,21. A. Preference index on day 7 in two intervals: 1-50 trials and 51-100 trials. **B.** Preference index on day 14 in two intervals: 1-50 trials and 51-100 trials. **C.** Preference index on day 21 in two intervals: 1-50 trials and 51-100 trials. On day 21, It was found a statistically significant reduction in the preference index on the first half of session in the CFA group compared to the Sham group, based on two-way ANOVA (F_1 : group $\times$ F_2 : trials), followed by Sidak *post hoc*. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): $n = 4$; Sham (blue): $n = 4$. * $p < 0.05$.

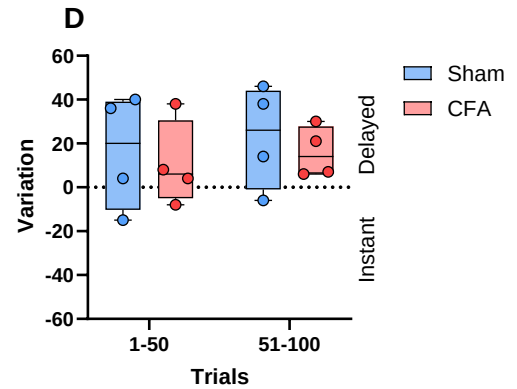
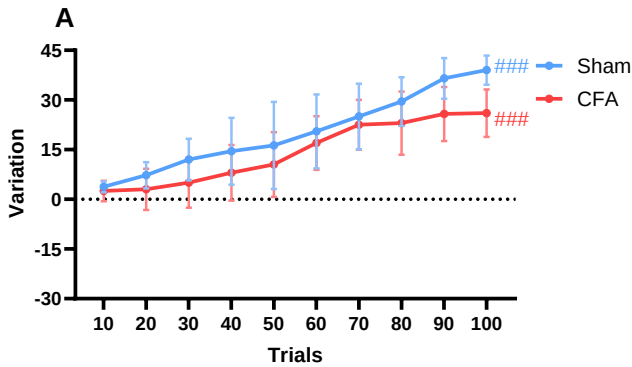
III.1.6 CFA group exhibited a lower cumulative preference compared to Sham group.

To thoroughly analyze any preference bias, the evaluation of variation/cumulative preference in DGt was conducted both throughout the session (**Figure III.7 A-C**) and every 50 trials (**Figure III-7 E-F**). In the first case, the existence of differences between experimental groups (using two-sample Kolmogorov-Smirnov (KS2)) and intragroup differences, (using one-sample Kolmogorov-Smirnov (KS)) over the 100 trials was analyzed for the three session days. Regarding **day 7**, no statistically significant differences were found between experimental groups (KS2=0.3, $p=0.67507$), but intragroup differences were observed (Sham: KS=0.99991, $p\approx 0$; CFA: KS=0.99379, $p\approx 0$). The same was also found on **day 14** (KS2= 0.4, $p=0.31285$; Sham: KS=1, $p=0$; CFA: KS=0.93319, $p=0$). On **day 21**, differences were observed between experimental groups (KS2=0.8, $p=0.00012$), as well as within the CFA and Sham groups throughout the session (Sham: KS=1, $p=0$; CFA: KS=0.49435, $p=0.008$).

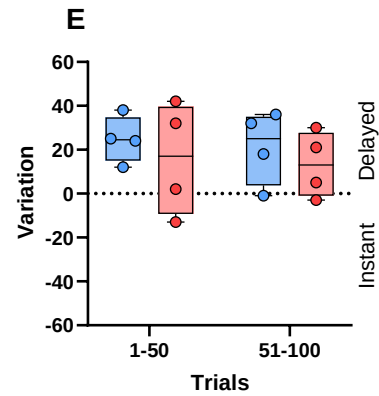
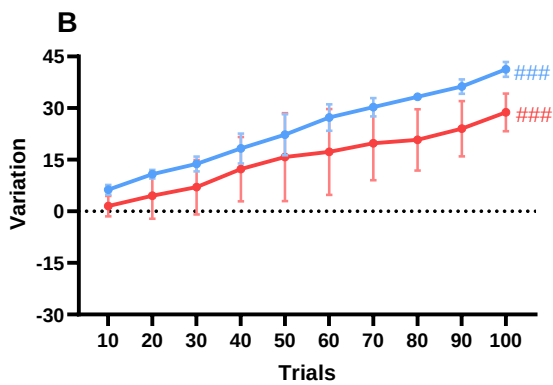
Subsequently, each session was divided into two equal blocks, and the variation for each of those blocks was calculated. Statistically significant differences were found between experimental groups on day 14, but not on days 7 and 21 (**day 7**: two-way ANOVA, $F_{(1,3)} = 2.571$, $p=0.9387$; $F_{1 \times F_2}$: $F_{(1,3)}=0.0039$, $p=0.9538$; **day 14**: two-way ANOVA, $F_{(1,3)} = 40.33$, $p=0.0079$; $F_{1 \times F_2}$: $F_{(1,3)}=0.0089$, $p=0.9780$. *Post hoc* analysis using Sidak test did not reveal any differences between the two experimental groups at a specific block of trials; **day 21**: KW=2.904, $p=0.4340$).

Therefore, during the session, differences between experimental groups were observed on day 21, with the CFA group showing less variation, indicating a greater preference for instant rewards. Regarding the data on session division by blocks, differences between experimental groups were found on day 14.

Day 7



Day 14



Day 21

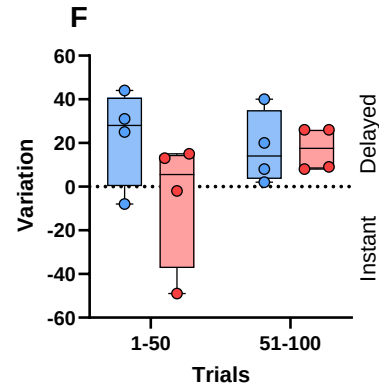
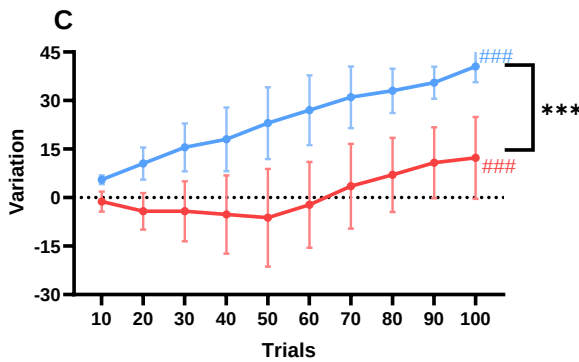


Figure III.6 – Variation/Cumulative preference on DGt. **A.** Variation throughout the session, on day 7. **B.** Variation throughout the session, on day 14. **C.** Variation throughout the session, on day 21. **D.** Variation before and after the 50th trial on day 7. **E.** Variation before and after the 50th trial. **F.** Variation before and after the 50th trial on day 21. Comparisons between groups and intragroup [A-C] throughout the session were based on two-sample Kolmogorov-Smirnov and one-sample Kolmogorov-Smirnov test respectively. Comparisons between experimental groups and trials blocks [E-F] were based on two-way on Kruskal-Wallis test (used to non-normal distributed data) and two-way ANOVA test (F1: group x F2: trials), followed by *post hoc* Sidak test. The values from graphs of left side are presented as mean $\pm$ SD. The data from right side is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): n = 4; Sham (blue): n = 4. *** $p=0.001$; ### $p<0.001$

III.1.7 On day 14, the CFA group showed fewer lever shifts in the first half of the session compared to the second half.

The number of lever shifts was also analyzed, as it could indicate a preference for a particular lever and, consequently, a greater or lesser valuation of that lever. The assessment of shifting was analyzed in different ways. Initially (**III.8 A-C**), each session was divided into 10 equal blocks, and the number of shifts for each experimental group was counted with each corresponding block. Subsequently, differences between the two groups were examined. It was found that there were no statistically significant differences between the CFA group and Sham group for each session day: **day 7**: $KS2=0.4$, $p=0.312865$; **day 14**: $KS2=0.1$, $p=1$; **day 21**: $KS2=0.2$, $p=0.974789$. Next, it was assessed whether there was homogeneity in the number of shifts within each experimental group throughout the session. Each experimental group demonstrated differences throughout the session, for each of the probe session days (**day 7**: Sham, $KS=0.97725$, $p\approx 0$; CFA, $KS=0.93319$, $p\approx 0$; **day 14**: Sham, $KS=0.89435$, $p=0$; CFA, $KS=0.95994$, $p\approx 0$; **day 21**: Sham, $KS=0.95994$, $p\approx 0$; CFA, $KS=0.98777$, $p\approx 0$).

Then, similar to the previous parameter assessed, each session was divided into 2 equal blocks, and the number of shifts was counted for both groups within each block. On **day 7 (Figure III.8 D)**, no statistically significant differences were found between experimental groups throughout the session ($KW=2.432$, $p=0.5198$). On **day 14 (Figure III.8 E)**, although the two groups showed similar performance (two-way ANOVA, $F_{(1,3)}=0.0795$, $p=0.7961$), differences were observed between the trial blocks of the session ($F_{(1,3)}=15.26$, $p=0.0298$. *Post hoc* analysis using Sidak test revealed that for CFA group, the number of shifts on the first half was lower than the second half ($p=0.046$). On **day 21 (Figure III.8 F)**, no differences were found between experimental groups and trial blocks (two-way ANOVA, $F_{(1,3)}=0.1195$, $p=0.7524$; $F_{(1,3)}= 2.382$, $p=0.2204$, respectively).

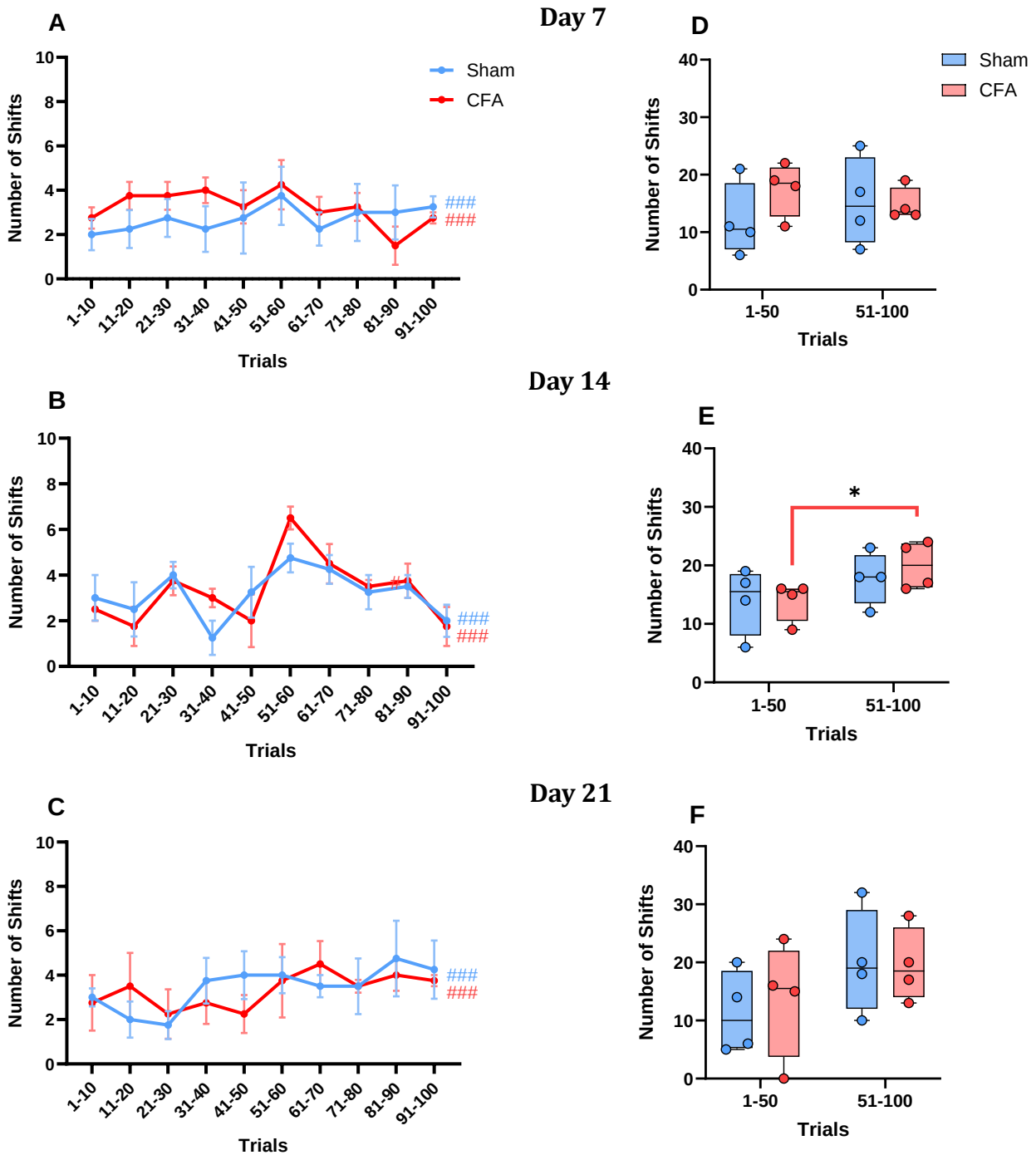


Figure III.7 – Shifting on Dgt. **A.** Number of shifts in blocks of 10 trials on day 7. **B.** Number of shifts in blocks of 10 trials on day 14. **C.** Number of shifts in blocks of 10 trials on day 21. **D.** Number of shifts before and after the 50th trial on day 7. **E.** Number of shifts before and after the 50th trial. **F.** Number of shifts before and after the 50th trial on day 21. Comparisons between groups and intragroup [A-C] throughout the session were based on two-sample Kolmogorov-Smirnov and one-sample Kolmogorov-Smirnov test respectively. Comparisons between experimental groups and trials blocks [E-F] were based on two-way ANOVA test (F1: group x F2: trials), followed by Sidak *post hoc* test. The values from graphs of left side are presented as mean $\pm$ SEM. The data from right side is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): n = 4; Sham (blue): n = 4. * $p < 0.05$; ### $p < 0.001$

III.1.8 Latency responses were not impacted in CFA group.

To investigate whether there were differences between experimental groups regarding response latencies, which could indicate more impulsive behavior, various types of latencies were evaluated. Initially, the mean of all latencies was assessed (**Figure III.8 A**), and no statistically significant differences were found between experimental groups throughout the three testing days, two-way ANOVA, $F_{1 \times F_2}$: $F_{(2,6)}=0.3491$, $p=0.7188$. Subsequently, the latencies of only the rewarded trials were evaluated (**Figure III.8 B**), and similarly, no statistically differences were found between experimental groups throughout the three testing days, two-way ANOVA, $F_{1 \times F_2}$: $F_{(2,6)}=0.4067$, $p=0.6829$. Within rewarded trials, the latencies for delayed rewarded trials and instant rewarded trials were evaluated separately (**Figure III.8 C** and **Figure III.8 D**, respectively). No significant differences were found in either case between experimental groups throughout the three testing days, two-way ANOVA, $F_{1 \times F_2}$: $F_{(2,3)}=2.187$, $p=0.2595$, and two-way ANOVA, $F_{1 \times F_2}$: $F_{(2,6)}=0.3621$, $p=0.7104$, respectively. It's important to clarify that, regarding, latency of delayed rewarded trials, on days 7 and 21, one animal from the Sham group, and on day 14, one animal from the CFA group had to be excluded from the evaluation of this parameter as they were never rewarded by the delayed lever. Similarly the same process was conducted for the latencies of the non-rewarded trials, and no statistically significant differences were found between experimental groups across the three testing days for: latency of non-rewarded trials (**Figure III.8 E**, two-way ANOVA, $F_{1 \times F_2}$: $F_{(2,6)}=0.2667$, $p=0.7745$), latency of no delayed rewarded trials (**Figure III.8 F**, two-way ANOVA, $F_{1 \times F_2}$: $F_{(2,6)}=2.585$, $p=0.1550$), and latency of no instant rewarded trials (**Figure III.8 G**, $F_{1 \times F_2}$: $F_{(2,6)}=0.0983$, $p=0.9078$).

No differences were found between experimental groups for the various types of latency, indicating that latency responses were not impacted in the CFA group.

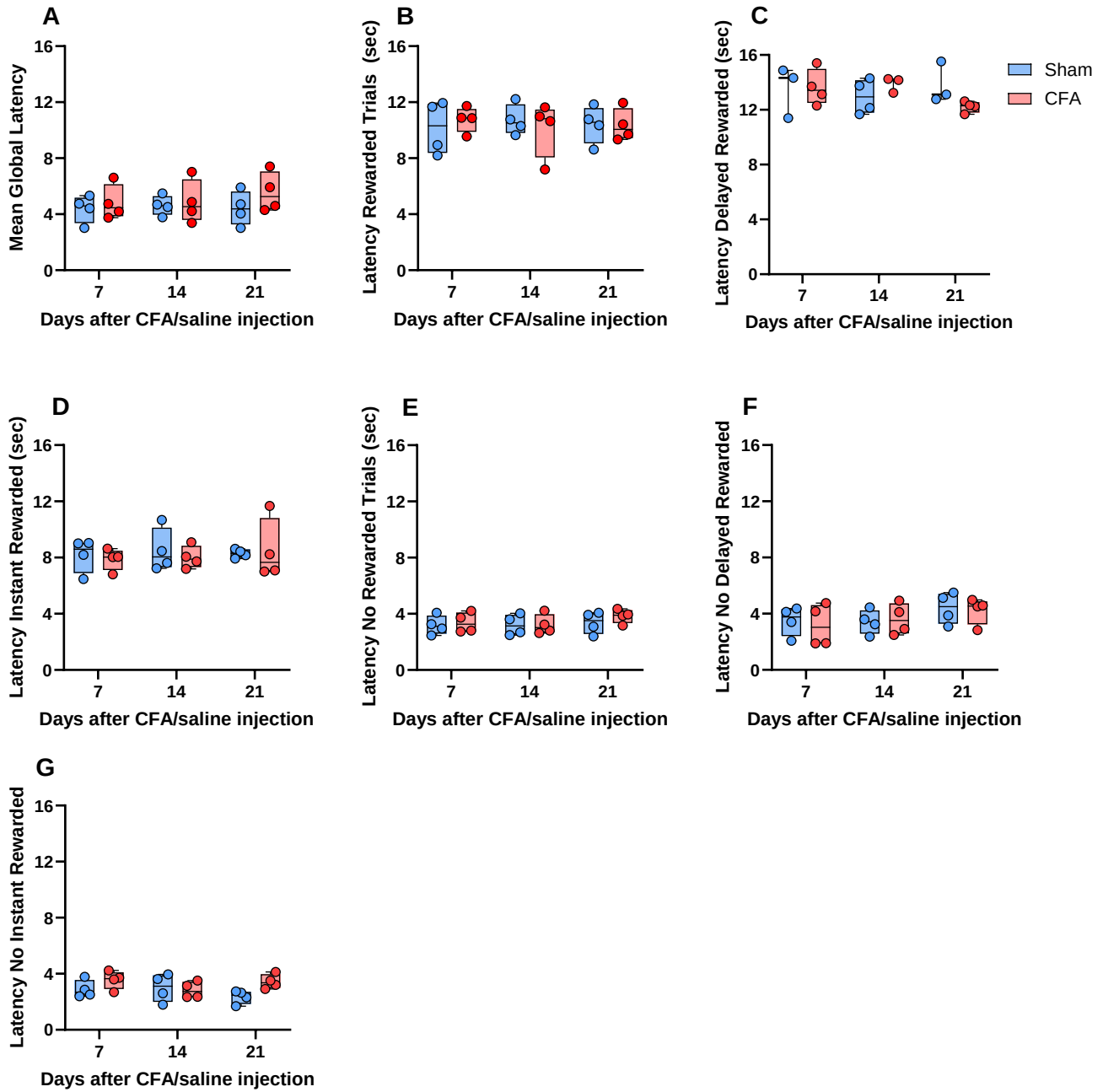


Figure III.8 – Latency responses on DGt. **A.** Mean of all latencies throughout the three days of probe sessions. **B.** Latency of all Rewarded trials across the three days of sessions. **C.** Latency of delayed rewarded trials throughout the three days of sessions. **D.** Latency of Instant Rewarded trials throughout the three days of probe sessions. **E.** Latency of all non-rewarded trials for the three days of sessions. **F** Latency of non-delayed rewarded trials throughout the three days of sessions. **G.** Latency of non- instant rewarded trials for the three days of sessions. The two groups exhibited similar latency responses based on two-way ANOVA test (F1: group x F2: trials /sessions). The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): n = 4 (3 on day 14 for latency of delayed rewarded trials); Sham (blue): n = 4 (3 on days 7 and 21 for latency of delayed rewarded trials).

III.1.9 Both experimental groups showed similar motor activity.

Given that CFA might have caused changes in locomotion, which could have compromised the animal's performance, motor activity was assessed by measuring the total distance traveled and average speed. Regarding the total distance traveled (**Figure III.9 A**), no statistically significant differences were observed between the two experimental groups and testing days (two-way ANOVA, $F_{(1,3)}=0.3062$, $p=0.6186$; $F_{1 \times F_2}$: $F_{(2,6)}=2.455$, $p=0.1664$). Similarly, on the average speed (**Figure III.9 B**) no statistically significant differences were found between experimental groups and testing sessions (KW=5.890, $p=0.3171$). Therefore, both experimental groups had similar motor activity.

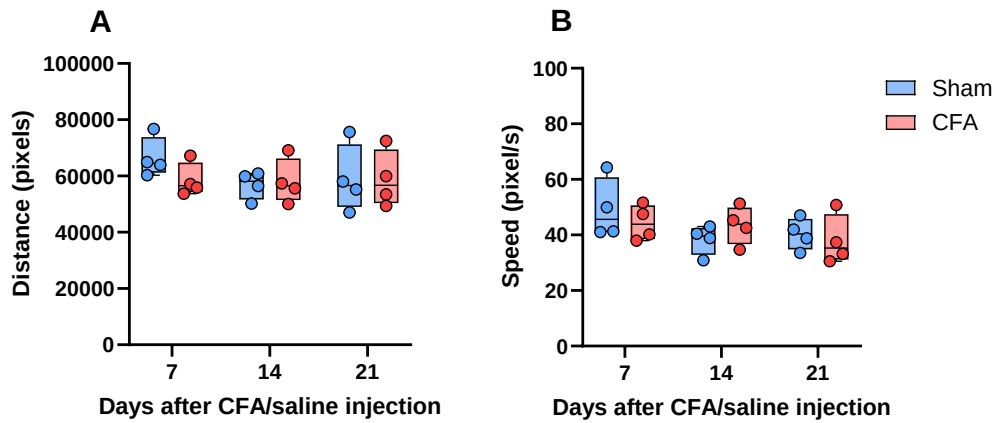


Figure III.9 – Motor responses on DGt. A. Total Distance traveled in pixel across the three probe sessions. **B.** Average speed in pixel/second. No differences in motor activity were found between experimental groups and days of session based on Kruskal-Wallis (used to non-normal distributed data) and two-way ANOVA test (F_1 : group x F_2 : sessions) test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. CFA (red): $n = 4$; Sham (blue): $n = 4$.

III.2 Pharmacological modulation of D2r on DGt

III.2.1 DGt Learning Curve

Similarly to the learning curve analysis performed with the previous set of animals, the percentage of completed trials and rewarded trials was evaluated, as well as the latency of rewarded trials and latency of non-rewarded trials (**Figure III.10**). As previously reported only animals that demonstrated an average of 90% completed trials in the last three sessions were selected to continue the experiment (no animals were discarded).

