Universidade do Minho
Escola de Medicina

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Chronic pain impact on decision-making – the role of the mesocorticolimbic system

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Chronic pain impact on decision-making – the role of the mesocorticolimbic system

Abstract

Chronic pain affects 20% of western countries' adults. Its etiology/pathophysiology