Completed trials rose from $88.500\% \pm 16.345$ on day 1 to $98.000\% \pm 1.852$ by day 10 (**Figure III.10 A**). The percentage of rewarded trials showed an increase from $31.500\% \pm 3.964$ on day 1 to $34.500\% \pm 9.055$ on the final day. Regarding response times (**Figure III.10 B**), the latency of rewarded trials decreased slightly from 10.190 ± 1.213 sec on the first day to 8.978 ± 0.808 sec by the end of sessions, while the latency of non-rewarded trials increased from 2.771 ± 0.557 sec on day 1 to 2.944 ± 0.233 sec on day 10. Furthermore, performance in the early phase of learning curve (first 3 sessions) was compared to the late phase (last 3 sessions). No statistical differences were found between these phases for any of the parameters. Completed trials (t-test, $t=1.747$; $df=2$; $p=0.2228$), rewarded trials (t-test, $t= 2.729$; $df=2$; $p=0.1121$), latency of non-rewarded trials (t-test, $t=1.850$; $df=2$; $p =0.2055$) and latency of rewarded trials (t-test, $t=1.260$; $df = 2$; $p=0.3347$).

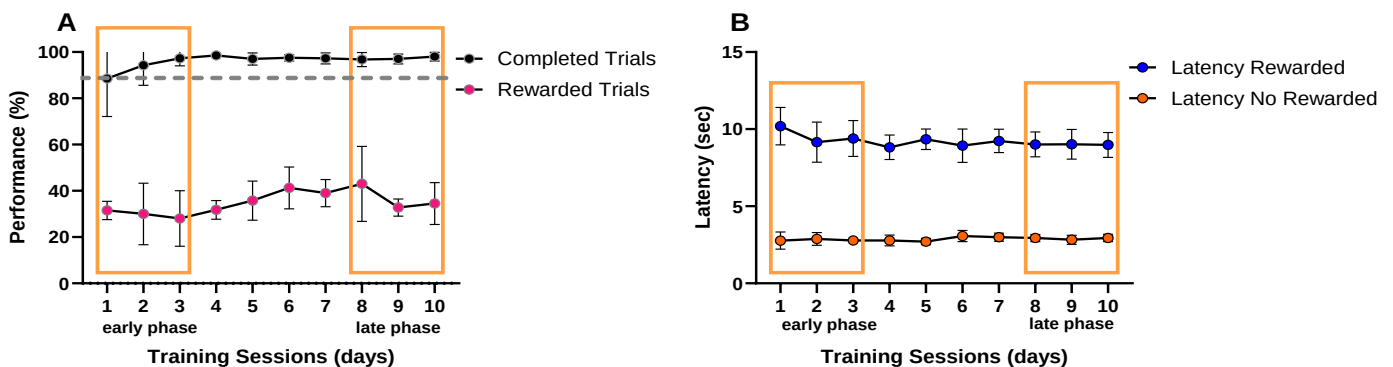


Figure III.10 – Learning curve of experiment 2. A. Over the 10-day learning curve, both the percentage of completed trials and rewarded trials showed an increase B. The latency of rewarded trials and latency of no rewarded trials showed a slight decrease and increase, respectively, indicating that the animals became more efficient, pressing the lever closer to 5 seconds. $n = 8$. Values are presented as mean $\pm$ SD.

III.2.2 Histological validation of lesion

As mentioned in section II.3, to understand the role of the OFC in DGt, eight animals received unilateral injections of quinolinic acid into the OFC. To confirm the precise injection of quinolinic acid, histological validations were performed (**Figure III.11**). The remaining images from the other animals were not adequate to be shown.

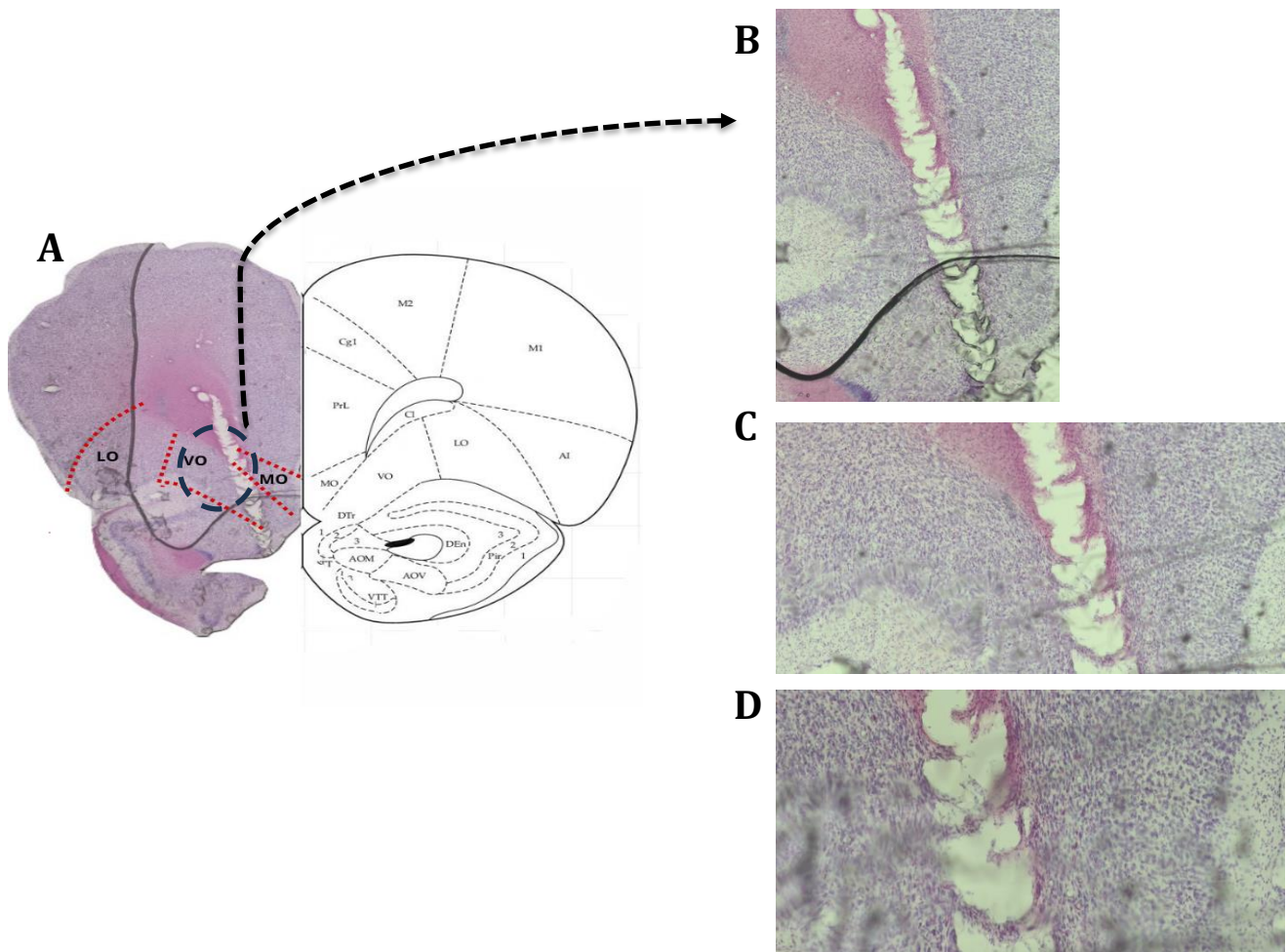


Figure III.11 – Histological validation of quinolinic acid lesion into OFC. **A.** Left half is a photograph from a lesioned rat (25x), right half is the respective diagram from Paxinos atlas (bregma: 3.70mm) **B.** Histological photograph (25x). **C.** Histological photograph (50x). **D.** Histological photograph (100x). LO: lateral orbital; VO: ventral orbital; MO: medial orbital; AI: agranular insular; AOM: anterior olfactory nucleus, medial; AOV: anterior olfactory nucleus, ventral; Cg1: cingulate cortex, area 1; Cl: claustrum; DEN: dorsal endopiriform nucleus; DTr: dorsal transition zone; M1: primary motor cortex; M2: secondary motor cortex; Pir: piriform cortex; PrL: prelimbic cortex; VTL: ventral tenia tecta.

III.2.3 Pain responses are not impacted by pharmacological modulation of D2r.

The impact of pharmacological modulation of D2r on nociceptive responses was assessed for each session day (**Figure III.12**). Statistically significant differences were found between experimental groups and pharmacological treatments on all days. On **day 7** (**Figure III.12 A**), $KW=18.99$, $p=0.0019$. *Post hoc* analysis using Dunn's test revealed that Sham group presented higher threshold compared to CFA group with the treatments saline ($p=0.009$), quinpirole ($p=0.041$), and raclopride ($p=0.0397$). On **day 14** (**Figure III.12 B**), $KW=18.35$, $p=0.028$, *Post hoc* analysis using Dunn's test revealed that Sham group presented higher threshold compared to CFA group with the treatments saline ($p=0.012$), quinpirole ($p=0.03$), and raclopride ($p=0.02$). Lastly, on **day 21** (**Figure III.12 C**), $KW=14.12$, $p=0.014$. *Post hoc* analysis using Dunn's test did not reveal any specific difference. This suggests that pharmacological modulation of D2r did not impact pain responses.

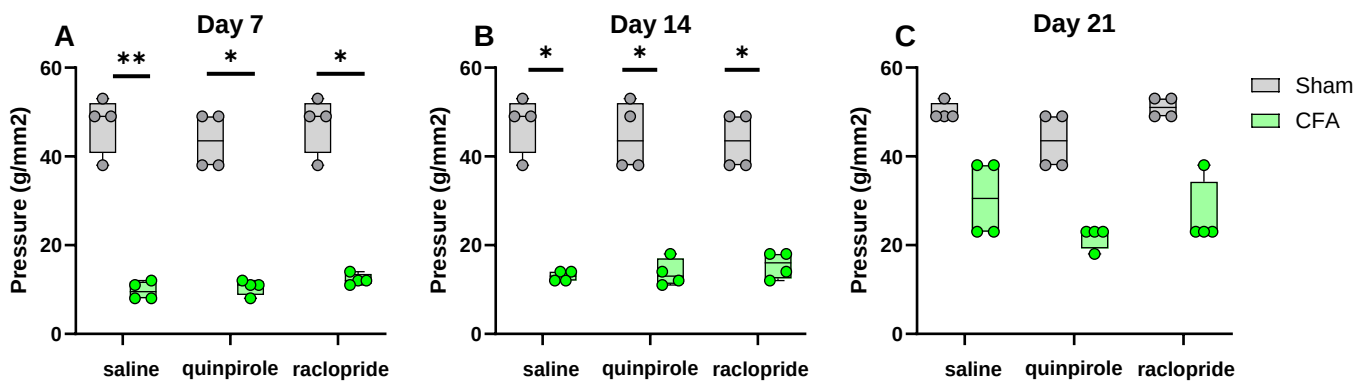


Figure III.12 – Mechanical sensitivity measured by the response to von frey filaments after pharmacological modulation of D2r. **A.** Mechanical threshold to noxious stimuli between Sham and CFA with pharmacological modulation of D2r on day 7. **B** Mechanical threshold to noxious stimuli between Sham and CFA with pharmacological modulation of D2r on day 14. **C.** Mechanical threshold to noxious stimuli between Sham and CFA with pharmacological modulation of D2r on day 21. Comparisons between experimental groups and treatments were based on Kruskal-Wallis test followed by *post hoc* Dunn's test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): $n = 4$; CFA (green): $n = 4$. * $p < 0.05$ ** $p < 0.01$.

III.2.4 Activation of D2r by quinpirole led to an increase of omissions.

Similar to the previous experiment, the performance was initially assessed by comparing the percentage of completed trials among experimental groups treated with saline, quinpirole, and raclopride for each testing day. Statistically significant differences were observed on days 14 and 21 between pharmacological treatments (F_2), but not between experimental groups (F_1). On day 7 (**Figure III.13 A**), $KW=9.250$, $p=0.099$. On day 14 (**Figure III.13 B**), two-way ANOVA, $F_1: F_{(1,3)}=3.959$, $p=0.1407$; $F_2: F_{(2,6)}=14.34$, $p=0.052$. *Post hoc* analysis using Sidak test revealed that CFA group treated with quinpirole had lower percentage of completed trials in comparison to CFA group treated with saline ($p=0.022$). On day 21 (**Figure III.13 C**), two-way ANOVA, $F_1: F_{(1,3)}=1.471$, $p=0.3120$; $F_2: F_{(2,6)}=6.988$, $p=0.027$. *Post hoc* analysis using Sidak test did reveal that Sham group treated with quinpirole had lower percentage of completed trials in comparison to Sham group treated with saline ($p=0.036$). Subsequently, the percentage of delayed rewarded trials, as well as percentage of non-rewarded delayed trials were analyzed (as the data is symmetrical, the results of instant rewarded and non-rewarded trials are the same to respective delayed's). Regarding, delayed rewarded trials statistically significant differences were found between experimental groups and treatments on days 7 and 14. On day 7 (**Figure III.13 D**), $KW=12.52$, $p=0.028$. *Post hoc* analysis with Dunn's test did not reveal any specific differences. On day 14 (**Figure III.13 E**), two-way ANOVA, $F_1: F_{(1,3)}=12.19$, $p=0.039$; $F_2: F_{(2,6)}=0.5670$, $p=0.5949$. *Post hoc* analysis with Sidak test revealed that CFA group treated with saline, quinpirole, and raclopride had a lower percentage of rewarded trials in comparison to Sham group treated with the respective treatment (saline: $p=0.001$; quinpirole: $p=0.02$; raclopride: $p=0.04$). Additionally, CFA group treated with quinpirole had a higher percentage of delayed rewards compared to CFA treated with saline ($p=0.042$). On day 21 (**Figure III.13 F**), two-way ANOVA, $F_1: F_{(1,3)}=2.976$, $p=0.1830$; $F_2: F_{(2,6)}=0.1829$, $p=0.8373$.

For the non-rewarded delayed trials, statistically significant differences were observed on day 14 between experimental groups, but not between treatments. On day 7 (**Figure III.13 G**), two-way ANOVA, $F_1: F_{(1,3)}=0.7912$, $p=0.4393$; $F_2: F_{(2,6)}=1.145$, $p=0.3792$. On day 14, (**Figure III.13 H**), two-way ANOVA, $F_1: F_{(1,3)}=12.22$, $p=0.039$; $F_2: F_{(2,6)}=2.768$, $p=0.1407$. *Post hoc* analysis using Sidak test did not reveal any specific differences. Lastly, on day 21 (**Figure III.13 I**), two-way ANOVA, $F_1: F_{(1,3)}=0.1310$, $p=0.7410$; $F_2: F_{(2,6)}=2.405$, $p=0.1710$.

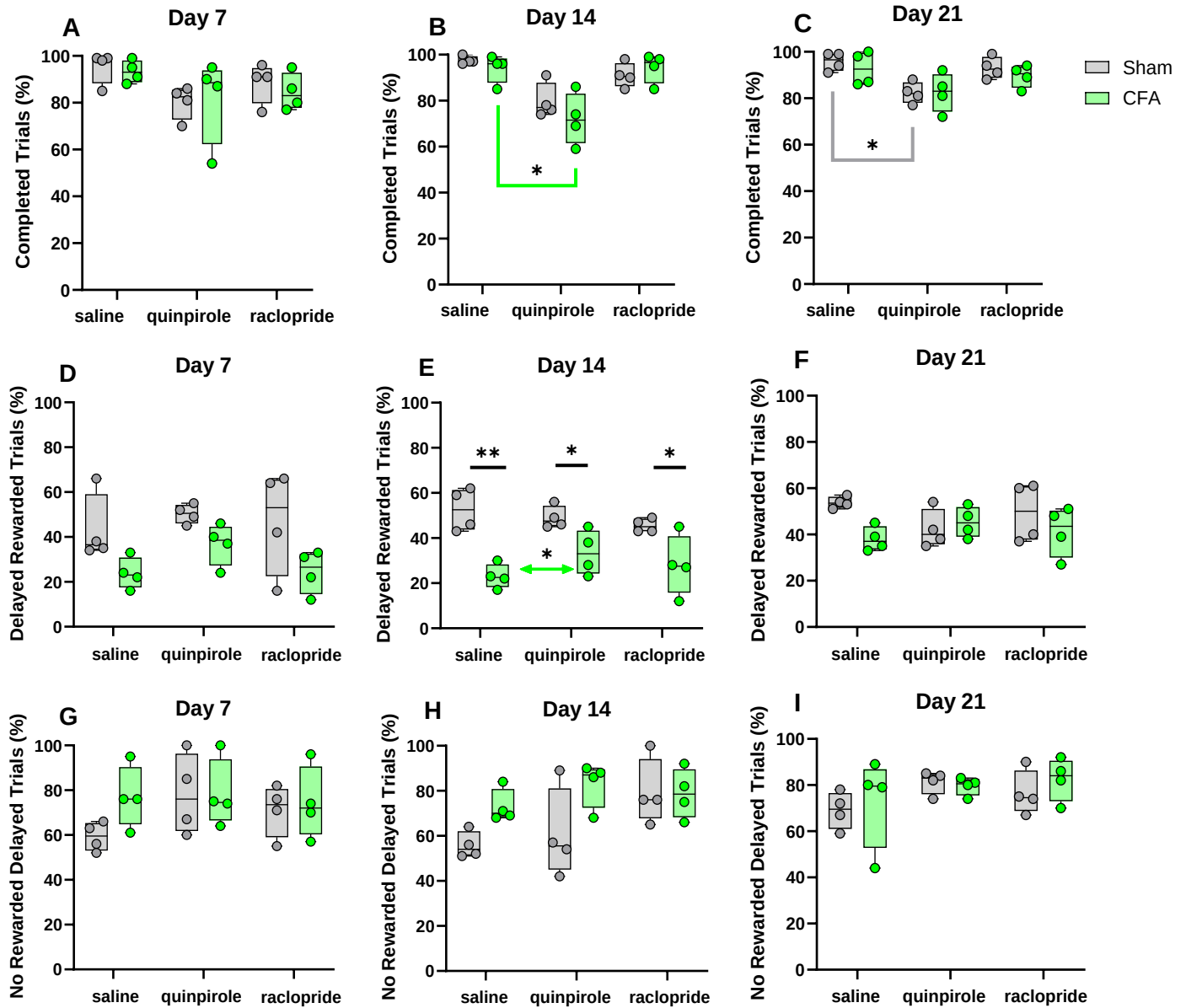


Figure III.13 – DGt performance completed trials, delayed rewarded and non-rewarded trials with D2r modulation. **A.** Percentage of completed trials between experimental groups with pharmacological modulation of D2r on day 7. **B.** On day 14. **C.** On day 21. **D** Percentage of delayed rewarded trials between experimental groups with pharmacological modulation of D2r on day 7. **E.** On day 14. **F.** On day 21. **G.** Percentage of delayed non- rewarded trials between experimental groups with pharmacological modulation of D2r on day 7. **H.** On day 14. **I.** On day 21. Comparisons between experimental groups and treatments were found based on Kruskal-Wallis test (used to non-normal distributed data) and two-way ANOVA test (F1: group x F2: treatments), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): n = 4; CFA (green): n = 4. * $p < 0.05$ ** $p < 0.01$.

III.2.5 On day 14, activation of D2r causes an increase in pellet consumption, whereas inhibition of D2r leads to a stabilization.

The number of pellets consumed was assessed following pharmacological modulation of D2r to identify potential differences, considering the dissimilarities observed in the percentage of delayed rewarded trials. Statistically differences were found between treatments on all testing days (**Figure III.14**). On **day 7**, two-way ANOVA, F_1 : $F_{(1,3)}=0.2539$, $p=0.6490$; F_2 : $F_{(2,6)}=12.98$, $p=0.0066$ (Sidak post hoc did not reveal any specific differences). On **day 14**, two-way ANOVA, F_1 : $F_{(1,3)}=13.21$, $p=0.0359$; F_2 : $F_{(2,6)}=10.73$, $p=0.0204$ (Sidak post hoc test revealed that CFA group treated with saline and quinpirole had a lower pellets consumption compared to Sham group treated with the same treatments (saline: $p=0.047$; quinpirole: $p=0.012$). Moreover, it was found that Sham group treated with quinpirole showed a higher pellets consumptions compared to Sham treated with saline ($p=0.020$). On **day 21**, two-way ANOVA, F_1 : $F_{(1,3)}=0.069$, $p=0.809$; F_2 : $F_{(2,6)}=8.958$, $p=0.015$ (Sidak *post hoc* test did not reveal any specific differences).

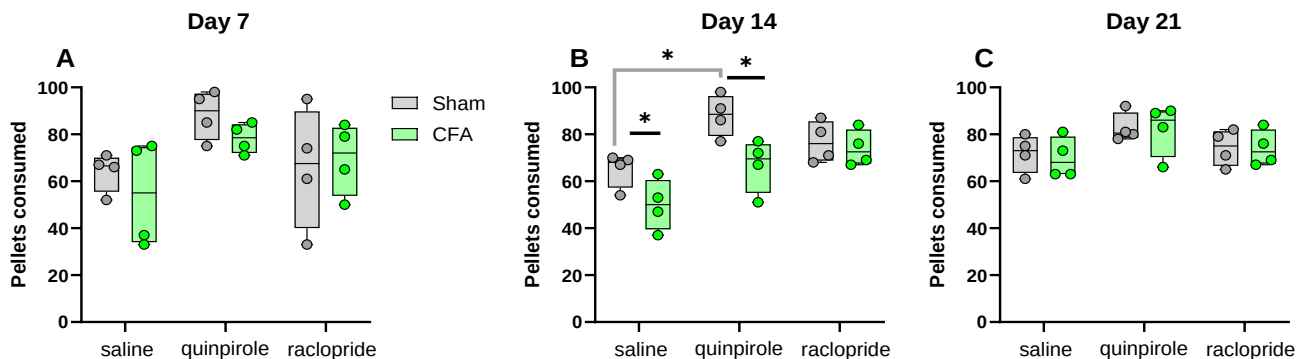


Figure III.14 – Total number of pellets consumed after pharmacological modulation of D2r. **A.** Total number of pellets consumed between experimental groups with pharmacological modulation of D2r on day 7. **B.** Total number of pellets consumed between experimental groups with pharmacological modulation of D2r on day 14. **C.** Total number of pellets consumed between experimental groups with pharmacological modulation of D2r on day 21. Comparisons between experimental groups and treatments were found based on two-way ANOVA test (F_1 : group $\times$ F_2 : treatments), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): $n = 4$; CFA (green): $n = 4$. * $p < 0.05$.

III.2.6 Neither activation nor inhibition of D2r impacts preference index.

To understand the animal's preference for a given lever and the corresponding reward, the preference index for each session day was also evaluated (**Figure III.15**). Statistically significant differences were found between experimental groups and treatments on day 14, but not on the other two test days. On **day 7**, two-way ANOVA, $F_1: F_{(1,3)}=7.327, p=0.073$; $F_2: F_{(2,6)}=2.716, p=0.1445$. On **day 14**, KW=13.47, $p=0.019$ (post hoc Dunn's test did not reveal any specific differences). On **day 21**, two-way ANOVA, $F_1: F_{(1,3)}=0.1151, p=0.7568$; $F_2: F_{(2,6)}=0.4311, p=0.6684$.

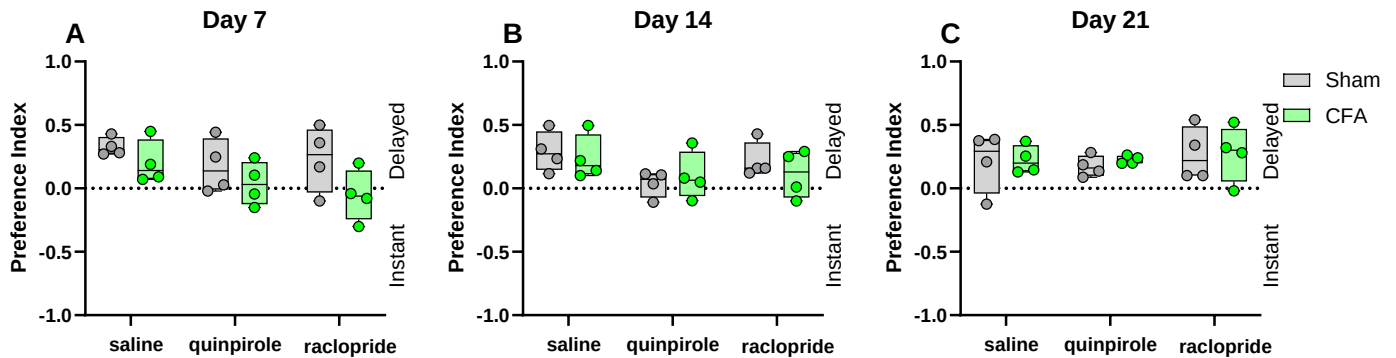


Figure III.15 –Preference Index on DGT after pharmacological modulation of D2r. **A.** Preference index between experimental groups with pharmacological modulation of D2r on day 7. **B.** On day 14. **C.** On day 21. Comparisons between experimental groups and treatments were found based on Kruskal-Wallis test (used to non-normal distributed data) and two-way ANOVA test (F_1 : group $\times$ F_2 : treatments), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): $n = 4$; CFA (green): $n = 4$.

III.2.7 D2r modulation showed no impact on performance across different halves of the session

In order to clarify the progression of preference for a given reward under the influence of D2r modulators, an evaluation of variation, (or cumulative preference), was performed both throughout each session, divided into 10 equal intervals, and in each half of the session. Regarding the first analysis (**Table III. 1-3**), the results are presented in the form of a table, rather than a graph, to avoid complicating its interpretation. On **day 7**, statistically significant differences were found within the two experimental groups, for each treatment (Sham- saline: $KS=1$, $p=0$; quinpirole: $KS=0.9997$, $p\approx 0$; raclopride: $KS=0.9999$, $p\approx 0$; CFA- saline: $KS=0.9599$, $p\approx 0$; quinpirole: $KS=0.8331$, $p\approx 0$; raclopride: $KS=0.7331$, $p\approx 0$). Furthermore, differences were also observed within Sham group between treatments saline and quinpirole ($KS_2=0.7$, $p=0.0068$), as well as between the saline and raclopride treatments ($KS_2=0.6$, $p=0.0310$). It was also observed differences between experimental groups for the treatment raclopride ($KS_2=0.7$, $p=0.0068$). On **day 14**, it was also observed statistically significant differences within the two experimental groups for each treatment (Sham- saline: $KS=0.9331$, $p\approx 0$; quinpirole: $KS=0.9772$, $p\approx 0$; raclopride: $KS=0.9999$, $p\approx 0$; CFA- saline: $KS=0.7331$, $p\approx 0$; quinpirole: $KS=0.8986$, $p\approx 0$; raclopride: $KS=0.9997$, $p\approx 0$). Additionally, differences were found within CFA group between treatments saline and quinpirole ($KS_2=0.6$, $p=0.031$), as well as between treatments saline and raclopride ($KS_2=0.7$, $p=0.0068$). It was also observed differences between experimental groups for the treatment saline ($KS_2=0.7$, $p=0.0068$). Lastly, on **day 21**, statistically significant differences were also observed were found within the two experimental groups, for each treatment (Sham- saline: $KS=0.8877$, $p\approx 0$; quinpirole: $KS=0.6937$, $p\approx 0$; raclopride: $KS=1$, $p=0$; CFA- saline: $KS=0.6986$, $p\approx 0$; quinpirole: $KS=0.9599$, $p\approx 0$; raclopride: $KS=0.8331$, $p\approx 0$). It was also observed differences within Sham group between treatments saline and raclopride ($KS_2=0.6$, $p=0.0310$).

Subsequently, as previously mentioned it was assessed differences of variation from each half of session between experimental groups and treatments (**Figure III.16**). Statistically significant differences were only found on the first half of day 7 between experimental groups (and not between treatments). On **day 7, first half**, two-way ANOVA, F_1 : $F_{(1,3)}=31.91$, $p=0.011$; F_2 : $F_{(2,6)}=0.058$, $p=0.9440$. *Post hoc* analysis with Sidak test revealed that between experimental groups, Sham treated with saline had a

higher cumulative preference value compared to CFA treated with saline, indicating a higher preference for delayed rewards ($p=0.02$). On **day 7 second half**, two-way ANOVA, $F_1: F_{(1,3)}=0.3943, p=0.5746$; $F_2: F_{(2,6)}=0.8409, p=0.4765$. On **day 14 first half**, two-way ANOVA, $F_1: F_{(1,3)}=2.445, p=0.2159$; $F_2: F_{(2,6)}=0.6567, p=0.5522$. On **day 14 second half**, two-way ANOVA, $F_1: F_{(1,3)}=8.266, p=0.0638$; $F_2: F_{(2,6)}=1.701, p=0.2601$. On **day 21 first half**, two-way ANOVA, $F_1: F_{(1,3)}=0.0001, p=0.9903$; $F_2: F_{(2,6)}=0.5667, p=0.5951$. Lastly, on the **second half of the day 21**, two-way ANOVA, $F_1: F_{(1,3)}=6, p=0.091$; $F_2: F_{(2,6)}=0.1083, p=0.8991$.

Table III.1. Variation throughout the session on day 7, after D2r modulation. Variation between experimental groups and treatments on day 7. Comparisons between experimental groups and treatments throughout the session were conducted using the two-sample Kolmogorov-Smirnov test, while the one-sample Kolmogorov-Smirnov test was employed for intratreatment comparisons within experimental groups. The data are presented as the mean of the 4 animals in each experimental group, for the respective treatment for each interval of trials. Sham: $n = 4$; CFA: $n = 4$. * $p<0.05$; ** $p<0.01$; ### $p<0.001$

Trials	Sham			CFA		
	Saline	Quinpirole	Raclopride	Saline	Quinpirole	Raclopride
10	6	3,5	4	2	0	1,5
20	10,25	7,5	6,75	2,75	1,5	6,5
30	16,5	10,5	10	3,25	4,25	9
40	22	15,5	16	1,75	5,25	10,5
50	25,75	17,25	17,25	4,25	7,25	10
60	28,5	16,25	18,25	9,75	9,75	11
70	30,5	17,5	22,75	13	12,25	7,75
80	30,75	15,75	23,75	17,75	12,5	5
90	28,75	15,25	18,25	22,75	10,5	-0,5
100	26,75	14,5	18,75	26,75	10	-3,5

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Table III.2. Variation throughout the session on day 14, after D2r modulation. Variation between experimental groups and treatments on day 14. Comparisons between experimental groups and treatments throughout the session were conducted using the two-sample Kolmogorov-Smirnov test, while the one-sample Kolmogorov-Smirnov test was employed for intratreatment comparisons within experimental groups. The data are presented as the mean of the 4 animals in each experimental group, for the respective treatment for each interval of trials. Sham: n = 4; CFA: n = 4. * $p < 0.05$; ** $p < 0.01$; ### $p < 0.001$

Trials	Sham			CFA		
	Saline	Quinpirole	Raclopride	Saline	Quinpirole	Raclopride
10	1,5	2	4,5	-1,25	0,5	3,5
20	4,25	5	7,75	-1	3	5,25
30	6	6,25	10,75	-3,25	4,75	6,75
40	8,5	9,5	15,5	-1,75	5,5	8,25
50	12,25	14,25	18,75	-2	6	8,25
60	12,25	15,5	19	0	8,5	10,25
70	14	14,75	19,25	2,75	10,5	13,25
80	16,5	15,75	18,5	5,5	10,5	13,5
90	14,5	11	18,75	9	11,5	12,75
100	16	8	14	9,5	11,5	10,75

* * **

Table III.3. Variation throughout the session on day 21, after D2r modulation. Variation between experimental groups and treatments on day 21. Comparisons between experimental groups and treatments throughout the session were conducted using the two-sample Kolmogorov-Smirnov test, while the one-sample Kolmogorov-Smirnov test was employed for intratreatment comparisons within experimental groups. The data are presented as the mean of the 4 animals in each experimental group, for the respective treatment for each interval of trials. Sham: n = 4; CFA: n = 4. * $p < 0.05$; ### $p < 0.001$

Trials	Sham			CFA		
	Saline	Quinpirole	Raclopride	Saline	Quinpirole	Raclopride
10	2,25	0	6	0,25	2	5
20	3	-1,5	9	0,25	1,75	5
30	4	1	9,25	1	2	6,25
40	3,5	2,5	12,5	3,25	2,75	5,75
50	1	5,5	11,5	3	6,25	9
60	5	9	16,5	5,25	8,75	11,5
70	10,25	10,5	19,5	6,5	12,75	11,75
80	12	14,5	20,25	5,75	16,25	15
90	15,75	14,25	23,75	7,25	16	19
100	19,5	14,25	25,25	9	16,75	24

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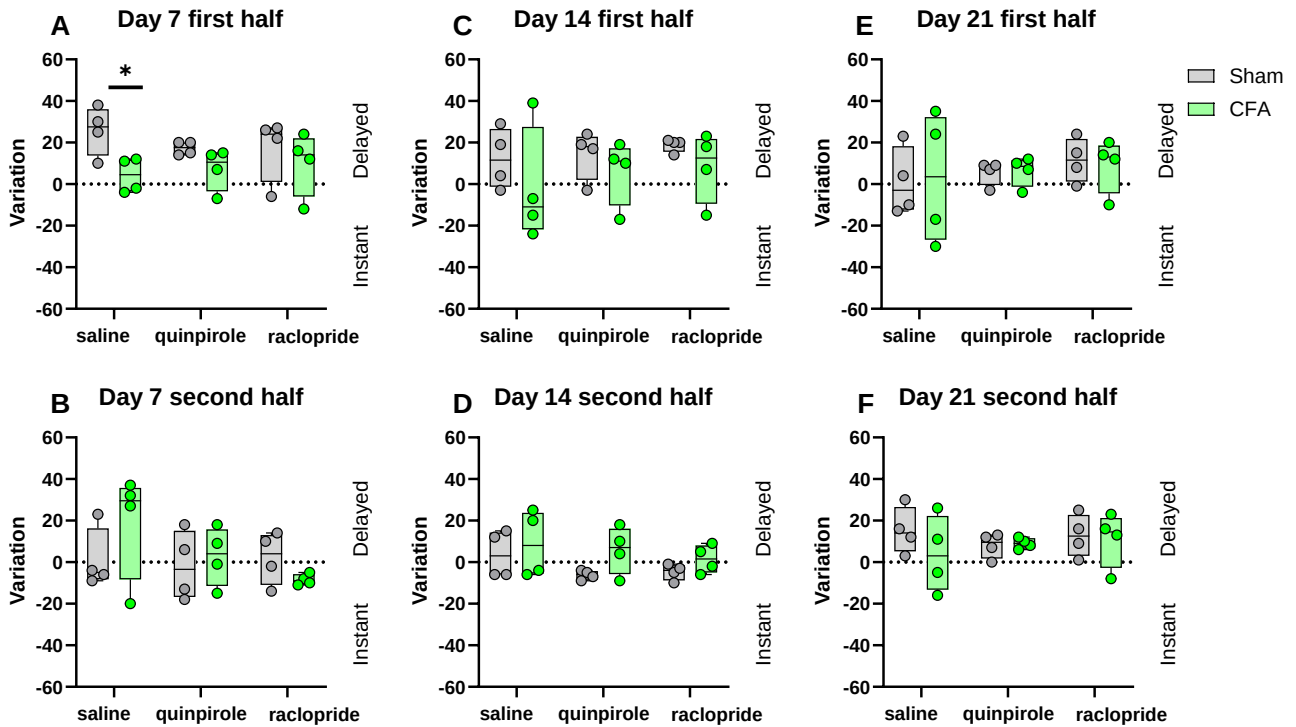


Figure III.16. – Variation/Cumulative preference between halves after pharmacological modulation of D2r on DGt. **A.** Variation value between experimental groups after pharmacological modulation of D2r on the first half of day 7. **B.** On the second half of day 7. **C.** Variation value between experimental groups after pharmacological modulation of D2r on the first half of day 14. **D.** On the second half of day 14. **E.** Variation value between experimental groups after pharmacological modulation of D2r on the first half of day 21. **F.** On the second half of day 21. Comparisons between experimental groups and treatments were based on two-way ANOVA test (F1: group x F2: treatments), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): n = 4; CFA (green): n = 4. * $p < 0.05$.

III.2.8 Activation of D2r increases shifting, while inhibition leads to the opposite effect.

The number of shifts was also evaluated in a similar manner to the variation, in order to understand the impact that pharmacological modulators of D2r have on the alternation of responses between levers. First, throughout the session of **day 7**, statistically significant differences were found, within the two experimental groups, for each treatment (Sham- saline: $KS=0.9772$, $p\approx 0$; quinpirole: $KS=0.9970$, $p\approx 0$; raclopride: $KS=0.9772$, $p\approx 0$; CFA- saline: $KS=0.9331$, $p\approx 0$; quinpirole: $KS=0.9991$, $p\approx 0$; raclopride: $KS=0.9877$, $p\approx 0$). Additionally, it was found differences within CFA group between treatments saline and quinpirole ($KS2=0.7$, $p=0.0068$). On **day 14**, it was also found statistically significant differences within the two experimental groups, for each treatment (Sham- saline: $KS=0.9937$, $p\approx 0$; quinpirole: $KS=0.9999$, $p\approx 0$; raclopride: $KS=0.9599$, $p\approx 0$; CFA- saline: $KS=0.9599$, $p\approx 0$; quinpirole: $KS=0.9937$, $p\approx 0$; raclopride: $KS=0.9772$, $p\approx 0$). It was also observed within Sham group differences between treatments saline and quinpirole ($KS2=0.6$, $p=0.0310$), as well, as between treatments saline and raclopride ($KS2=0.7$, $p=0.0068$), and within CFA group differences between saline and quinpirole treatments ($KS2=0.7$, $p=0.0068$). On **day 21**, similarly, it was observed differences within the two experimental groups, for each treatment (Sham- saline: $KS=0.9970$, $p\approx 0$; quinpirole: $KS=0.9970$, $p\approx 0$; raclopride: $KS=0.9599$, $p\approx 0$; CFA- saline: $KS=0.9331$, $p\approx 0$; quinpirole: $KS=0.9997$, $p\approx 0$; raclopride: $KS=0.9599$, $p\approx 0$). Furthermore, it was found differences within Sham group between saline and quinpirole treatments ($KS2=0.6$, $p=0.0310$) and between saline and raclopride ($KS2=0.8$, $p=0.0012$). Lastly, within CFA group differences were observed between rats treated with saline and quinpirole ($KS2=0.6$, $p=0.0310$).

Then, the number of shifts of each half (of session) was analyzed between experimental groups and treatments (**Figure III.17**). Statistically significant differences between treatments and experimental groups were found on every half except, the first half of day 7. On **day 7, first half**, two-way ANOVA, F_1 : $F_{(1,3)}=4.348$, $p=0.1284$; F_2 : $F_{(2,6)}=2.499$, $p=0.1620$. On **the second half of day 7**, two-way ANOVA, F_1 : $F_{(1,3)}=1.027$, $p=0.3854$; F_2 : $F_{(2,6)}=7.579$, $p=0.022$ (Sidak *post hoc* test revealed that between experimental groups, Sham treated with saline had higher number of shifts compared to CFA treated with saline ($p=0.011$). Additionally, it was found that within CFA group, rats treated with quinpirole had a higher number of shifts compared to rats treated with

saline ($p=0.0018$). On the **first half of day 14**, two-way ANOVA, $F_1: F_{(1,3)}=0.1176$, $p=0.7543$; $F_2: F_{(2,6)}=12.54$, $p=0.0072$. Post hoc analysis using Sidak test revealed that within CFA group, rats treated with quinpirole had a higher number of shifts compared to rats treated with saline ($p=0.049$). On the **day 14, second half** two-way ANOVA, $F_1: F_{(1,3)}=1.785$, $p=0.2738$; $F_2: F_{(2,6)}=37.60$, $p=0.0004$. Post hoc analysis using Sidak test revealed that within Sham group, rats treated with saline had a higher number of shifts compared to rats treated with raclopride, and a lower number of shifts compared to treatment quinpirole ($p=0.0126$, and $p=0.024$, respectively). On **day 21, first half**, two-way ANOVA, $F_1: F_{(1,3)}=0.0162$, $p=0.9066$; $F_2: F_{(2,6)}=52.62$, $p=0.0002$ (post hoc analysis using Sidak test revealed that within CFA group, rats treated with quinpirole showed a higher number of shifts compared to rats treated with saline ($p=0.0082$). Lastly, on the **second half of day 21**, two-way ANOVA, $F_1: F_{(1,3)}=0.8649$, $p=0.4210$; $F_2: F_{(2,6)}=17.80$, $p=0.003$. Post hoc analysis using Sidak test revealed that Sham group treated with saline had a higher shifting compared to Sham treated with raclopride ($p=0.037$), and CFA group treated with saline had a higher shifting compared to CFA treated with raclopride ($p=0.041$).

Altogether, these findings suggest that quinpirole increases the number of shifts, while raclopride decreases it.

Table III.4. Number of shifts throughout the session on day 7, after D2r modulation. Variation between experimental groups and treatments on day 7. Comparisons between experimental groups and treatments throughout the session were conducted using the two-sample Kolmogorov-Smirnov test, while the one-sample Kolmogorov-Smirnov test was employed for intratreatment comparisons within experimental groups. The data are presented as the mean of the 4 animals in each experimental group, for the respective treatment for each interval of trials. Sham: n = 4; CFA: n = 4. *** $p < 0.001$; ### $p < 0.001$

Trials	Sham			CFA		
	Saline	Quinpirole	Raclopride	Saline	Quinpirole	Raclopride
1-10	2	3,25	3	2,5	5,25	3,25
11-20	2,25	5,75	2,75	3,5	5	3,25
21-30	3	4,25	3,25	3,5	5	3,5
31-40	2,75	4,25	2	3,25	4,75	3,75
41-50	3,5	2,75	2,25	2,75	4,5	2,75
51-60	4,75	5	3,5	3,25	6	3,75
61-70	4	5	3	2	5	3,25
71-80	2,75	3,75	2,25	1,5	4	3,5
81-90	4	2,75	2,5	2	3,75	2,75
91-100	4,75	3,5	2	1,75	4,25	2,25

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Table III.5. Number of shifts throughout the session on day 14, after D2r modulation. Variation between experimental groups and treatments on day 14. Comparisons between experimental groups and treatments throughout the session were conducted using the two-sample Kolmogorov-Smirnov test, while the one-sample Kolmogorov-Smirnov test was employed for intratreatment comparisons within experimental groups. The data are presented as the mean of the 4 animals in each experimental group, for the respective treatment for each interval of trials. Sham: n = 4; CFA: n = 4. * $p < 0.05$; ** $p < 0.01$; ### $p < 0.001$

Trials	Sham			CFA		
	Saline	Quinpirole	Raclopride	Saline	Quinpirole	Raclopride
1-10	2,5	4,25	1,75	2,25	5,5	2,75
11-20	3,75	4,5	2,25	2,75	5	2,25
21-30	3,5	4,75	2,75	2,5	5,25	3,25
31-40	4	4,75	2	2,75	5,25	2,25
41-50	2,75	3,75	2,5	3	3,5	3,25
51-60	4,25	5	2,25	4,25	4	2,25
61-70	2,5	4,5	2	1,75	3	2
71-80	3,75	4	1,75	2,75	3,5	2,25
81-90	2,75	3,75	2	2,25	2,5	2
91-100	3,25	4,75	3,25	2,75	4	2,25

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Table III.6. Number of shifts throughout the session on day 21, after D2r modulation. Variation between experimental groups and treatments on day 21. Comparisons between experimental groups and treatments throughout the session were conducted using the two-sample Kolmogorov-Smirnov test, while the one-sample Kolmogorov-Smirnov test was employed for intratreatment comparisons within experimental groups. The data are presented as the mean of the 4 animals in each experimental group, for the respective treatment for each interval of trials. Sham: n = 4; CFA: n = 4. * $p < 0.05$; ** $p < 0.01$; ### $p < 0.001$

Trials	Sham			CFA		
	Saline	Quinpirole	Raclopride	Saline	Quinpirole	Raclopride
1-10	3,75	5,25	2	2,25	6,5	1,75
11-20	3,75	5,75	2,75	3,75	6	2,5
21-30	4,25	4,75	3	2	5,25	3
31-40	3	4	2,5	1,75	6,25	3,5
41-50	4,75	4,25	1,75	2,5	3,5	4,25
51-60	4,5	5,5	2,25	4,25	4,5	2
61-70	2,75	5,75	2	2,75	4	2
71-80	3,5	4	2,5	3,5	3,75	2,25
81-90	3,5	2,75	2,25	3	3,5	3,25
91-100	3,25	4	3	3,5	3,75	2,25

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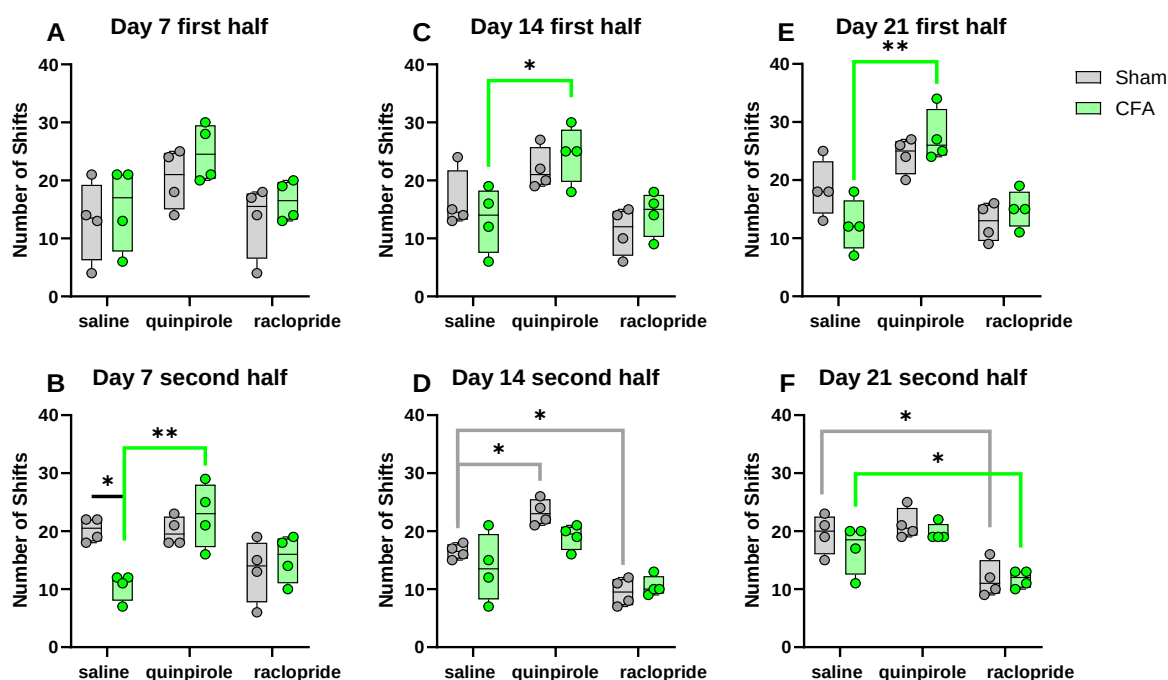
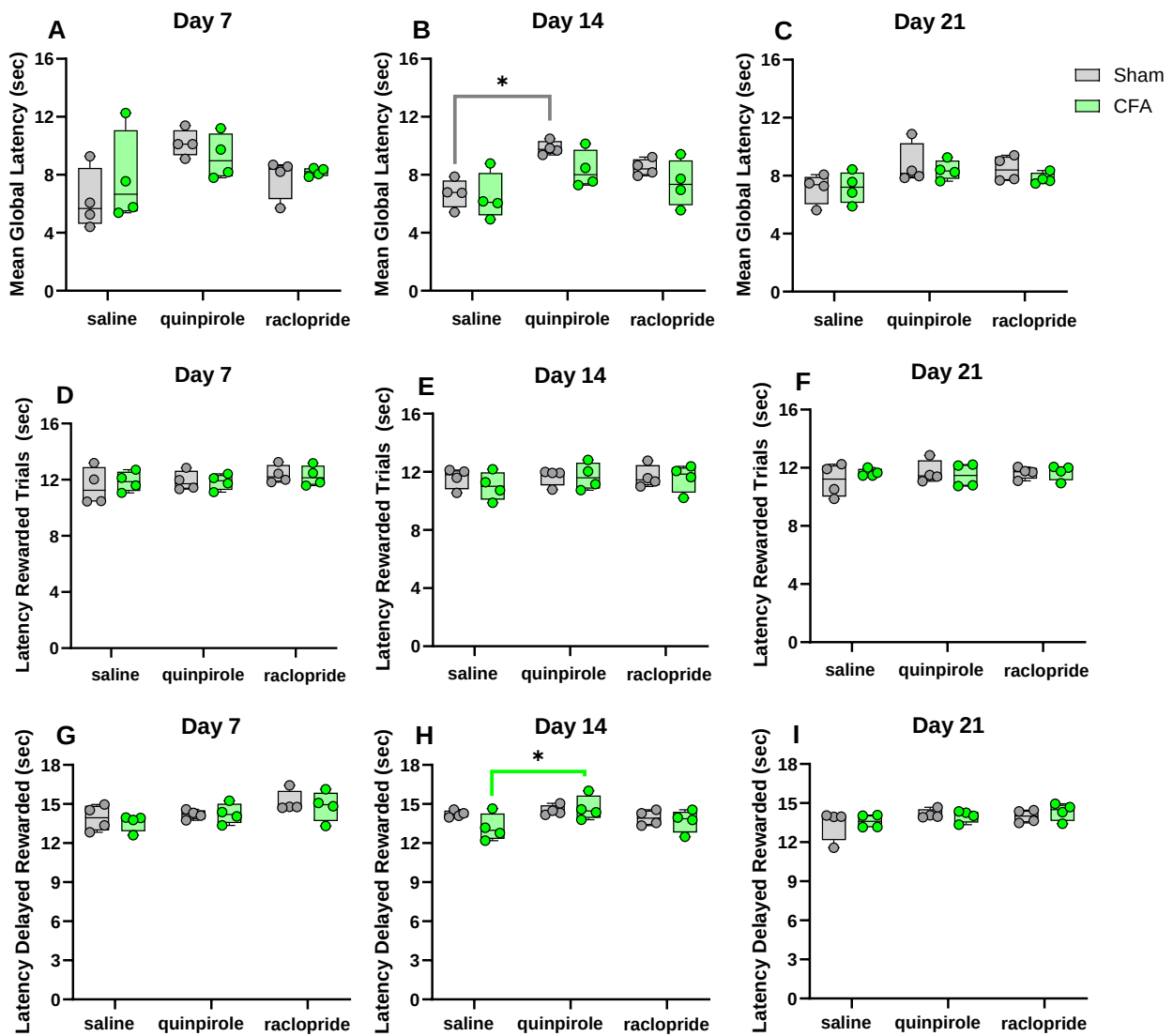


Figure III.17 – Number of shifts between halves after pharmacological modulation of D2r. A. Total number of shifts between experimental groups with pharmacological modulation of D2r on the first half of day 7. B. On the second half of day 7. C. Total number of shifts between experimental groups with pharmacological modulation of D2r on the first half of day 14. D. On the second half of day 14. E. Total number of shifts between experimental groups with pharmacological modulation of D2r on the first half of day 21. F. On the second half of day 21. Comparisons between experimental groups and treatments were based on two-way ANOVA test (F1: group x F2: treatments) The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): n = 4; CFA (green): n = 4. * $p < 0.05$, ** $p < 0.01$.

III.2.9 Activation of D2r leads to an increase of latency responses.

Similarly to the previous experiment, various latencies were evaluated to understand how the modulation of D2r impacted them (**Figure III.18**). These included: average latency of all trials, average latency of all rewarded trials, average latency of instant rewarded trials, average latency of delayed rewarded trials, average of latency of non-rewarded trials, average of latency of non-rewarded delayed trials and to conclude average of latency of non-rewarded instant trials. Statistically significant differences were found between experimental groups and treatments on global latency, latency of delayed rewarded trials, latency of non-rewarded trials, latency of non-rewarded delayed and instant trials. Regarding **global latency (Figure III.18 A-C)**, on **day 7**: KW=8.341, $p=0.1384$; on **day 14**: two-way ANOVA, $F_1: F_{(1,3)}=7.094, p=0.0761$; $F_2: F_{(2,6)}=10.72, p=0.0105$ (Sidak *post hoc* test revealed that Sham treated with quinpirole had a higher latency than Sham treated with saline ($p=0.033$)). On **day 21**, KW=8.661, $p=0.1233$. Regarding **latency of rewarded trials (Figure III.18 D-F)**, on **day 7**: two-way ANOVA, $F_1: F_{(1,3)}=0.2265, p=0.667$ $F_2: F_{(2,6)}=0.8254, p=0.4824$. On **day 14**: two-way ANOVA, $F_1: F_{(1,3)}=3.473, p=0.1593$ $F_2: F_{(2,6)}=0.2420, p=0.7920$. On **day 21**: two-way ANOVA, $F_1: F_{(1,3)}=0.0365, p=0.8606$ $F_2: F_{(2,6)}=0.4412, p=0.662$. Concerning **latency of delayed rewarded trials (Figure III.19 G-I)**, on **day 7**: KW=8.166, $p=0.1473$; on **day 14**: two-way ANOVA, $F_1: F_{(1,3)}=9.737, p=0.05$ $F_2: F_{(2,6)}=1.819, p=0.2412$ (Sidak *post hoc* test revealed that CFA treated with quinpirole had higher latency than CFA treated with saline ($p=0.041$)). On **day 21**, KW=4.625, $p=0.4634$. About, latency of instant rewards (**Figure III.18 J-L**), on **day 7**: two-way ANOVA, $F_1: F_{(1,3)}=0.9227, p=0.4061$ $F_2: F_{(2,6)}=0.0436, p=0.9576$; on **day 14**: two-way ANOVA, $F_1: F_{(1,3)}=0.1172, p=0.7547$ $F_2: F_{(2,6)}=0.4614, p=0.6510$; on **day 21**: two-way ANOVA, $F_1: F_{(1,3)}=0.0096, p=0.9279$ $F_2: F_{(2,6)}=0.0545, p=0.9475$. Regarding **latency of non-rewarded trials (Figure III.18 M-O)**, on **day 7**, two-way ANOVA, $F_1: F_{(1,3)}=0.0372, p=0.8593$ $F_2: F_{(2,6)}=9.967, p=0.0124$ (Sidak *post hoc* test revealed that Sham treated with quinpirole and CFA treated with quinpirole had a higher latency than their counterpart treated with saline ($p=0.02$ and $p=0.04$, respectively)). On **days 14 and 21**, two-way ANOVA, $F_1: F_{(1,3)}=1.236, p=0.3474$ $F_2: F_{(2,6)}=6.619, p=0.030$, and $F_1: F_{(1,3)}=0.003, p=0.9579$ $F_2: F_{(2,6)}=6.584, p=0.031$, respectively. Sidak *post hoc* test did not reveal any specific differences. **Latency of non-rewarded delayed trials (Figure III.18 P-R)**, on **day 7**: two-way ANOVA, $F_1: F_{(1,3)}=0.0832, p=0.7917$ $F_2: F_{(2,6)}=7.784, p=0.0215$. (Post hoc analysis using Sidak test

revealed that within Sham group, rats treated with quinpirole had a higher latency response compared to rats treated with saline ($p=0.032$), on **day 14**: two-way ANOVA, F_1 : $F_{(1,3)}=1.315$, $p=0.3346$ F_2 : $F_{(2,6)}=3.926$, $p=0.0813$; on day 21: two-way ANOVA, F_1 : $F_{(1,3)}=0.0626$, $p=0.8186$ F_2 : $F_{(2,6)}=5.903$, $p=0.0383$ (*Post hoc* analysis using Sidak test did not reveal any specific differences). To conclude, regarding latency of non-rewarded instant trials (**Figure III.18 S-V**), on **day 7**: two-way ANOVA, F_1 : $F_{(1,3)}=0.3633$, $p=0.5892$ F_2 : $F_{(2,6)}=3.152$, $p=0.1169$; **day 14**: two-way ANOVA, F_1 : $F_{(1,3)}=0.4245$, $p=0.5612$ F_2 : $F_{(2,6)}=9.23$, $p=0.025$ (*Post hoc* analysis using Sidak test revealed that within Sham group, rats treated with quinpirole had a higher latency response compared to rats treated with saline ($p=0.0208$); **day 21**: two-way ANOVA, F_1 : $F_{(1,3)}=0.0273$, $p=0.8783$ F_2 : $F_{(2,6)}=3.419$, $p=0.2821$. These results suggest that quinpirole led to an increase in latency response.



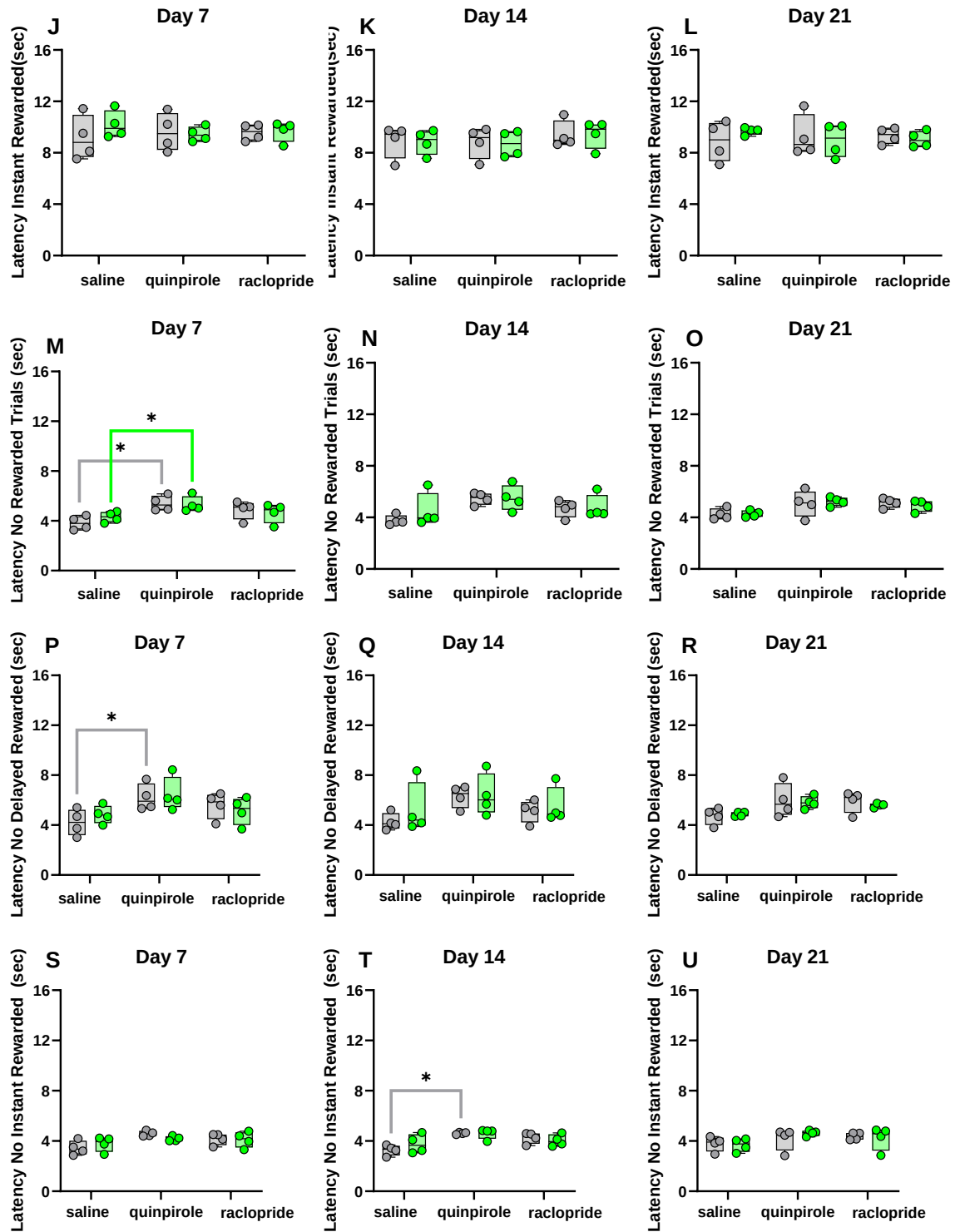


Figure III.18 – Latency responses on DGt after pharmacological modulation of D2r. A. Mean latency of all trials between experimental groups with pharmacological modulation of D2r on day 7. B. On day 14. C. On day 21. D. Latency of rewarded trials between experimental groups with pharmacological modulation of D2r on day 7. E. On day 14. F. On day 21. G. Latency of instant rewarded trials between experimental groups with pharmacological modulation of D2r on day 7. H. On day 14. I. On day 21. J. Latency of instant of delayed rewarded trials between experimental groups with pharmacological modulation of D2r on day 7. K. On day 14. L. On day 21. M. Latency of non-rewarded trials between experimental groups with pharmacological modulation of D2r on day 7. N. On day 14. O. On day 21. P. Latency of non-rewarded delayed trials between experimental groups with pharmacological modulation of D2r on day 7. Q. On day 14. R. On day 21. S. Latency of non-rewarded instant trials between experimental groups with pharmacological modulation of D2r on day 7. T. On day 14. U. On day 21. Comparisons between experimental groups and treatments were based on Kruskal-Wallis test (used to non-normal distributed data), and two-way ANOVA test (F1: group x F2: treatments). The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): n = 4; CFA (green): n = 4. *p<0.05

III.2.10 Motor activity is not impacted by modulation of D2r.

To conclude, the total distance traveled and average speed after systemic injection of D2r pharmacological modulators were also evaluated for each test day (**Figure III.19**). No statistically significant differences were observed neither treatments, nor experimental groups for both distance and average speed. On **day 7** (**Figure III.19 A,D**), for distance two-way ANOVA, $F_1: F_{(1,3)}=2.564, p=0.6825$; $F_2: F_{(2,6)}=2.564, p=0.1564$; and for speed: two-way ANOVA, $F_1: F_{(1,3)}=0.8344, p=0.4284$; $F_2: F_{(2,6)}=1.625, p=0.2730$. On **day 14** (**Figure III.19 B,E**), for distance two-way ANOVA, $F_1: F_{(1,3)}=5.532, p=0.1001$; $F_2: F_{(2,6)}=4.090, p=0.0758$; and for speed two-way ANOVA, $F_1: F_{(1,3)}=0.6118, p=0.4912$; $F_2: F_{(2,6)}=3.953, p=0.080$. Lastly, on **day 21** (**Figure III.19 C,F**), for distance: two-way ANOVA, $F_1: F_{(1,3)}=2.929, p=0.1855$; $F_2: F_{(2,6)}=4.090, p=0.075$; and for speed: two-way ANOVA, $F_1: F_{(1,3)}=0.7167, p=0.4594$; $F_2: F_{(2,6)}=0.6902, p=0.5373$.

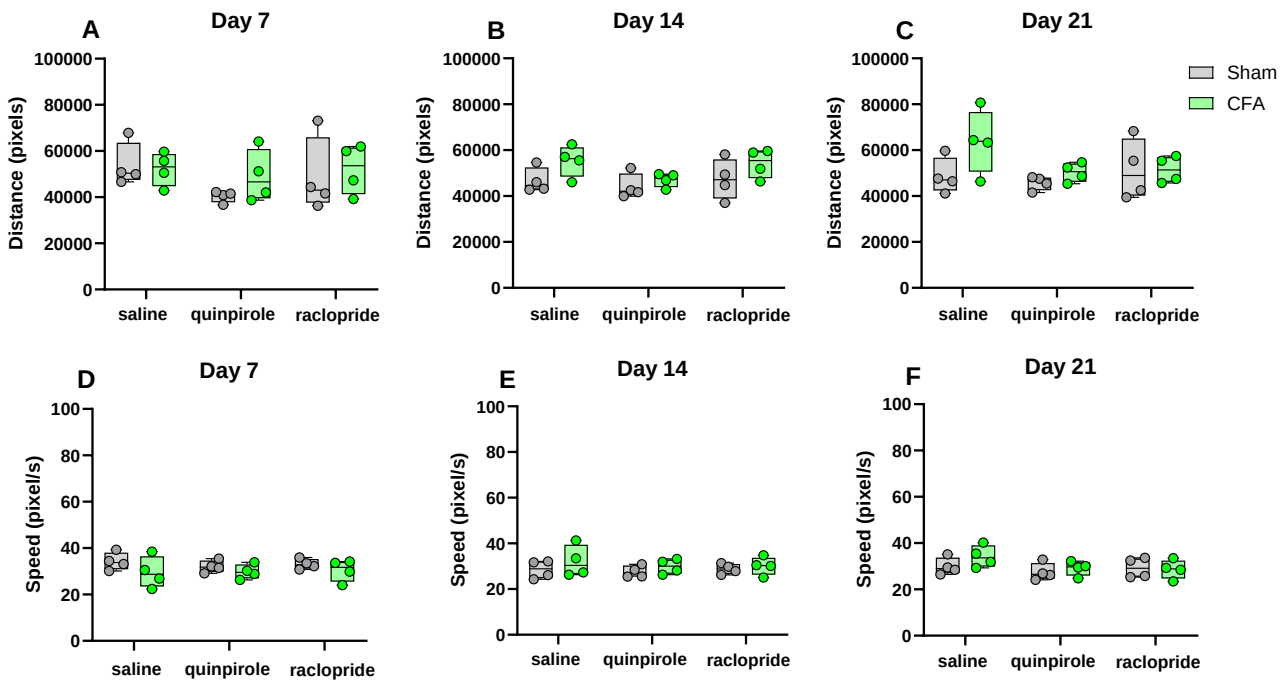


Figure III.19 – Motor responses on DGt. **A.** Total Distance traveled between experimental groups with pharmacological modulation of D2r on day 7. **B.** Total Distance traveled between experimental groups with pharmacological modulation of D2r on day 14. **C.** Total Distance traveled between experimental groups with pharmacological modulation of D2r on day 21. **D.** Average speed between experimental groups with pharmacological modulation of D2r on day 7. **E.** Average speed between experimental groups with pharmacological modulation of D2r on day 14. **F.** Average speed between experimental groups with pharmacological modulation of D2r on day 21. Comparisons between experimental groups and treatments were based on two-way ANOVA test (F_1 : group x F_2 : treatments) test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): $n = 4$; CFA (green): $n = 4$.

III.3 Role of OFC on DGt

III.3.1 DGt Learning Curve

As in the previous two experiments, the performance of the learning curve in this study was assessed based on the following parameters: the percentage of completed trials and rewarded trials was evaluated, as well as the latency of rewarded trials and latency of non-rewarded trials (**Figure III.20**). Only animals that demonstrated an average of 90% completed trials in the last three sessions were selected to continue the experiment (no animals were discarded).

Completed trials rose from $90.938\% \pm 11.991$ on day 1 to $98.500\% \pm 1.862$ by day 10 (**Figure III.20 A**). The percentage of rewarded trials showed a decrease from $34.375\% \pm 7.145$ on day 1 to $31.250\% \pm 11.024$ on the final day. Regarding response latencies (**Figure III.20 B**), the latency of rewarded trials decreased slightly from 9.734 ± 1.919 sec on the first day to 8.762 ± 1.108 sec by the end of sessions, while the latency of non-rewarded trials increased from 2.605 ± 0.537 sec on day 1 to 2.868 ± 0.400 sec on day 10. Furthermore, performance in the early phase of learning curve (first 3 sessions) was compared to the late phase (last 3 sessions). No statistical differences were found between these phases for any of the parameters. Completed trials (t-test, $t=1.552$; $df=2$; $p=0.2608$), rewarded trials (t-test, $t=1.616$; $df=2$; $p=0.2475$), latency of non-rewarded trials (t-test, $t=2.641$; $df=2$; $p=0.1184$) and latency of rewarded trials (t-test, $t=1.472$; $df=2$; $p=0.2788$).

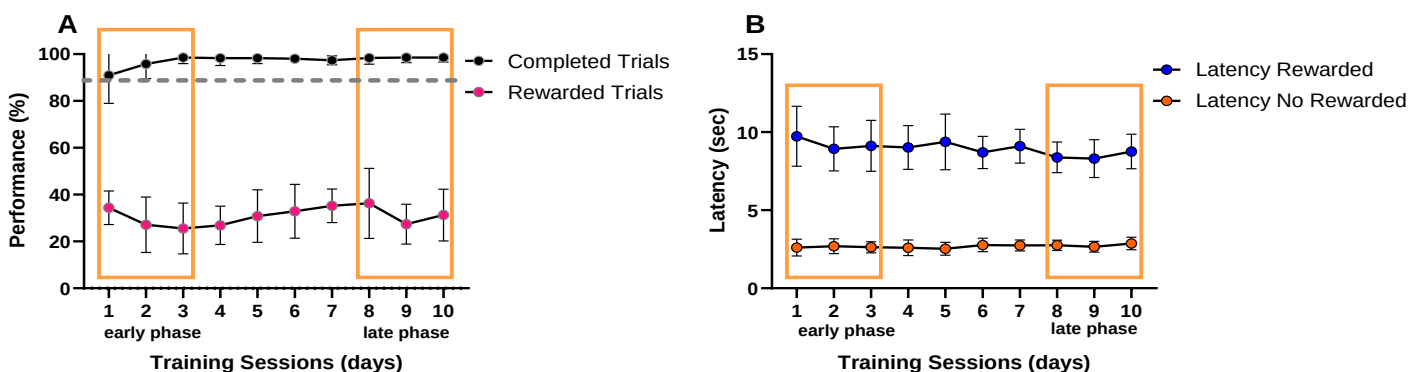


Figure III.20 - Learning curve of experiment 3. **A.** Over the 10-day learning curve, the percentage of completed trials showed an increase, whereas the percentage of rewarded trials a slight decrease. **B.** The latency of rewarded trials and latency of no rewarded trials showed a slight decrease and increase, respectively, indicating that the animals became more efficient, pressing the lever closer to 5 seconds. $n = 16$. Values are presented as mean $\pm$ SD.

III.3.2 OFC lesion does not impact mechanical threshold to pain.

To address the impact of OFC lesion in response to noxious stimuli, lesioned animals (sham and CFA) were compared to their counterpart, but not lesioned on each testing day (**Figure III.21**). On **day 7**, statistically significant differences were found between experimental groups and lesion, KW=13.29, $p=0.0001$. *Post hoc* analysis using Dunn's test revealed that within non-lesioned rats, Sham group had a higher mechanical threshold to elicit pain ($p=0.029$), and within lesioned rats, Sham group had also a higher mechanical threshold to elicit pain ($p=0.008$). On **day 14**, statistically significant differences were found between experimental groups and lesion, KW=13.03, $p=0.0001$. *Post hoc* test using Dunn's test revealed that within non-lesioned rats, Sham group had a higher mechanical threshold to elicit pain ($p=0.011$), and within lesioned rats, Sham group had a higher mechanical threshold to elicit pain ($p=0.021$). On **day 21**, statistically significant differences were found between experimental groups and lesion, KW=12.27, $p=0.0004$. *Post hoc* test using Dunn's test revealed that within non-lesioned rats, Sham group had a higher mechanical threshold to elicit pain ($p=0.031$). These results suggest that the lesion in OFC did not impact pain responses.

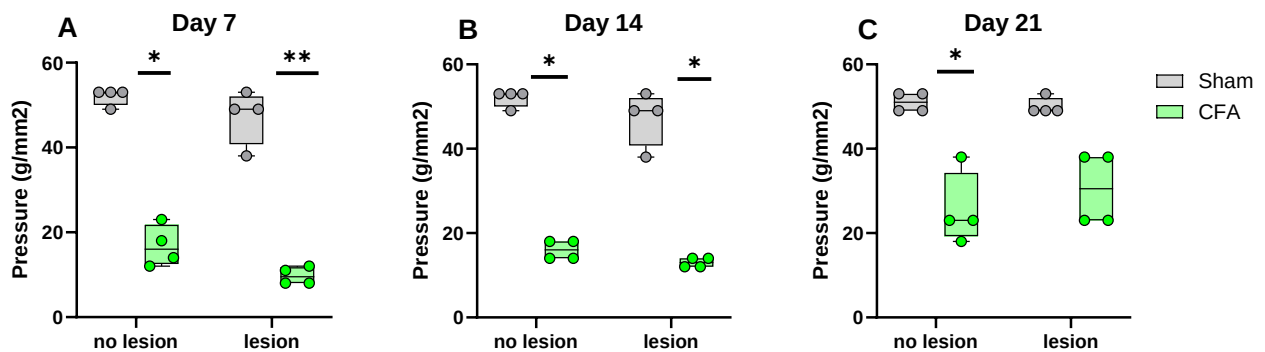


Figure III.21 – Mechanical sensitivity measured by the response to von frey filaments. **A.** Mechanical threshold to noxious stimuli between experimental groups (Sham and CFA) without (left) and with (right) OFC lesion on day 7. **B.** Mechanical threshold to noxious stimuli between experimental groups (Sham and CFA) without (left) and with (right) OFC lesion on day 14. **C.** Mechanical threshold to noxious stimuli between experimental groups (Sham and CFA) without (left) and with (right) OFC lesion on day 21. Comparisons between experimental groups and lesion were based on Kruskal-Wallis test followed by *post hoc* Dunn's test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: $n = 4$, non-lesioned: $n = 4$ CFA (green): $n = 4$, non-lesioned: $n = 4$.

III.3.3 Lesioned sham rat present higher choice to delayed rewards.

The impact of an OFC lesion on DGt performance was initially assessed by the percentage of completed trials, delayed rewarded trials, and non-rewarded delayed trials on each testing day (**Figure III.22**). Regarding completed trials, no statistically significant differences were found between experimental group and lesion, on any of testing days (**day 7**: KW=3.546, $p=0.3411$; **day 14**: KW=6.042, $p=0.099$; **day 21**: KW=5.356, $p=0.1570$). Subsequently, the percentage of delayed rewarded trials was analyzed, and, unlike the previously parameter, statistically differences were found on every testing day (**day 7**: KW=9.019, $p=0.012$. *Post hoc* analysis using Dunn's test revealed that within Sham group, lesioned rats had higher percentage of delayed rewards ($p=0.032$); **day 14**: KW=8.906, $p=0.014$, *Post hoc* analysis using Dunn's test revealed that within Sham group, lesioned rats had higher percentage of delayed rewards ($p=0.019$), and lesioned CFA had lower percentage of delayed rewards compared to lesioned Sham ($p=0.034$); **day 21**: two-way ANOVA, F1: $F_{(1,3)}=5.38$, $p=0.0049$; F2: $F_{(1,3)}=13.82$, $p=0.032$. *Post hoc* analysis using Sidak test revealed that within Sham and CFA groups, lesioned rats had higher percentage of delayed rewards ($p=0.003$ and $p=0.047$, respectively), and lesioned CFA had lower percentage of delayed rewarded trials compared to lesioned Sham ($p=0.019$)). Lastly, the percentage of non-rewarded delayed trials was assessed, and statistically differences were found solely on day 14 between experimental groups and lesion (**day 7**: KW=6.141, $p=0.096$; **day 14**: two-way ANOVA, F1: $F_{(1,3)}=15.84$, $p=0.028$; F2: $F_{(1,3)}=20.96$, $p=0.019$. *Post hoc* analysis using Sidak test revealed that within Sham group, lesioned rats had a lower percentage of delayed non-rewarded trials ($p=0.007$), and lesioned CFA had a higher percentage of delayed non-rewarded trials compared to lesioned Sham ($p=0.02$); **day 21**: two-way ANOVA, F1: $F_{(1,3)}=1.125$, $p=0.3668$; F2: $F_{(1,3)}=0.2666$, $p=0.6417$. Therefore, on every session, lesioned Sham consistently showed a higher percentage of delayed rewarded trials compared to their non-lesioned counterpart, and a lower percentage of non-delayed rewarded trials on day 14.

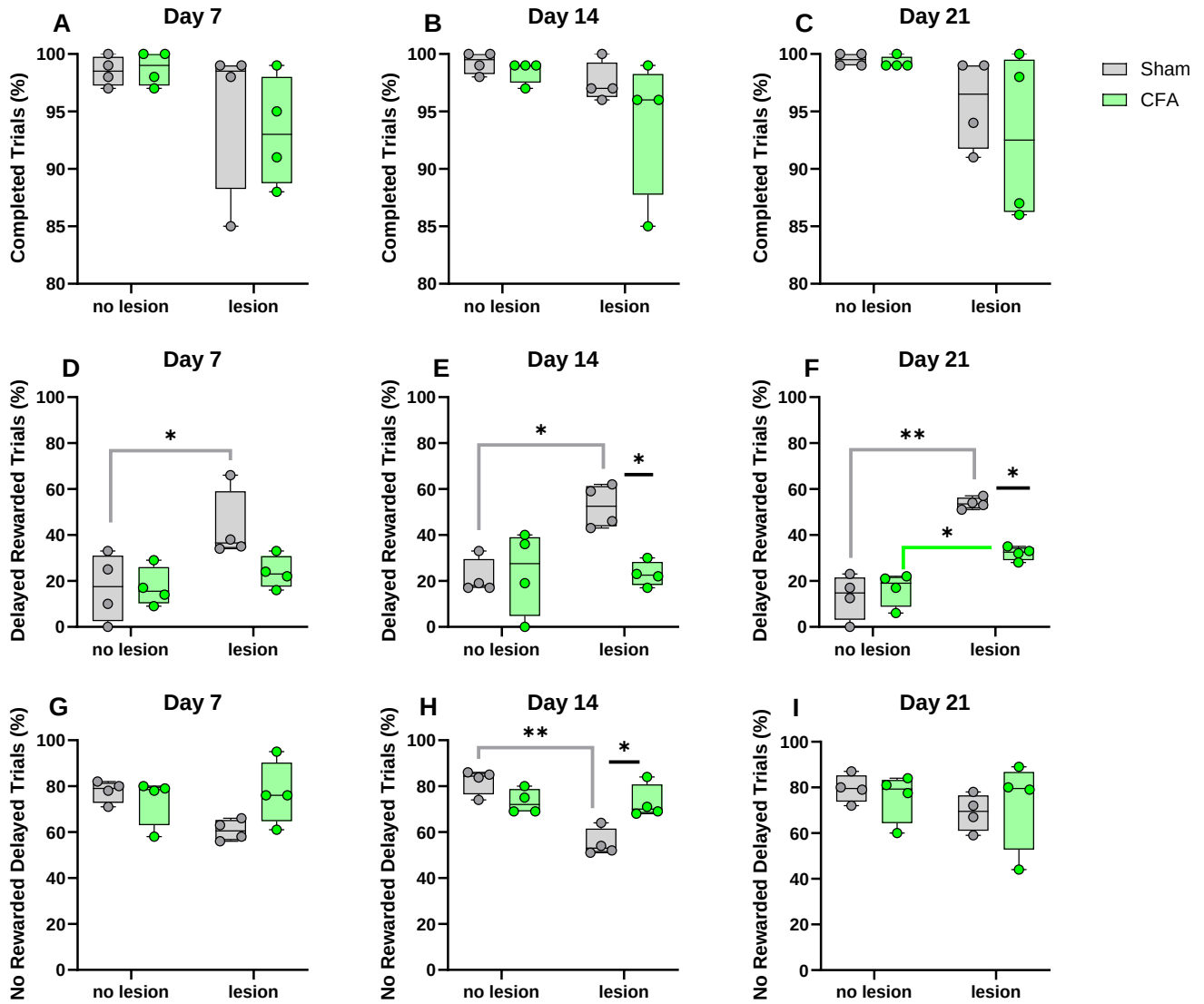


Figure III.22 – DGT performance assessed by rewarded trials and non-rewarded trials of OFC lesioned rats. **A.** Percentage of completed trials between experimental groups without (left) and with (right) OFC lesion on day 7. **B.** On day 14. **C.** On day 21. **D** Percentage of delayed rewarded trials between experimental groups without (left) and with (right) OFC lesion on day 7. **E.** On day 14. **F.** On day 21. **G.** Percentage of non-rewarded delayed trials between experimental groups without (left) and with (right) OFC lesion on day 7. **H.** On day 14. **I.** On day 21. Comparisons between experimental groups and lesion were based on Kruskal-Wallis test, followed by *post-hoc* Dunn's test (used to non-normal distributed data) and two-way ANOVA test (F1: groups x F2: lesion), followed by *post hoc* Sidak test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: n = 4, non-lesioned: n = 4 CFA (green): n = 4, non-lesioned: n = 4. * $p < 0.05$, ** $p < 0.01$.

III.3.4 Lesioned Sham rats consumed more pellets.

The total number of pellets was also evaluated between experimental groups with and without OFC lesion on every testing day (**Figure III.23**). Statistically significant differences were found solely between lesioned and non-lesioned animals within the same experimental group. On **day 7**, two-way ANOVA, $F_1: F_{(1,3)}=0.024, p=0.884$; $F_2: F_{(1,3)}=12.26, p=0.039$. *Post hoc* analysis using Sidak test revealed that within Sham group, lesioned rats had a higher consumption of pellets ($p=0.039$). On **day 14**, two-way ANOVA, $F_1: F_{(1,3)}=0.8571, p=0.422$; $F_2: F_{(1,3)}=9.91, p=0.05$. *Post hoc* analysis using Sidak test revealed that within Sham group, lesioned rats had a higher consumption of pellets ($p=0.04$). To conclude, on **day 21**, two-way ANOVA, $F_1: F_{(1,3)}=0.6424, p=0.4815$; $F_2: F_{(1,3)}=84.9, p=0.002$. *Post hoc* analysis using Sidak test revealed that within Sham and CFA groups, lesioned rats had a higher consumption of pellets compared to their non-lesioned counterparts ($p=0.017$, and $p=0.049$, respectively). Therefore, lesioned Sham rats showed a higher consumption of pellets compared to non-lesioned Sham.

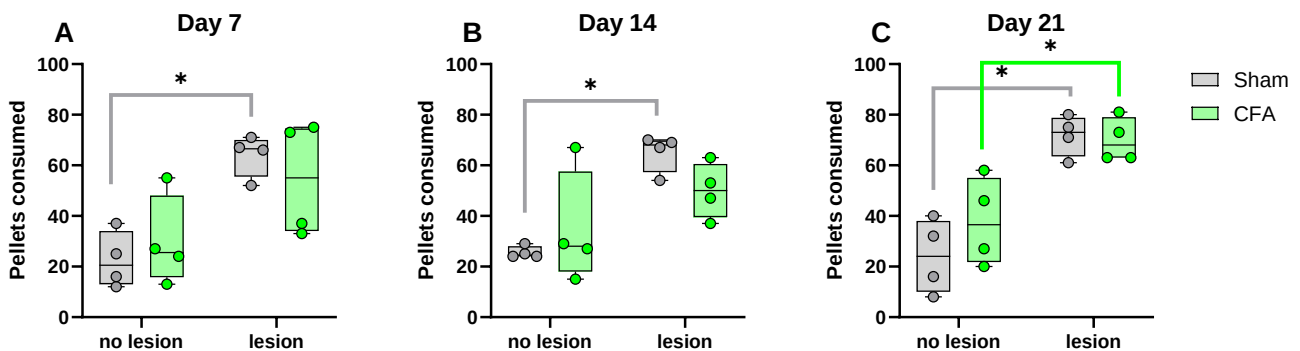


Figure III.23 – Pellets consumption on DGt of OFC lesioned rats. **A.** Total number of pellets consumed between experimental groups without (left) and with (right) OFC lesion on day 7. **B.** On day 14. **C.** On day 21. Comparisons between experimental groups and lesion were based on two-way ANOVA test (F_1 : group x F_2 : lesion), followed by *post hoc* Sidak test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: $n = 4$, non-lesioned: $n = 4$ CFA (green): $n = 4$, non-lesioned: $n = 4$. * $p < 0.05$.

III.3.5 Lesioned and non-lesioned rats present similar preference index.

To understand the animal's preference for a given lever and the corresponding reward, the preference index for each session day was also evaluated, similarly to the previous experiment (**Figure III.24**). No statistically significant differences were found on any testing days between experimental groups and lesion. On **day 7**, two-way ANOVA, $F_1: F_{(1,3)}=0.3604, p=0.5906$; $F_2: F_{(1,3)}=6.335, p=0.086$; on **day 14**, two-way ANOVA, $F_1: F_{(1,3)}=2.414, p=0.218$; $F_2: F_{(1,3)}=4.056, p=0.1374$. Lastly, on **day 21**, two-way ANOVA, $F_1: F_{(1,3)}=0.5046, p=0.5287$; $F_2: F_{(1,3)}=5.164, p=0.1077$. Despite the increased percentage of delayed rewarded trials in the lesioned Sham rats, their preference index remains similar to non-lesioned Sham and CFA group, regardless of the presence of lesion.

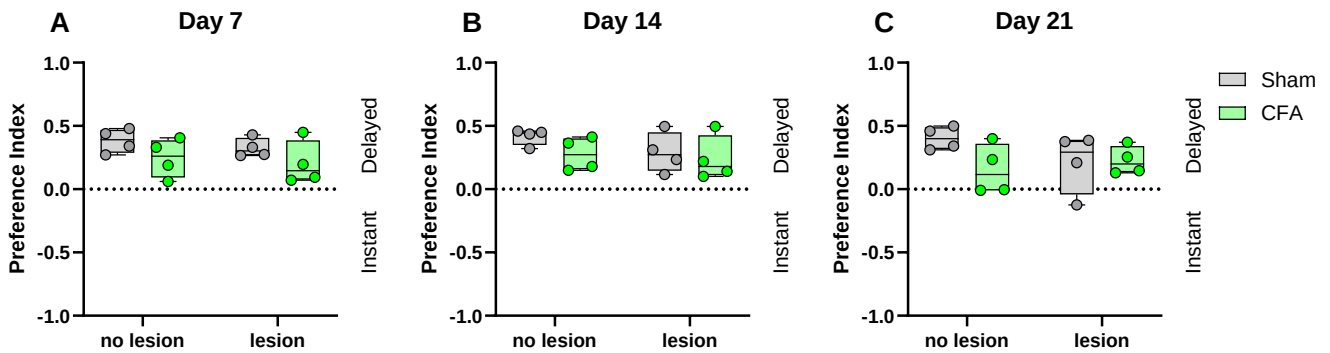


Figure III.24 – Preference index across days 7,14,21 of OFC lesioned rats. **A.** Preference index between experimental groups without (left) and with (right) OFC lesion on day 7. **B.** On day 14. **C.** On 21. No statistically significant differences were found between experimental groups and lesion presence based on two-way ANOVA (F_1 : group x F_2 : lesion). The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: $n = 4$, non-lesioned: $n = 4$ CFA (green): $n = 4$, non-lesioned: $n = 4$.

III.3.6 On days 14 and 21, lesioned Sham exhibits a lower cumulative preference throughout the session compared to non-lesioned Sham.

To understand how variation of preference progresses in lesioned rats throughout each session and whether the presence of a lesion has any impact, the variation across the session was evaluated by dividing it into 10 equal intervals. Subsequently, each session was split into two equal halves, and differences in variation between lesioned and non-lesioned animals were assessed. Regarding the first approach (**Figure III.26**), on **day 7** statistically significant differences were only found within the two experimental groups, for lesioned and non-lesioned (Sham- non-lesion: $KS=0.9999$, $p\approx 0$; lesion: $KS=1$, $p=0$; CFA- no lesion: $KS=0.9937$, $p\approx 0$; $KS=0.9599$, $p\approx 0$). On **day 14**, statistically significant differences were observed within the two experimental groups, for lesioned and non-lesioned (Sham- non-lesion: $KS=1$, $p=0$; lesion: $KS=0.9331$, $p\approx 0$; CFA- no lesion: $KS=0.93319$, $p\approx 0$; lesion: $KS=0.7331$, $p\approx 0$). Additionally, it was also observed differences within Sham groups between lesioned and non-lesioned rats ($KS^2=0.7$, $p=0.0068$), and between lesioned CFA and lesioned Sham ($KS^2=0.6$, $p=0.0310$). Lastly, on **day 21**, statistically significant differences were observed within the two experimental groups, for lesioned and non-lesioned (Sham- non-lesion: $KS=1$, $p=0$; lesion: $KS=0.8877$, $p\approx 0$; CFA- no lesion: $KS=0.49435$, $p=0.008$; lesion: $KS=0.6986$, $p\approx 0$). It was also found differences within Sham and CFA groups between lesioned and non-lesioned rats ($KS^2=0.7$, $p=0.0068$ and $KS^2=0.6$, $p=0.0310$, respectively), and between non-lesioned rats ($KS^2=0.8$, $p=0.00012$).

Regarding to the second approach (**Figure III.25**) (differences in each half), on the **first half of the day 7**, two-way ANOVA, F_1 : $F_{(1,3)}=0.0512$, $p=0.8355$; F_2 : $F_{(1,3)}=10.91$, $p=0.0456$, *Post hoc* analysis using Sidak test revealed that lesioned Sham had a higher variation compared to lesioned CFA, indicating a higher preference to delayed rewards ($p=0.027$). On the **second half of day 7**, two-way ANOVA, F_1 : $F_{(1,3)}=0.3488$, $p=0.5964$; F_2 : $F_{(1,3)}=23.86$, $p=0.0164$. *Post hoc* analysis using Sidak test did not reveal any specific differences. On **day 14, first and second halves** no statistically significant differences were found neither between experimental groups nor lesion (two-way ANOVA, F_1 : $F_{(1,3)}=4.483$, $p=0.1151$; F_2 : $F_{(1,3)}=0.6462$, $p=0.4866$, and two-way ANOVA, F_1 : $F_{(1,3)}=1.040$, $p=0.3828$; F_2 : $F_{(1,3)}=6.093$, $p=0.0902$, respectively. Lastly, and similarly to the previous testing day no statistically significant differences were observed neither in **first half** nor **second half of day 21**, two-way ANOVA, F_1 : $F_{(1,3)}=0.2357$,

$p=0.6606$; F_2 : $F_{(1,3)}=2.379$, $p=0.2207$, and two-way ANOVA, F_1 : $F_{(1,3)}=0.7788$, $p=0.4425$; F_2 : $F_{(1,3)}=0.4145$, $p=0.5655$, respectively. These results suggest that throughout sessions 14, and 21, lesioned Sham present a lower value of variation, indicating a higher pressing of instant lever.

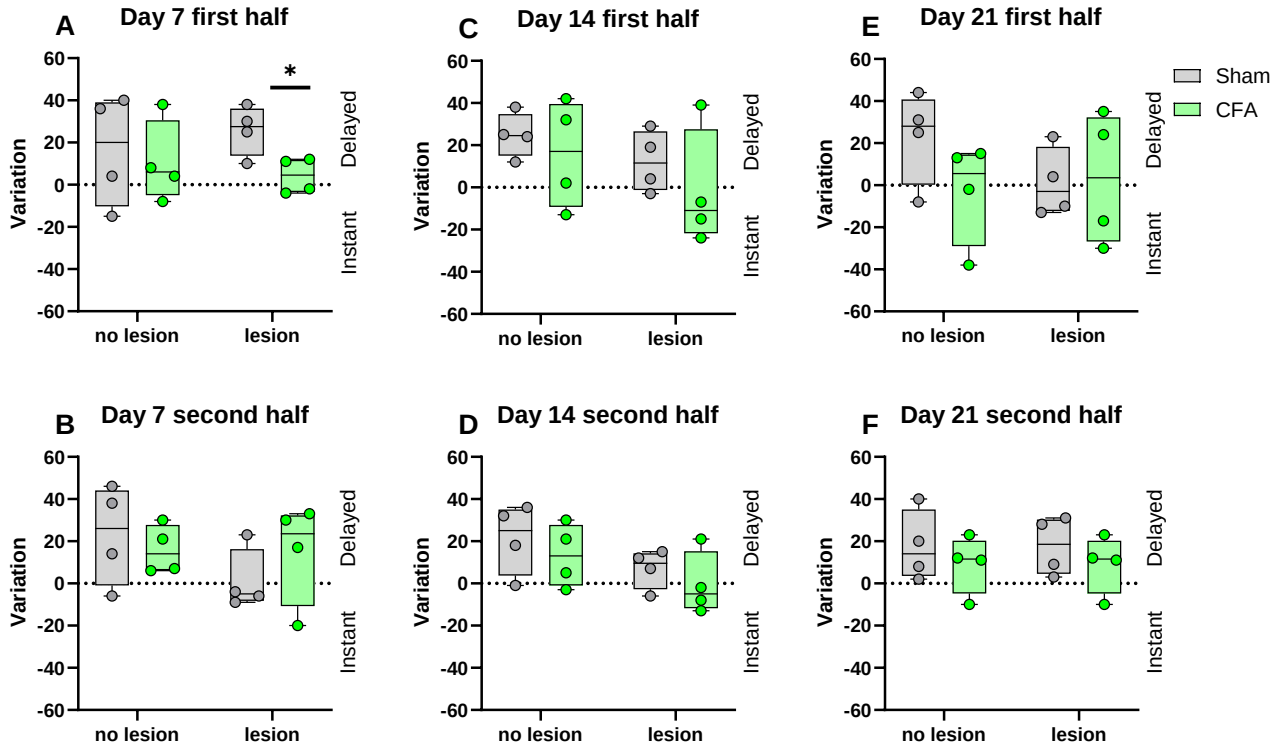


Figure III.25 –Variation/Cumulative preference between halves of session of OFC lesioned rats. **A.** Variation value between experimental groups without (left) and with (right) OFC lesion on the first half of day 7. **B.** On the second half of day 7. **C.** Variation value between experimental groups without (left) and with (right) OFC lesion on the first half of day 14. **D.** On the second half of day 14. **E.** Variation value between experimental groups without (left) and with (right) OFC lesion on the first half of day 21. **F.** On the second half of day 21. Comparisons between experimental groups and treatments were based on two-way ANOVA test (F_1 : group x F_2 : lesion), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: $n = 4$, non-lesioned: $n = 4$ CFA (green): $n = 4$, non-lesioned: $n = 4$. * $p < 0.05$.

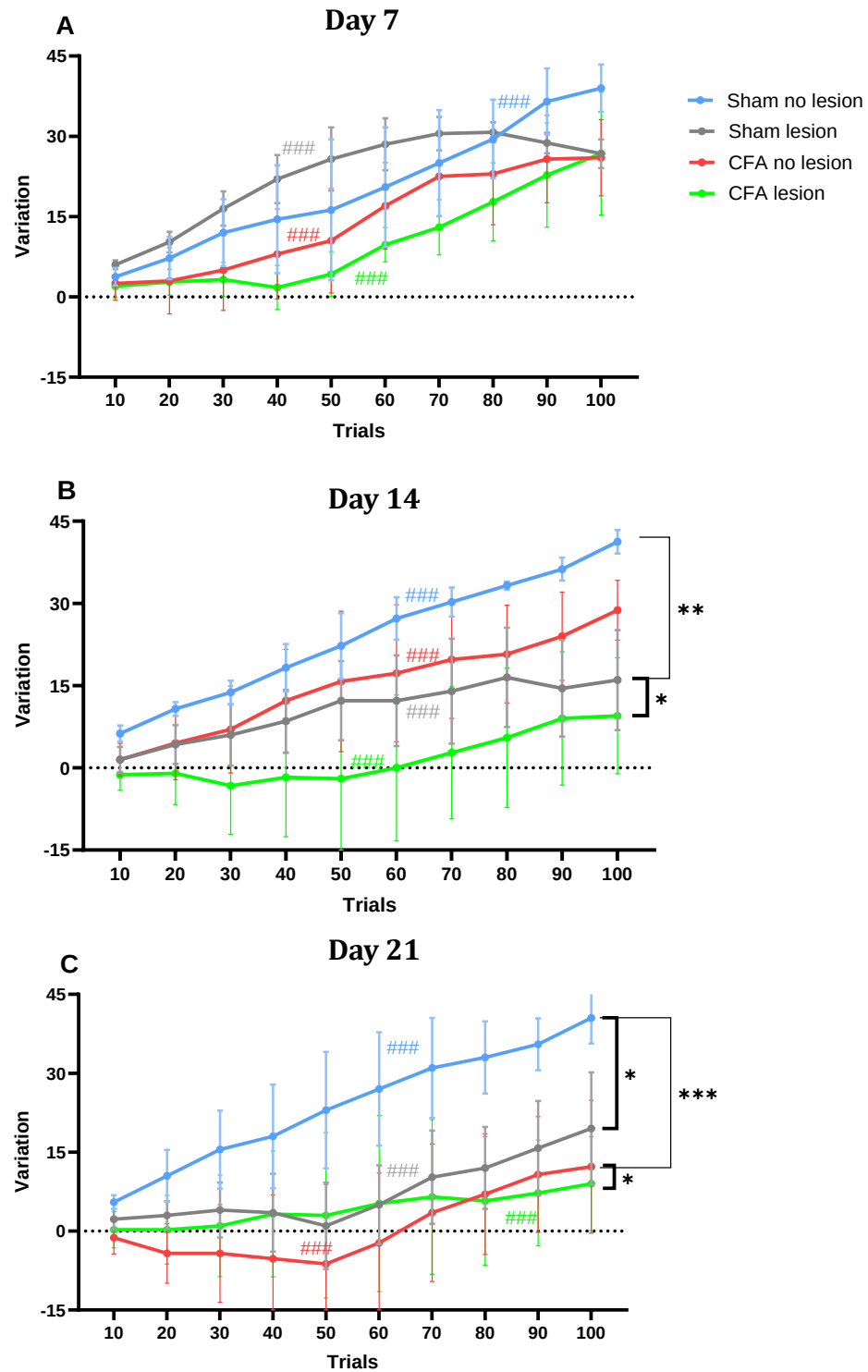


Figure III.26 – Variation/Cumulative preference throughout the session of OFC lesioned rats on DGt. A. Variation throughout the session, on day 7. B. Variation throughout the session, on day 14. C. Variation throughout the session, on day 21. Comparisons between groups and intragroup (with and without lesion) throughout the session were based on two-sample Kolmogorov-Smirnov and one-sample Kolmogorov-Smirnov test respectively. The values from graphs are presented as mean $\pm$ SD. Sham, no-lesion (blue): $n=4$; Sham lesioned (gray): $n=4$; CFA no lesion (red): $n=4$; CFA lesioned (green): $n=4$. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; ### $p<0.001$

III.3.7 Lesioned and non-lesioned rats share similarities in shifting.

The impact of OFC lesion in number of shifts was also assessed throughout the session (**Figure III.27**) and every fifty trials (**Figure III.28**). On **day 7**, no statistically significant differences were found between lesioned Sham and lesioned CFA rats ($KS2=0.4$, $p=0.3128$), neither intragroup between lesioned and non-lesioned (Sham: $KS2=0.4$, $p=0.3128$; CFA: $KS2=0.3$, $p=0.6750$), but statistically significant differences were observed within the two experimental groups, for lesioned and non-lesioned (Sham- non-lesion: $KS=0.9772$, $p\approx 0$; lesion: $KS=0.9772$, $p\approx 0$; CFA- no lesion: $KS=0.9331$, $p\approx 0$; lesion: $KS=0.9599$, $p\approx 0$). On **day 14**, similarly to the previous day, differences were only observed within the two experimental groups, for lesioned and non-lesioned (Sham- non-lesion: $KS=0.8943$, $p\approx 0$; lesion: $KS=0.9937$, $p\approx 0$; CFA- no lesion: $KS=0.9599$, $p\approx 0$; lesion: $KS=0.9599$, $p\approx 0$). Between lesioned Sham and lesioned CFA, it was found a $KS2=0.3$, $p=0.6750$, and intragroup between lesioned and non-lesioned, Sham: $KS2=0.2$, $p=0.9478$, and CFA: $KS2=0.4$, $p=0.3128$. Lastly, on **day 21**, no statistically significant differences were found between lesioned Sham and lesioned CFA rats ($KS2=0.4$, $p=0.3128$), neither intragroup between lesioned and non-lesioned (Sham: $KS2=0.3$, $p=0.6750$; CFA: $KS2=0.2$, $p=0.9747$). However, statistically significant differences were observed within the two experimental groups, for lesioned and non-lesioned (Sham- non-lesion: $KS=0.9599$, $p\approx 0$; lesion: $KS=0.9970$, $p\approx 0$; CFA- no lesion: $KS=0.9877$, $p\approx 0$; lesion: $KS=0.9331$, $p\approx 0$).

Subsequently, differences in each half of session were assessed between experimental groups with and without lesion. On the **first half of the day 7**, two-way ANOVA, F_1 : $F_{(1,3)}=2.567$, $p=0.2074$; F_2 : $F_{(1,3)}=0.0245$, $p=0.8854$. On the **second half of day 7**, $KW=7.506$, $p=0.041$. Post hoc analysis using Dunn's test revealed that lesioned Sham present a higher number of shifting compared to lesioned CFA rats ($p=0.0191$.) On **day 14, first and second halves** no statistically significant differences were found neither between experimental groups nor lesion ($KW=1.461$, $p=0.7241$, and two-way ANOVA, F_1 : $F_{(1,3)}=1.482$, $p=0.3105$; F_2 : $F_{(1,3)}=7.771$, $p=0.0686$, respectively. Similarly to the previous testing day no statistically significant differences were observed neither in **first half** nor **second half** of **day 21**, two-way ANOVA, F_1 : $F_{(1,3)}=0.6187$, $p=0.4889$; F_2 : $F_{(1,3)}=0.5933$, $p=0.4973$, and two-way ANOVA, F_1 : $F_{(1,3)}=0.0851$, $p=0.7815$; F_2 :

$F_{(1,3)}=0.3394$, $p=0.6011$, respectively. These results suggest that lesioned and non-lesioned rats present similar number of shifts on DGt.

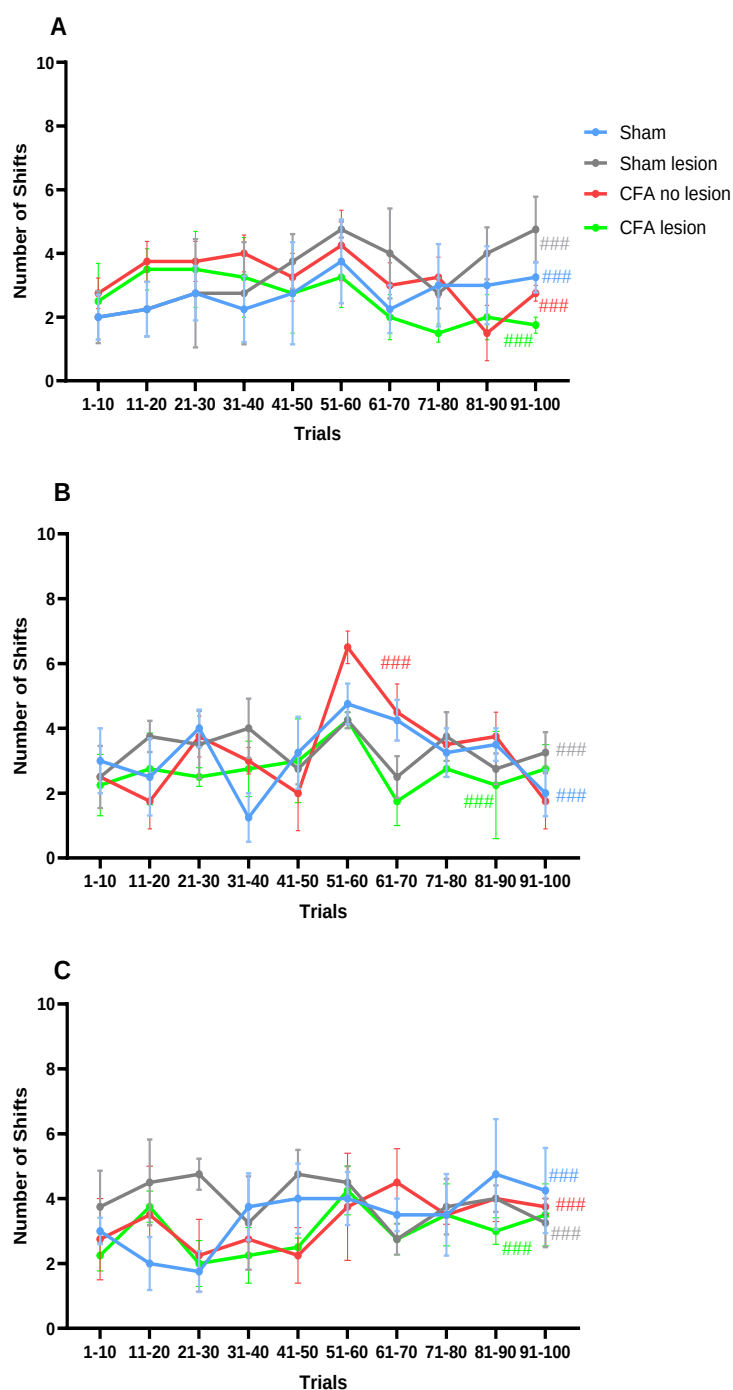


Figure III.27 – Shifting throughout the session of OFC lesioned rats on DGt. **A.** Number of shifts in blocks of 10 trials throughout the session of day 7. **B.** Number of shifts in blocks of 10 trials throughout the session of day 14. **C.** Number of shifts in blocks of 10 trials throughout the session of day 21. Comparisons between groups and intragroup (with and without lesion) throughout the session were based on two-sample Kolmogorov-Smirnov and one-sample Kolmogorov-Smirnov test respectively.) The values from graphs are presented as mean $\pm$ SD. Sham, no-lesion (blue): $n=4$; Sham lesioned (gray): $n=4$; CFA no lesion (red): $n=4$; CFA lesioned (green): $n=4$. ### $p<0.001$

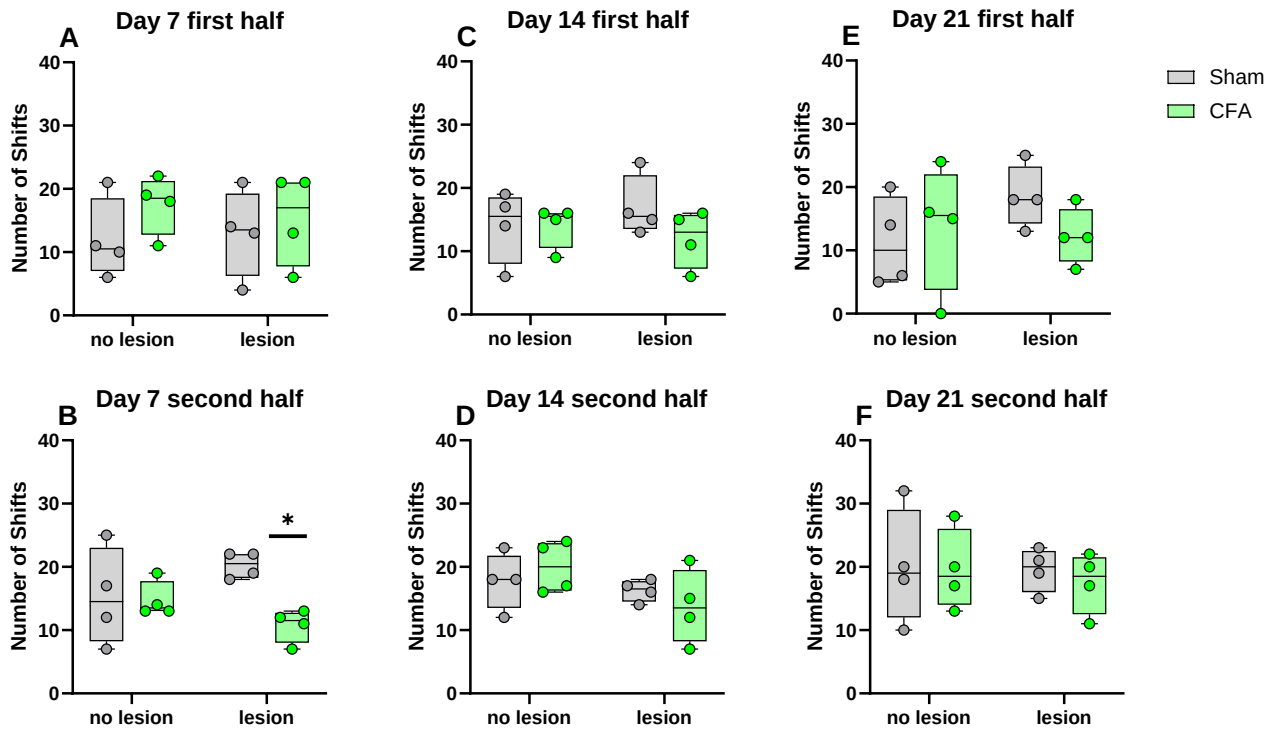
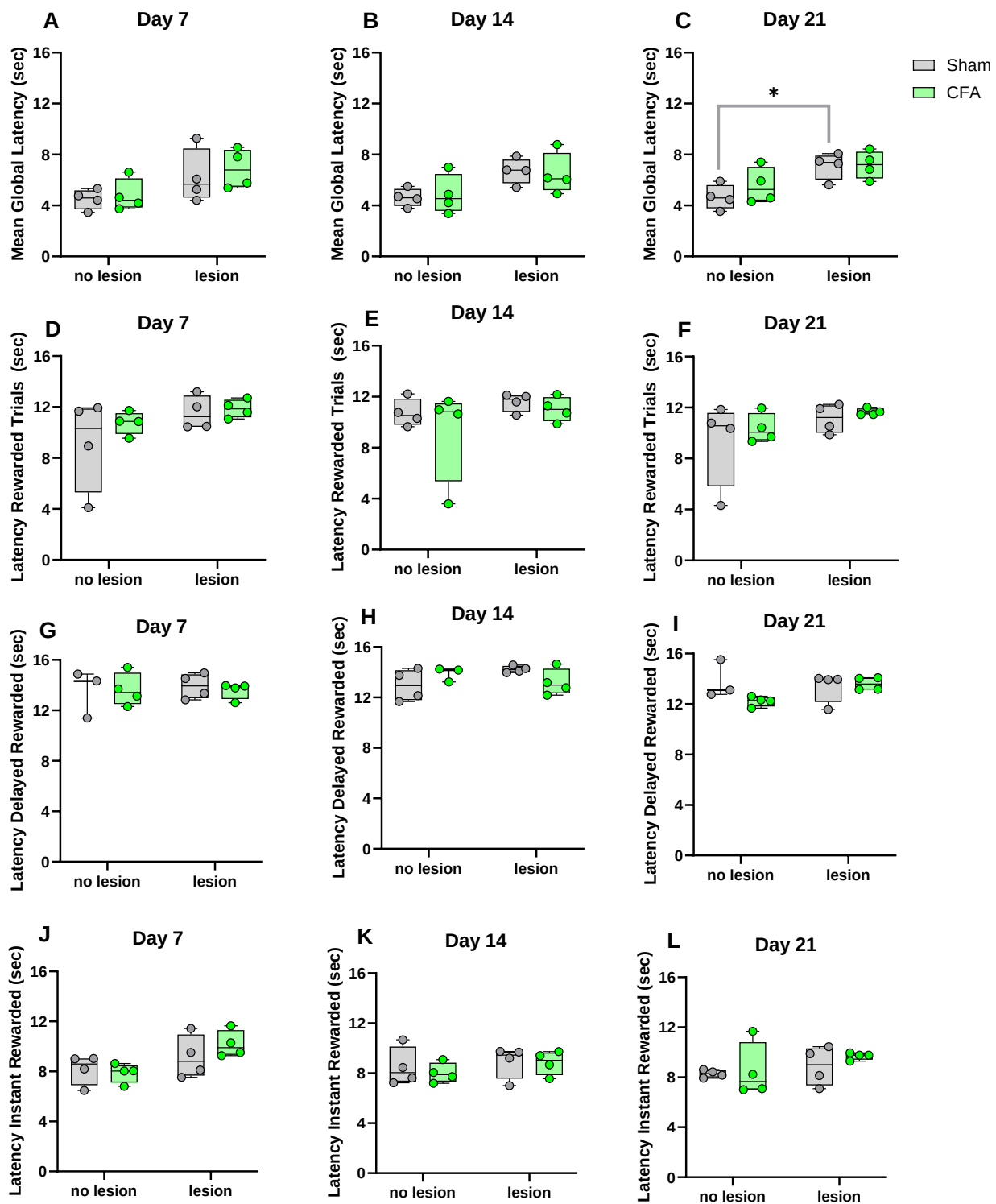


Figure III.28 – Number of shifts between halves of OFC lesioned rats. **A.** Number of shifts between experimental groups without (left) and with (right) OFC lesion on the first half of day 7. **B.** On the second half of day 7. **C.** Number of shifts between experimental groups between experimental groups without (left) and with (right) OFC lesion on the first half of day 14. **D.** On the second half of day 14 **E.** Number of shifts value between experimental groups without (left) and with (right) OFC lesion on the first half of day 21 **F.** On the second half of day 21. Comparisons between experimental groups and treatments were based on two-way ANOVA test (F1: group x F2: lesion), followed by Sidak *post hoc* test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: n = 4, non-lesioned: n = 4 CFA (green): n = 4, non-lesioned: n = 4. * $p < 0.05$.

III.3.8 OFC lesioned rats present similar latency response to non-lesioned rats.

In order to understand whether the OFC lesion had any impact on response latencies in the DGt, various types of latencies were evaluated for each testing day. (**Figure III.29**). Regarding the **global latencies**, no statistical differences were found on days 7 and 14, but were found on day 21 (**day 7**: $F_{(1,3)}=0.6472$, $p=0.4804$; F_2 : $F_{(1,3)}=3.310$, $p=0.1664$; **day 14**: $F_{(1,3)}=0.0899$, $p=0.7839$; F_2 : $F_{(1,3)}=4.075$, $p=0.1368$; **day 21**: $F_{(1,3)}=0.4210$, $p=0.5624$; F_2 : $F_{(1,3)}=24.04$, $p=0.0162$. Post hoc analysis using Sidak test revealed that within Sham group, lesioned rats presented higher latency than non-lesioned, $p=0.04$). About **latency of rewarded trials**, no statistical differences were observed on any of testing days (**day 7**: $F_{(1,3)}=2.175$, $p=0.1989$; F_2 : $F_{(1,3)}=1.763$, $p=0.2762$; **day 14**: $KW=1,478$, $p=0,7260$; **day 21**: $F_{(1,3)}=0.8016$, $p=0.4366$; F_2 : $F_{(1,3)}=2.091$, $p=0.2439$). Similarly, no statistical differences were found neither in latency of delayed rewarded trials nor latency of instant rewarded trials for any of testing days (**latency delayed rewarded trials- day 7**: $KW=0.3792$, $p=0.9543$; **day 14**: $F_{(1,3)}=0.0983$, $p=0.7744$; F_2 : $F_{(1,3)}=0.1026$, $p=0.7698$; **day 21**: $KW=5.942$, $p=0.2762$; **latency instant rewarded trials- day 7**: $F_{(1,3)}=4.575$, $p=0.1220$; F_2 : $F_{(1,3)}=0.5633$, $p=0.5074$; **day 14**: $F_{(1,3)}=0.4802$, $p=0.5382$; F_2 : $F_{(1,3)}=2.439$, $p=0.2163$; **day 21**: $F_{(1,3)}=3.005$, $p=0.1814$; F_2 : $F_{(1,3)}=0.8235$, $p=0.4311$). Regarding **latency of non-rewarded trials**, no statistical differences were found on any of testing days, **day 7**: $F_{(1,3)}=1.971$, $p=0.255$; F_2 : $F_{(1,3)}=6.924$, $p=0.0782$; **day 14**: $KW=4.257$, $p=0.2482$; **day 21**: $F_{(1,3)}=9.848$, $p=0.054$; F_2 : $F_{(1,3)}=0.6713$, $p=0.4726$. However, within latency of non-rewarded trials, statistical differences were found in latency of non-rewarded delayed and instant trials on days 7 and 21, respectively. About the first, on **day 7**: $F_{(1,3)}=0.059$, $p=0.8226$; F_2 : $F_{(1,3)}=24.09$, $p=0.0162$. Post hoc analysis using Sidak test did not reveal any specific differences; **day 14**: $KW=4,125$, $p=0,2638$; **day 21**: $F_{(1,3)}=0.0956$, $p=0.7773$; F_2 : $F_{(1,3)}=8.497$, $p=0.0617$. To conclude, regarding **latency of non-rewarded instant trials**, **day 7**: $F_{(1,3)}=1.104$, $p=0.3706$; F_2 : $F_{(1,3)}=3.064$, $p=0.1783$; **day 14**: $F_{(1,3)}=0.2663$, $p=0.6414$; F_2 : $F_{(1,3)}=2.424$, $p=0.2173$; **day 21**: $F_{(1,3)}=4.804$, $p=0.1161$; F_2 : $F_{(1,3)}=10.88$, $p=0.045$. Post hoc analysis using Sidak test did not reveal any specific differences. These results suggest that the OFC lesion did not impact latency response in DGt.



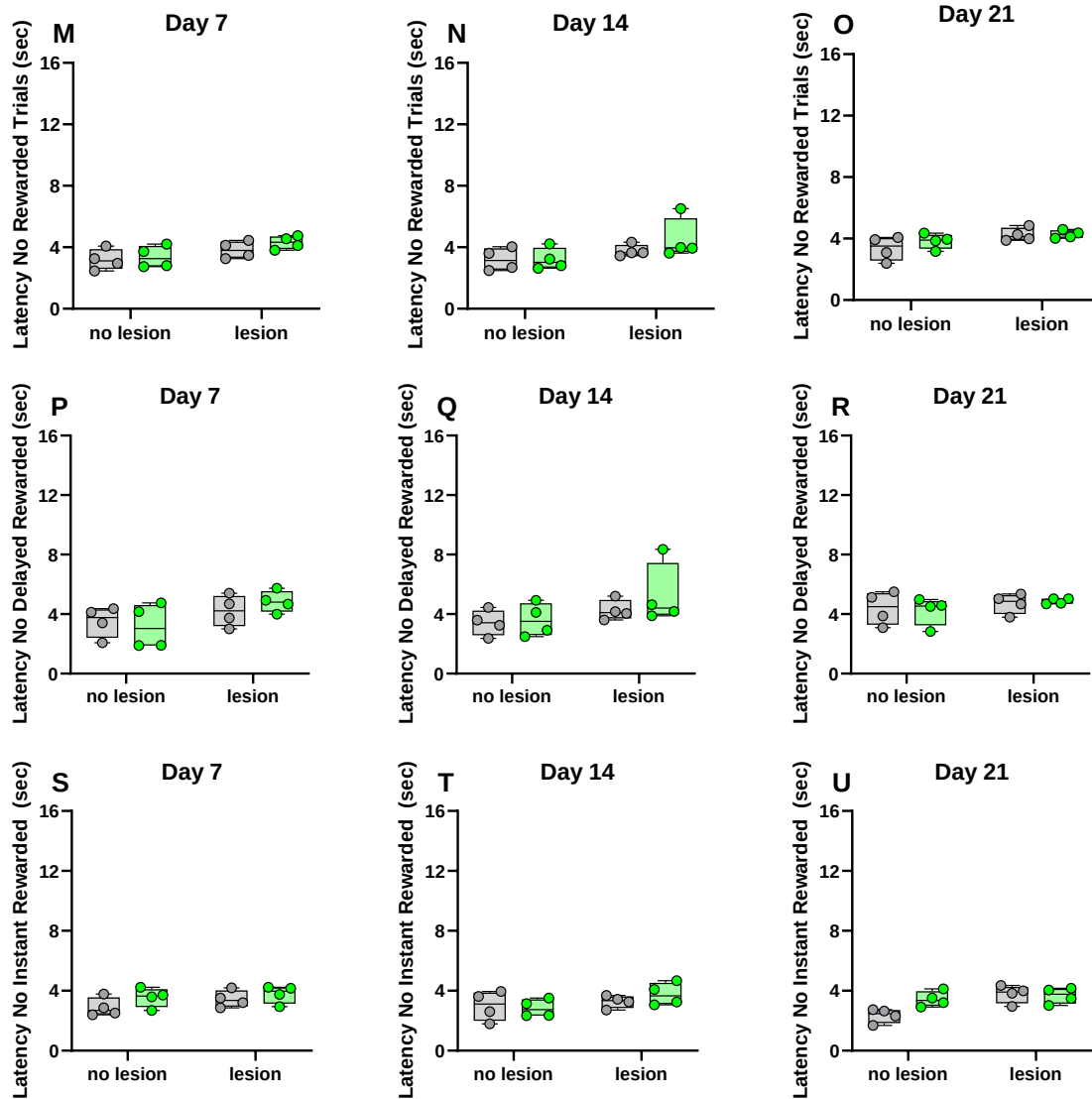


Figure III.29 – Latency responses after OFC lesion on DGt. **A.** Mean latency of all trials between experimental groups without (left) and with (right) OFC lesion on day 7. **B.** On day 14. **C.** On day 21. **D.** Latency of all rewarded trials between experimental groups without (left) and with (right) OFC lesion on day 7. **E.** On day 14. **F.** On day 21. **G.** Latency of instant rewarded trials between experimental groups without (left) and with (right) OFC lesion on day 7. **H.** On day 14. **I.** On day 21. **J.** Latency of delayed rewarded trials between experimental groups without (left) and with (right) OFC lesion on day 7. **K.** On day 14. **L.** On day 21. **M.** Latency of non-rewarded trials between experimental groups without (left) and with (right) OFC lesion on day 7. **N.** On day 14. **O.** On day 21. **P.** Latency of non-rewarded delayed trials between experimental groups without (left) and with (right) OFC lesion on day 7. **Q.** On day 14. **R.** On day 21. **S.** Latency of non-rewarded instant trials between experimental groups without (left) and with (right) OFC lesion on day 7. **T.** On day 14. **U.** On day 21. Comparisons between experimental groups and lesion presence were based on Kruskal-Wallis test (used to non-normal distributed data), and two-way ANOVA test (F1: group x F2: lesion). The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): n = 4; CFA (green): n = 4. *p<0.05

III.3.9 Motor responses are not impacted by OFC lesion.

Lastly, motor activity was also assessed by measuring the total distance traveled and the average speed for each testing day (**Figure III.30**). No statistically significant differences were found between experimental groups with and without lesions. For total distance: on **day 7**, two-way ANOVA, $F_1: F_{(1,3)}=5.277, p=0.1053$; $F_2: F_{(1,3)}=3.227, p=0.1703$; **day 14**, two-way ANOVA, $F_1: F_{(1,3)}=7.504, p=0.0714$; $F_2: F_{(1,3)}=2.436, p=0.2165$; **day 21**, two-way ANOVA, $F_1: F_{(1,3)}=0.2092, p=0.6785$; $F_2: F_{(1,3)}=1.229, p=0.3659$. Regarding, average speed: on **day 7**, two-way ANOVA, $F_1: F_{(1,3)}=4.812, p=0.1159$; $F_2: F_{(1,3)}=0.4145, p=0.5655$; **day 14**, two-way ANOVA, $F_1: F_{(1,3)}=5.298, p=0.1048$; $F_2: F_{(1,3)}=0.6925, p=0.4664$; **day 21**, two-way ANOVA, $F_1: F_{(1,3)}=5.116, p=0.1087$; $F_2: F_{(1,3)}=0.1042, p=0.7680$. These results suggest that OFC lesion did not impact motor responses.

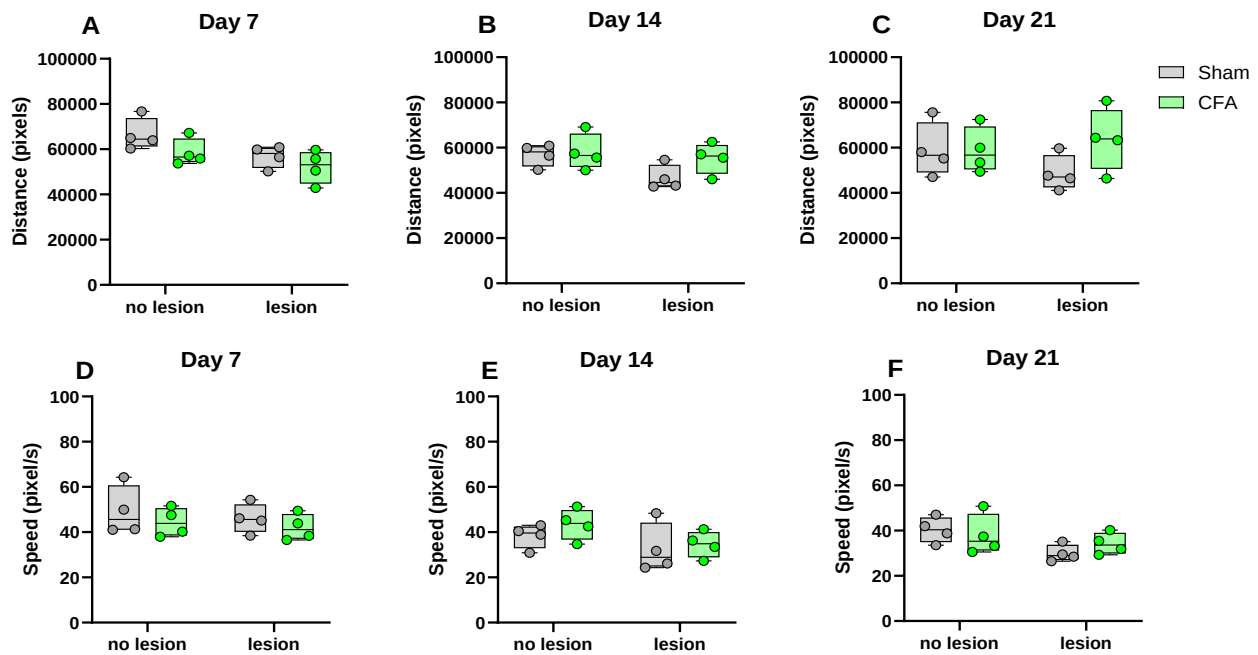


Figure III.30 – Motor responses of OFC lesioned rats on DGt. **A.** Total Distance traveled between experimental groups without (left) and with (right) OFC lesion on day 7. **B.** On day 14. **C.** On day 21. **D.** Average speed in pixel/second between experimental groups without (left) and with (right) OFC lesion on day 7. **E.** On day 14. **F.** On day 21. No differences in motor activity were found between experimental groups and lesion presence based on two-way ANOVA test (F_1 : group x F_2 : lesion) test. The data is depicted using a Box and whiskers plot, based in the median and minimum/maximum values. Each data point represents a rat. Sham (gray): lesioned: $n = 4$, non-lesioned: $n = 4$ CFA (green): $n = 4$, non-lesioned: $n = 4$.

IV. Discussion

This study, initially focused on examining the differences between rats experiencing pain, induced by CFA, and rats without pain in a DGt. Subsequently, the research aimed to determine whether the use of pharmacological modulators of D2r would alter task performance, and later, investigate the role of the OFC in the performance of the same task. The results showed that while no differences were found between CFA-group and Sham-group in the percentage of rewarded trials (either instant or delayed), differences were observed in the percentage of non-rewarded trials, contributing to differences between experimental groups in cumulative preference /variation throughout the session. However, it was found that rats with OFC lesions exhibited a higher percentage of delayed rewarded trials and, interestingly, also instant non-rewarded trials. This resulted in lower variation in the lesioned animals compared to the non-lesioned ones. Regarding the modulation of D2r, it was observed that the activation of D2 receptor, by systemic injection of quinpirole was associated with an increased in the number of incomplete trials, as well as a slight increase in delayed rewarded trials in the CFA-group, leading to an increase in latency time. Moreover, both activation and inhibition of D2 receptors were linked to an increase and decrease, respectively, in the number of shifts.

IV.1 Performance in DGt.

Chronic pain induces alterations in the brain's reward processing, leading to imbalances in decision-making between delayed and instant options, with a bias toward the latter [42, 43]. This occurs due to a reduced appreciation of the value of delayed rewards linked to a decline in cognitive control, resulting in a shift toward instant gratification [43]. This was indeed observable in the first experiment of this study, where the CFA group showed a clear preference for the instant lever over the delayed one on all testing days. However, this behavior was also observed in the Sham group, resulting in no significant differences between experimental groups in delayed and instant rewarded trials. One possible explanation could be a lack of understanding of the DGt. But the fact

that significant differences were observed in non-rewarded trials between experimental groups suggests that Sham rats did understand the value of each lever. Yet, they exhibited difficulty in waiting the necessary time to receive the reward. This may be attributed to the complexity of the task itself, particularly regarding the time required to receive the delayed reward. In other tasks designed to study delayed gratification, these animals were able to wait without any issue [3, 15]. The key difference lies in the waiting time, for example, (in one of those tasks), the waiting time for the animal is proportional to the number of pellets they are rewarded with [3]. In contrast, in this experiment, the waiting time is fixed for each lever, being ten seconds for the delayed option. It might have been interesting to explore how the animals would behave if there had been a third lever, rewarding them somewhere between seven or eight and ten seconds, with two pellets, to clarify if it was problem of waiting duration, and if it would be easier for them to be rewarded. Additionally, another possible cause could have been due to the learning phase. Apart from the error I am responsible for regarding the number of trials conducted with some animals, the no distinction of the value between the two levers could contribute to the difficulty to understand the time required to be rewarded, at least for the first testing day. However, the fact that there were three testing days, and a preference for the delayed lever in non-rewarded trials, underlies that the animals had to be rewarded with 3 pellets before to have that behavior, meaning that they had to wait the time needed. A lack of motivation to wait the required time for the reward could also be a contributing factor. Despite being under food restriction, the large number of instant rewarded trials could be sufficient to reduce their willingness to wait the necessary ten seconds. What is clear is the existence of a difference in the non-rewarded trials, which becomes more apparent when examining the cumulative preference /variation data throughout each testing day. In this measure, the Sham group shows higher values than the CFA group, further supporting a greater choice for delayed non-rewarded trials in the Sham group, as the rewarded trials were similar between experimental groups. Therefore, there is distinction between experimental groups, although it is more subtle than expected, as the anticipated outcome was higher percentage of delayed rewarded trials for the Sham group.

However, this was observed in the Sham rats with a lesion in the OFC. Interestingly, these animals exhibited a behavior opposite to the Sham without a lesion, both in rewarded and non-rewarded trials. Specifically, they showed a higher percentage of delayed rewarded trials and a higher percentage of instant non-rewarded trials. Why this difference? These lesioned animals clearly had no issues waiting at least ten seconds

to be rewarded with three pellets, something that was not observed in the non-lesioned Sham. Besides the difference in the learning curve, where these lesioned animals had a homogeneous number of trials, the only clear difference is the lesion in the OFC area. The OFC is crucial in reward processing and decision-making, as it integrates information from various brain areas, being responsible to encode and update the value of outcomes [21, 110]. So, it plays a key role in situations similar to the DGt, where there is a confrontation between two choices with different delays and reward magnitudes. The lesion caused by quinolinic acid targets the excitatory glutamatergic neuronal population, leading to neuronal death as quinolinic acid is an agonist of NMDA receptor [111]. This disrupts the normal functioning of the OFC, affecting these animals' ability to evaluate the two levers. This might explain why these lesioned Sham rats exhibited opposite choices in rewarded and non-rewarded trials. However, something surprising is that these animals were able to wait at least ten seconds, showing even higher latency responses compared to non-lesioned Sham. The lesion coordinates target the LO region, which has been associated with cognitive control, when reward-associated responses require suppression, making it crucial for pursuing delayed larger rewards [32, 33]. Some studies have observed that lesion in this orbital area result in increased impulsive behavior, which contradicts what was observed in this study [33]. One possible explanation could be the nature of the lesion itself. Unlike this study, where a unilateral lesion was performed, those studies that observed increased impulsive behavior, involved a bilateral lesion. Since the lesioned Sham rats had a higher percentage of delayed rewards, this indicate that the unilateral lesion didn't increase impulsivity. However, despite the absence of this impulsive behavior, it was observed that the lesion compromised the animal's ability to encode and update the value of the lever. This explains the higher percentage of delayed rewards and instant non-rewarded trials. These animals demonstrated an inadequate integration of responding for the larger reward. Therefore, as noted in the literature, these animals exhibited problems with associative learning, being unable to associate a lever with a specific value based on the reward received [29, 112]. However, an interesting question is why these animals struggle to wait 5 seconds to be rewarded instantly but could wait 10 seconds for the delayed reward. These rats have difficulty assigning a value to each lever associated with different rewards, but why do they 'miss' the delivery time one lever and not the other? One possible explanation could be due the role of OFC in computing temporal information about rewards [28, 113]. A study has shown that the OFC is crucial for understanding the temporal context of outcome related rewards [28].

In this case, the lesion may have caused the animal to inadequately estimate the delay associated with one lever, leading to failure in timing with one lever but not the other. On the other hand, non-lesioned CFA rats had a similar performance to those with lesions in both rewarded and non-rewarded trials. Persistent inflammatory pain causes cognitive alterations by disrupting the functioning of certain brain regions, including the OFC [40, 82]. These changes, as previously stated, ultimately contribute to a preference for instant rewards. So, in pain syndromes, the functions of the OFC are overshadowed by the impact of pain. Consequently, whether an (OFC) lesion is present or not, it might lead to a similar outcome in both situations, that it is a preferred choice to instant rewards.

Despite the differences previously mentioned in the Sham group between rats with and without lesions, the preference indices in Sham (and CFA) were similar, indicating that the number of delayed rewarded trials of lesioned was similar to the number of delayed non-rewarded trials of non-lesioned. In the DGt setup, at the 50th trial, there was a switch in the number of pellets associated by the levers. As previously stated, OFC plays a crucial role in mediating behavioral flexibility in response to changes in stimulus-reward associations [114]. So, the OFC is essential in those situations, and it has been shown that lesions in the orbital area result in deficits in reversal learning[110]. Interestingly, focusing on the cumulative preference data before and after the 50th trial, it is evident that in four sessions, the lesioned animals exhibited completely opposite behavior. Given that the levers swapped reward values, this means the animals continued to favor the same lever in both halves, which could indicate a subtle impairment in reversal learning. One explanation for why the effects were not more pronounced could be that the lesion was not bilateral. In various studies targeting impairments in reversal learning, bilateral lesions are typically used rather than unilateral ones [115-117]. Additionally, although the LO area is involved in these types of cognitive functions, other subareas such as the DLO and VO are also highly significant [17]. Overall, these results highlight the critical role of the OFC in reward processing, and behavioral flexibility, with lesions in this area leading to specific deficits that impact the ability to integrate and update reward values effectively. The presence of chronic pain complicates these processes, possibly overshadowing the functions of OFC and leading to similar behavior in both lesioned and non-lesioned animals.

Lastly, this study aimed to understand the effect of pharmacological modulation through systemic injection of quinpirole and raclopride on performance. Quinpirole is highly selective for D2 and D3 receptors and is commonly used as an agonist in

behavioral tasks requiring behavioral flexibility [118, 119]. On the other hand, raclopride is an antagonist with high affinity for D2r, making it useful for studying the effects of D2r inhibition without significant off-target effect [120], and being widely used in D2r studies. [121]. D2r play a critical role in cost-benefit decision-making, and disruption of D2 dopaminergic transmission can disrupt this balance, biasing choices toward instant rewards. The first noteworthy observation was that the animals treated with quinpirole showed a higher rate of omissions compared to those treated with saline (control). This is consistent with other studies, which have found that animals injected either locally or systemically with quinpirole exhibit reduced task engagement [53, 66, 122]. Quinpirole activates D2r, which play an inhibitory role in dopaminergic transmission [60, 123]. Dopaminergic activity can be represented as an inverted U-shape, meaning that at both extremes, represented by very low or very high doses, there is a compromise in dopaminergic activity [76, 81]. So, overexpression of D2r could disrupt the balance of dopamine signaling, affecting cognitive functions, including attention. This was observed in a study where animals injected with quinpirole had difficulties completing trials in a five-choice serial reaction time task [53].

Interestingly, animals treated with quinpirole exhibited slightly different performances depending on the presence or absence of pain. While animals with pain showed slight increase in delayed rewarded trials, the opposite occurred in animals without pain. This somewhat contradicts what is stated in the literature, which asserts that injections of D2r agonists contributes to a preference for rewards with longer delays [1, 79]. So, what might have caused these results? D2r activation by quinpirole inhibits dopamine release [123]. This inhibitory effect reduces overall dopamine signaling, which has influence in decision-making, including in cost-benefits decisions [124, 125]. Given the OFC lesion, that already compromises the ability to evaluate reward outcomes and make decisions based on them, the additional reduction in dopamine could further impair the cognitive processes that support delayed gratification. As a result, these animals have an increase choice for instant rewards. Conversely, in animals with both pain and orbital lesions the opposite was observed. Chronic pain is known to disrupt dopaminergic signaling, leading to lower dopamine levels [87, 90, 91]. In this altered state, quinpirole could trigger a compensatory response that stabilizes dopamine levels, thereby supporting a choice for delayed rewards. The differential effects of quinpirole on delayed rewarded choices in OFC lesioned rats could be understood through dopaminergic modulation along with altered neurobiology in chronic pain. However, this did not result in

differences in preference indices or variation throughout the sessions, but in terms of temporal responses, animals treated with quinpirole exhibited slightly higher response latencies. Something that became evident was the number of shifts between levers. Animals treated with quinpirole showed an increase in shifting, while those treated with raclopride exhibited a decrease. This is consistent with the findings in the literature [66, 76]. This result can be explained by the sensitivity to reward reception. Treatment with quinpirole results in increase lever switching due to a decrease in reward sensitivity, in other words, regardless of the receipt of reward the rewards hold little value for the animals, leading them to switch levers more frequently [66]. On the other hand, raclopride increases sensitivity to reward reception, resulting in the animals sticking to one lever when they are rewarded [76]. This indicates that D2r are crucial in modifying decision biases, particularly in situations that require monitoring reward outcomes and adapting the behavior accordingly. It's important to note that although D2r were the focus of this work, D1r also involved a role in these cognitive functions [126]. In matter of fact, both D1r and D2r work together in choices that require behavioral flexibility [127-129]. D2r are responsible for the animal's exploration of different options, while D1r allow for the stabilization of an effective option [76, 130]. Thus, D2r alone are not solely responsible, as, modulating D2r can also affect D1r activity [81].

This study highlights the complex role of D2r modulation in decision making and behavioral flexibility in the context of pain and OFC lesions. Systemic administration of quinpirole led to a decrease in delayed rewarded trials in non-pain animals, while the opposite was found in pain animals, possibly due to compensatory dopamine mechanisms. Additionally, quinpirole increased shifting between levers, whereas systemic raclopride injection stabilized behavior by increasing reward sensitivity. These findings underscore the role of D2r on cognitive functions, with the possibly the interaction of D1r.

IV.2 Motor activity.

Motor activity, measured by total distance and average speed, was similar across the three testing days between experimental groups. Usually, the CFA-induced model is applied to the joint (persistent monoarthritic inflammatory pain), however, due to the

locomotion limitations it causes, this study employed a variation where CFA was injected into the animal's hindpaw [131, 132]. This approach is less aggressive and does not impact motor activity, thereby avoiding any compromise in performance [133, 134]. This is particularly beneficial, as it ensures that any observable differences in DGt are likely due to cognitive changes associated with reward processing and decision-making, rather than impairments in movement.

Similar results were observed between rats with and without OFC lesions, as well between those injected with D2r pharmacological modulators. While the OFC is not directly responsible for motor control, it may influence motor actions indirectly through its role in value-based decision-making which guides the selection and prioritization of actions [135, 136]. However, studies involving unilateral and bilateral OFC lesions do not report any impact on task engagement due to reduced motor activity [137, 138]. This may be due to the presence of intact areas that play other roles in this goal-directed behavior circuit, such as the DLPFC and NAc [135, 139]. Furthermore, the regulation of motor activity is mainly controlled by other brain regions, including basal ganglia and motor cortex, which are responsible for initiating and regulating movement [140, 141]. The similar locomotion patterns observed in both lesioned and non-lesioned rats indicate that the OFC does not play a direct role in motor activity.

Regarding the pharmacological modulators of D2r, the dose used (0.05mg/kg) was selected to avoid motor impairments that could affect the performance in the DGt [108]. Quinpirole, in a dose-dependent manner, decreases motor activity [142]. Some studies report a significant decrease in traveled distance even at this dosage [143]. One possible explanation could be the time between the injection and the start of behavioral testing. In this study, animals waited 20 minutes before beginning the task, ensuring that quinpirole's effects were stable before behavioral evaluation. As for raclopride, the results are consistent with the literature, as no differences were observed at these dosages compared to the control treatment [108, 144].

Taken together, motor activity was not affected in any of the experiments, indicating that motor activity was not the reason for observed differences between experimental groups in the performance of DGt.

IV.3 Nociception.

Although the persistent inflammatory pain model typically exhibits longer-lasting effects due to CFA-induced hypersensitivity, with a peak occurring between 5 to 7 days followed by a decrease in pain, significant differences were observed between experimental groups across the three testing days [46, 50, 145]. Despite a slight progression between the second and third testing days (within CFA group), it is noteworthy that on days 14 and 21, animals injected with CFA still had a relatively low pain threshold compared to Sham animals. Similar observations have been reported in studies using CFA, though in those studies the rats were injected CFA into the joint rather than the hindpaw, which is expected to produce a greater impact [146]. In other studies where CFA was injected into the hindpaw but with a double dose, similar results were observed [147]. One possible explanation for the difference results between experimental groups on the three testing days could be due to the stress experienced by the animals, which might exacerbate pain [148, 149]. Although the animals were given 15-20 minutes acclimatization period before any testing to reduce stress, some animals might still have been stressed. Another possibility could be errors in measuring threshold levels. Certain animal movements may have led to inaccuracies, however several repetitions were performed. Additionally, two animals developed visible sores from frequent licking and biting on the injected site, which could have led to heightened sensitivity in the area and a lower threshold measured by the von Frey test. The other two animals, despite not showing physical signs of the same problem, might also have experienced increased sensitivity in the same area.

Later, the effect of OFC lesions on pain responses was assessed. The results revealed that lesioned animals, regardless of whether they were injected with CFA or not, exhibited similar pain thresholds to those without lesions, suggesting that the OFC lesion did not significantly influence the sensory pain response. This finding is consistent with the known role of the OFC in pain, which is involved in the cognitive and emotional dimensions of pain rather than its direct sensory processing [150, 151]. The Von Frey test measures peripheral nociceptive response, reflecting the ability of the nervous system to detect and respond to mechanical stimuli, that is mainly processed through other brain regions, including somatosensory cortex [152-154]. Therefore, these findings suggest that the similar pain thresholds between lesioned and non-lesioned animals indicate that the

OFC does not play a significant role in the sensory aspect of pain, which highlights distinct functions of different brain regions in the overall experience of pain.

The absent of differences between pharmacological treatments aligns with the literature, indicating that the dose used (0.05 mg/kg) does not impact pain responses [108].

V. Conclusions and future perspectives.

This work aimed to validate the preference for instant rewards in chronic pain syndromes and explore the interaction between chronic pain, the OFC, and dopaminergic modulation of D2r in delayed gratification. Chronic pain was found to bias decision-making toward instant gratification. Surprisingly, the Sham group also exhibited a similar bias, possibly due to task complexity or motivation factors, rather than poor understanding. Nevertheless, cumulative preferences differed between experimental groups. OFC lesions revealed unexpected results, with lesioned Sham animals showing a higher percentage of delayed rewarded trials and instant non-rewarded, suggesting that unilateral OFC lesions may impair the ability to update reward values, compromising association learning, but without increasing impulsivity. Chronic pain further influenced these behaviors, as both lesioned and non-lesioned animals preferred instant rewards, indicating that impact of pain on OFC overshadow the impairment of OFC lesion, leading to similar outcomes. It was also observed lightly the OFC's role in reversal learning, as lesioned animals displayed distinct behaviors between the two halves of session. Regarding D2r modulation, quinpirole increased shifting behavior, while raclopride had the opposite effect, likely due to changes in reward sensitivity, without impacting the overall indices of preference. Also, motor activity remained unaffected by OFC lesions or systemic pharmacological treatments, confirming that the observed behavioral changes were cognitive rather than motor impairments. Lastly, similar pain thresholds between lesioned and non-lesioned suggest that the OFC's role in pain does not involve sensory perception.

However, this study has some limitations. One major problem is the small number of animals, with only eight animals per experiment, which significantly limits statistical power and the conclusions that can be drawn. Additionally, due to this small n , there was no control group for the surgical procedure of the lesion. Another limitation is the use of systemic administration for D2r modulation, which affects the entire brain rather than only the OFC. This broad action complicates the ability to isolate the specific impact of D2r modulation on the OFC in delayed gratification. A last caveat could be the use of unilateral surgeries, which may lead to partial effects in behavioral responses. With that said, it would be beneficial in the future to replicate this work with a larger sample size and include bilateral OFC lesions. Additionally, using local injections of quinpirole and

raclopride into the OFC could provide more precise insights, which can then be compared to the behavioral results with systemic injections. Regarding the task itself, it may be interesting to explore tasks that employ a variable delay proportional to the animal's waiting time, rather than a fixed delay. This would allow a more flexible measurement of how the animals evaluate reward (magnitude) over time. Lastly, optogenetics could be a crucial tool for understanding the OFC-BLA-NAc circuit's role in delayed gratification, in the context of chronic pain. This neural network regulates impulsive behavior by influencing the pursuit of delayed and larger rewards. By selectively targeting the OFCs projections to the NAc and BLA, this would help understand how chronic pain disrupts the balance between instant and delayed reward processing, offering insights of the root of this impaired cognitive control. Furthermore, combining it with dLight could provide real-time monitoring of dopamine dynamics within this pathway during a delayed gratification task.

Chronic pain alters decision-making, leading to impulsive behaviors and consequently, a preference for instant rewards over delayed, which compromises adherence to treatments that require long duration and negatively affect the quality of life. This shift is linked to a dysfunction in D2r and brain areas such as OFC, that works together with NAc and BLA, to evaluate and pursue delayed rewards. Understanding these circuit and targeting d D2r by modulating them may offer potential treatments that restore balanced decision-making in chronic pain individuals, improving their well-being and overall quality of life.

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VII. Supplementary materials

Annex I

Acid Acetone (5 min) → Distilled H₂O → Thionin stain (30 sec) → Distilled H₂O → Xylene → Entellan

Figure VII.1 - Thionine staining protocol adapted by Antonio Avelino. This protocol commences by immersing the slides in acid acetone for a duration of 5 minutes, followed by a swift rinse with distilled water, and subsequent staining with thionine for 30 seconds. Then the slices are rinsed one more with distilled water, gently dried with filter paper, and then place in a greenhouse at 37°C. Once adequately dried, xylene is applied to the slices. Afterwards, the mounting medium – Entellan – is applied.

Annex II

A) Example of Opencontrol output (session.txt)

First 50 trials from a given session:

Trial: 1 Time: 50507,5 - Response: Lever B - NO REWARD - Latency (sec): 8,703
 Trial: 2 Time: 50526,203 - Response: Lever A - NO REWARD - Latency (sec): 2,51499999999866
 Trial: 3 Time: 50538,718 - Response: Lever A - NO REWARD - Latency (sec): 2,90699999999924
 Trial: 4 Time: 50551,625 - Response: Lever A - REWARD(1) - Latency (sec): 6,171
 Trial: 5 Time: 50567,796 - Response: Lever A - NO REWARD - Latency (sec): 2,4999999999979
 Trial: 6 Time: 50580,296 - Response: Lever B - NO REWARD - Latency (sec): 6,39099999999791
 Trial: 7 Time: 50596,687 - Response: Lever B - NO REWARD - Latency (sec): 3,25000000000192
 Trial: 8 Time: 50609,937 - Response: Lever A - NO REWARD - Latency (sec): 1,54700000000192
 Trial: 9 Time: 50621,484 - Response: Lever B - NO REWARD - Latency (sec): 2,95300000000326
 Trial: 10 Time: 50634,437 - Response: Lever B - NO REWARD - Latency (sec): 2,68800000000192
 Trial: 11 Time: 50647,125 - Response: Lever A - NO REWARD - Latency (sec): 1,843
 Trial: 12 Time: 50658,968 - Response: Lever B - NO REWARD - Latency (sec): 3,32799999999924
 Trial: 13 Time: 50672,296 - Response: Lever B - NO REWARD - Latency (sec): 1,7039999999979
 Trial: 14 Time: 50684 - Response: Lever B - NO REWARD - Latency (sec): 7,984
 Trial: 15 Time: 50701,984 - Response: Lever B - NO REWARD - Latency (sec): 2,89100000000326
 Trial: 16 Time: 50714,875 - Response: Lever B - NO REWARD - Latency (sec): 4,609
 Trial: 17 Time: 50729,484 - Response: Lever A - NO REWARD - Latency (sec): 1,59400000000326
 Trial: 18 Time: 50741,078 - Response: Lever A - REWARD(1) - Latency (sec): 6,01499999999866
 Trial: 19 Time: 50757,093 - Response: Lever B - NO REWARD - Latency (sec): 8,26599999999925
 Trial: 20 Time: 50775,359 - Response: Lever A - NO REWARD - Latency (sec): 1,46900000000326
 Trial: 21 Time: 50786,828 - Response: Lever B - REWARD(3) - Latency (sec): 12,2969999999987
 Trial: 22 Time: 50809,125 - Response: Lever A - NO REWARD - Latency (sec): 1,656
 Trial: 23 Time: 50820,781 - Response: Lever B - NO REWARD - Latency (sec): 6,07799999999732
 Trial: 24 Time: 50836,859 - Response: Lever B - NO REWARD - Latency (sec): 4,14100000000326
 Trial: 25 Time: 50851 - Response: Lever A - NO REWARD - Latency (sec): 2,562
 Trial: 26 Time: 50863,562 - Response: Lever B - NO REWARD - Latency (sec): 6,85900000000192
 Trial: 27 Time: 50880,421 - Response: Lever B - NO REWARD - Latency (sec): 2,8749999999979

Trial: 28 Time: 50893,296 - Response: Lever B - NO REWARD - Latency (sec): 1,9069999999979
Trial: 29 Time: 50905,203 - Response: Lever B - NO REWARD - Latency (sec): 1,31199999999866
Trial: 30 Time: 50916,515 - Response: Lever B - NO REWARD - Latency (sec): 3,92200000000058
Trial: 31 Time: 50930,437 - Response: Lever A - NO REWARD - Latency (sec): 1,46900000000192
Trial: 32 Time: 50941,906 - Response: Lever A - NO REWARD - Latency (sec): 3,43699999999732
Trial: 33 Time: 50955,343 - Response: Lever A - NO REWARD - Latency (sec): 3,21899999999924
Trial: 34 Time: 50968,562 - Response: Lever A - NO REWARD - Latency (sec): 1,95300000000192
Trial: 35 Time: 50980,515 - Response: Lever B - NO REWARD - Latency (sec): 2,31300000000058
Trial: 36 Time: 50992,828 - Response: Lever B - NO REWARD - Latency (sec): 8,81199999999866
Trial: 37 Time: 51011,64 - Response: Lever A - NO REWARD - Latency (sec): 1,37500000000058
Trial: 38 Time: 51023,015 - Response: Lever B - NO REWARD - Latency (sec): 6,61000000000058
Trial: 39 Time: 51039,625 - Response: Lever B - NO REWARD - Latency (sec): 8,5
Trial: 40 Time: 51058,125 - Response: Lever A - REWARD(1) - Latency (sec): 8,89
Trial: 41 Time: 51077,015 - Response: Lever A - NO REWARD - Latency (sec): 4,62500000000058
Trial: 42 Time: 51091,64 - Response: Lever A - NO REWARD - Latency (sec): 1,48500000000058
Trial: 43 Time: 51103,125 - Response: Lever B - NO REWARD - Latency (sec): 3,14
Trial: 44 Time: 51116,265 - Response: Lever A - NO REWARD - Latency (sec): 4,96900000000058
Trial: 45 Time: 51131,234 - Response: Lever B - NO REWARD - Latency (sec): 3,09400000000326
Trial: 46 Time: 51144,328 - Response: Lever A - NO REWARD - Latency (sec): 3,04699999999866
Trial: 47 Time: 51157,375 - Response: Lever B - NO REWARD - Latency (sec): 2
Trial: 48 Time: 51169,375 - Response: Lever A - REWARD(1) - Latency (sec): 6,031
Trial: 49 Time: 51185,406 - Response: Lever A - NO REWARD - Latency (sec): 2,59399999999732
Trial: 50 Time: 51198 - Response: Lever B - NO REWARD - Latency (sec): 1,843

B) Example of Bonsai output (bonsai_file.txt)

First 5 seconds from a given session:

(171,25, 280)
(279,9394, 48,81818)
(276,7604, 54,19792)
(264,1566, 78,94949)
(249,0651, 91,18893)
(235,2799, 106,4378)
(225,3285, 122,9242)
(226,7315, 120,0117)
(224,5245, 127,2585)
(224,6044, 125,3882)
(226,4051, 124,8788)
(224,773, 129,5822)
(223,0359, 144,2958)
(218,4669, 150,1957)
(212,3119, 154,5018)
(207,614, 159,9695)
(202,7755, 161,1126)
(201,6848, 159,7133)
(199,3919, 156,5583)
(197,8753, 159,0726)
(198,5949, 159,042)
(198,0385, 160,1371)
(198,7649, 157,9592)
(200,591, 163,2768)
(201,6018, 156,4191)
(202,6102, 154,2269)

(202,0097, 155,4991)
 (201,8903, 154,9852)
 (200,7258, 156,832)
 (200,183, 155,4325)
 (201,9231, 154,7478)
 (200,4116, 155,58)
 (201,7377, 154,7308)
 (200,0136, 156,6942)
 (199,6667, 157,8803)
 (193,9519, 166,9805)
 (186,2711, 184,334)
 (181,1876, 201,0245)
 (178,9686, 210,4583)
 (178,7646, 216,465)
 (174,8827, 230,3703)
 (173,4293, 237,5526)
 (172,6362, 242,0315)
 (170,4496, 252,1931)
 (169,4614, 256,4369)
 (167,611, 261,4231)
 (165,1328, 263,7328)
 (165,4885, 264,1422)
 (164,5038, 265,4079)
 (164,9912, 264,8095)
 (164,9201, 265,2025)
 (164,7823, 265,4276)
 (163,3238, 268,5578)
 (161,8294, 269,7944)
 (163,7109, 269,5971)
 (165,6338, 270,3685)
 (167,7337, 271,5279)
 (169,0674, 270,8785)
 (168,2409, 271,0593)
 (167,2281, 271,4136)
 (167,4556, 269,3786)
 (166,673, 269,4867)
 (166,8406, 267,141)
 (167,8803, 265,2166)
 (169,0228, 262,4374)
 (169,3451, 262,4486)
 (169,0887, 262,9859)
 (168,4855, 267,6267)
 (169,1119, 272,6694)
 (169,2582, 277,9555)
 (170,8257, 284,1723)
 (172,4173, 287,4962)
 (174,1483, 290,9041)
 (177,7414, 291,3026)

Annex III

A) Learning Curve: Latencies

```

import os

def process_file(file_path):
    with open(file_path, 'r') as file:
        lines = file.readlines()

    latencies = []

    for line in lines:
        if "Latency (sec):" in line:
            # Replace commas with dots for proper conversion to float
            latency_str = line.split("Latency
(sec):") [1].strip().replace(',', '.')

            # Remove non-numeric characters, including the hyphen
            latency_str = ''.join(char for char in latency_str if
char.isdigit() or char == '.')

            # Convert to float and round
            if latency_str:
                latency = round(float(latency_str), 4)
                latencies.append(latency)
            else:
                latencies.append("")
        elif "Omission|Start" in line:
            # If the line indicates an "Omission" trial, insert a
blank space
            latencies.append("")

    return latencies

def calculate_average(filename):
    latencies = process_file(filename)

    # Lists to store latencies greater than and less than 5 seconds
    total_values = []
    values_gt_5 = []
    values_lt_5 = []

    # Iterate through extracted latencies
    for latency in latencies:
        if isinstance(latency, float):
            # Append to the total values list
            total_values.append(latency)

            # Categorize into values greater than and less than 5
seconds
            if latency > 5:
                values_gt_5.append(latency)
            elif latency < 5:
                values_lt_5.append(latency)

    # If no valid latency values found, return zero averages
    if not total_values:

```

```

print("Error: No valid latency values found.")
return {
    "latency_avg_all": 0,
    "latency_avg_gt_5": 0,
    "latency_avg_lt_5": 0
}

# Calculate average latency values for all, greater than 5, and
less than 5 seconds
average_all = sum(total_values) / len(total_values)
average_gt_5 = sum(values_gt_5) / len(values_gt_5) if values_gt_5
else 0
average_lt_5 = sum(values_lt_5) / len(values_lt_5) if values_lt_5
else 0

# Return a dictionary containing the average latency values
return {
    "latency_avg_all": average_all,
    "latency_avg_gt_5": average_gt_5,
    "latency_avg_lt_5": average_lt_5
}

if __name__ == "__main__":
    # Input File
    file_path = "session.txt"

    try:
        # Calculate average latency values
        result = calculate_average(file_path)

        # Print the results
        print("Latency Average of All Trials:",
result["latency_avg_all"])
        print("Latency Average of Trials with Latencies Greater than 5
Seconds:", result["latency_avg_gt_5"])
        print("Latency Average of Trials with Latencies Less than 5
Seconds:", result["latency_avg_lt_5"])

    except FileNotFoundError:
        # Handle file not found error
        print(f"File not found at {file_path}. Please ensure the file
exists and the path is correct.")

```

B) Probe Sessions: Latencies

```

import os

def process_file(file_path):
    # Open the file and read all lines
    with open(file_path, 'r') as file:
        lines = file.readlines()

    # Initialize lists to store different types of latencies
    total_latencies = []
    delayed_reward_latencies = []
    immediate_reward_latencies = []
    rewarded_latencies = []
    no_reward_latencies = []

```

```

latencies = []

# Iterate through each line in the file
for line in lines:
    # Check if the line contains information about latency
    if "Latency" in line:
        # Extract the latency value from the line
        latency_str = line.split("Latency
(sec):") [1].strip().replace(',', '.')

        # Convert the latency value to float and handle any errors
        try:
            latency = float(latency_str)
        except ValueError:
            print(f"Invalid latency value: {latency_str}. Skipping
this line.")
            continue

        # Add the latency to the appropriate lists based on reward
type
        total_latencies.append(latency)
        if "REWARD(3)" in line:
            delayed_reward_latencies.append(latency)
            rewarded_latencies.append(latency)
        elif "REWARD(1)" in line:
            immediate_reward_latencies.append(latency)
            rewarded_latencies.append(latency)
        elif "NO REWARD" in line:
            no_reward_latencies.append(latency)

        # Append the latency to the general latencies list
        latencies.append(latency)
        # Check for "Omission" trials and add a blank space in the
latencies list
        elif "Omission|Start" in line:
            latencies.append("")

    # Calculate latency averages for different conditions
    latency_avg_all_trials = sum(total_latencies) /
len(total_latencies) if total_latencies else 0
    latency_avg_delayed_reward = sum(delayed_reward_latencies) / len(
delayed_reward_latencies) if delayed_reward_latencies else 0
    latency_avg_immediate_reward = sum(immediate_reward_latencies) /
len(
immediate_reward_latencies) if immediate_reward_latencies else
0
    latency_avg_rewarded = sum(rewarded_latencies) /
len(rewarded_latencies) if rewarded_latencies else 0
    latency_avg_no_reward = sum(no_reward_latencies) /
len(no_reward_latencies) if no_reward_latencies else 0

    # The return statement should be indented within the function
    return {
        "latency_avg_all_trials": latency_avg_all_trials,
        "latency_avg_delayed_reward": latency_avg_delayed_reward,
        "latency_avg_immediate_reward": latency_avg_immediate_reward,
        "latency_avg_rewarded": latency_avg_rewarded,
        "latency_avg_no_reward": latency_avg_no_reward,
        "latencies": latencies
    }

```

```

if __name__ == "__main__":
    # Input and output file paths
    input_file_path = "session.txt" # Replace with the actual path to
    your input file
    output_file_path = "all_latencies.txt" # Change the output file
    name if needed

    try:
        result = process_file(input_file_path)

        # Write all latencies to the output file
        with open(output_file_path, 'w') as output_file:
            for latency in result["latencies"]:
                if isinstance(latency, float):
                    output_file.write(f"{latency:.4f}\n")
                else:
                    output_file.write("\n")

        # Print latency averages for different conditions
        print("Latency Average of All Trials:",
result["latency_avg_all_trials"])
        print("Latency Average of Delayed Reward Trials (REWARD 3):",
result["latency_avg_delayed_reward"])
        print("Latency Average of Immediate Reward Trials (REWARD
1):", result["latency_avg_immediate_reward"])
        print("Latency Average of Rewarded Trials (REWARD 1 + REWARD
3):", result["latency_avg_rewarded"])
        print("Latency Average of No Rewarded Trials:",
result["latency_avg_no_reward"])

    except FileNotFoundError:
        # The except block should also be indented correctly
        print(f"File not found at {input_file_path}. Please ensure the
file exists and the path is correct.")

```

C) Probe Sessions: Variation

```

def get_initial_value():
    while True:
        try:
            initial_value = float(input("Enter the initial value: "))
            return initial_value
        except ValueError:
            print("Invalid input. Please enter a numerical value.")

def get_lever_values():
    while True:
        lever_a = input("Enter the value for Lever A (-1 or 1): ")
        lever_b = input("Enter the value for Lever B (-1 or 1): ")

        if lever_a in ['-1', '1'] and lever_b in ['-1', '1']:
            lever_a = int(lever_a)
            lever_b = int(lever_b)
            if lever_a == -1 and lever_b == 1 or lever_a == 1 and
lever_b == -1:

```

```

        return lever_a, lever_b
    else:
        print("Invalid lever values. Lever A and Lever B must
be opposites.")
    else:
        print("Invalid input. Lever A and Lever B must be either -
1 or 1.")

def read_file(filename):
    with open(filename, 'r') as file:
        lines = file.readlines()
    return lines

def compute_cumulative_preference(lines, lever_a, lever_b,
initial_value):
    cumulative_value = initial_value
    cumulative_values = []

    for line in lines:
        if 'Lever A' in line:
            cumulative_value += lever_a
        elif 'Lever B' in line:
            cumulative_value += lever_b

        cumulative_values.append(int(cumulative_value)) # Convert to
int to remove decimal points

    return cumulative_values

def main():
    filename = 'session.txt' # Specify your file name here
    initial_value = get_initial_value()
    lever_a, lever_b = get_lever_values()
    lines = read_file(filename)
    cumulative_values = compute_cumulative_preference(lines, lever_a,
lever_b, initial_value)

    print("Cumulative values evolution:")
    for value in cumulative_values:
        print(value)

if __name__ == "__main__":
    main()

```

D) Probe Sessions: Shifting

```

def process_file(file_path):
    with open(file_path, 'r') as file:
        lines = file.readlines()

    lever_order = []
    shifts = 0
    prev_lever = None

    for line in lines:
        if "Response: Lever" in line:
            lever = line.split("Response: Lever ")[1][0]

            if lever == "A":
                lever_order.append(1)  # Lever associated with
REWARD(1)
            elif lever == "B":
                lever_order.append(2)  # Lever associated with
REWARD(3)

            if prev_lever is not None and lever != prev_lever:
                shifts += 1

            prev_lever = lever

        elif "Omission" in line:
            lever_order.append(0)  # Omission trial, no lever pressed

    return lever_order, shifts

if __name__ == "__main__":
    input_file_path = "session.txt"  # Replace with the actual path to
your input file
    output_file_path = "order_of_levers.txt"  # Change the output file
name if needed

    lever_order, shifts = process_file(input_file_path)

    with open(output_file_path, 'w') as output_file:
        for lever in lever_order:
            output_file.write(str(lever) + '\n')

    print("Shifts between levers:", shifts)

```

E.1) Probe Sessions: Average Speed and Distance

```
def read_coordinates_from_file(file_path):
    with open(file_path, 'r') as file:
        # Read all lines from the file
        lines = file.readlines()
        # Create an empty list to store inner coordinates
        inner_coordinates = []
        # Iterate through each line in the file
        for line in lines:
            # Remove parentheses and split the line into x and y
            values = line.replace('(', ' ').replace(')', ' ').replace('_', ',').split(',')

            # Handle different coordinate formats
            if len(values) == 4:
                # (x, xdecimal_part, y, ydecimal_part) format
                x = int(values[0])
                decimal_x = int(values[1])
                y = int(values[2])
                decimal_y = int(values[3])
                inner_coordinates.append((x, decimal_x, y, decimal_y))
            elif len(values) == 3:
                # Check if the second value exceeds the range for X
                if int(values[1]) > 500:
                    # (x, xdecimal_part, y) format
                    x = int(values[0])
                    decimal_x = int(values[1])
                    y = int(values[2])
                    inner_coordinates.append((x, decimal_x, y, 0))
                else:
                    # (x, y, ydecimal_part) format
                    x = int(values[0])
                    y = int(values[1])
                    decimal_y = int(values[2])
                    inner_coordinates.append((x, 0, y, decimal_y))

            # Return the list of inner coordinates
            return inner_coordinates

# Specify the file
file_path = 'bonsai_file.txt'

# Read the coordinates using the provided function
coordinates = read_coordinates_from_file(file_path)

# Create a new document with separate columns for x and y coordinates
with open('formatted_coordinates.txt', 'w') as new_file:
    # Iterate through each set of coordinates
    for x, decimal_x, y, decimal_y in coordinates:
        # Write the formatted coordinates to the new file
        new_file.write(f'{x}.{decimal_x:04d}{y}.{decimal_y:04d}\n')
```

E.2) Probe Sessions: Average Speed and Distance

```

% Input files can't have headers!

clear all

resol = 15; % frames per second

[file_name, filepath1] = uigetfile({'*.txt'}, 'Select TXT data
file...');
    cd(filepath1);
    data = load(file_name);
    data_length = length(data);

% Calculations
diff_xy = diff(data).^2;
diff_xy_sum = sum(diff_xy,2);
sequentialDistances = sqrt(sum(diff_xy,2));

total_distance = sum(sequentialDistances);
    resol_sec = data_length/resol;
mean_velocity = total_distance/resol_sec;

disp('Total distance (pixels): ')
    total_distance
disp('Mean velocity (pixel/sec): ')
    mean_velocity

% Print tracking vector
hold on
    plot(data(:,1),data(:,2)); title('Tracking vector');
ylabel('posY(px)'); xlabel('posX(px)')
hold off

```

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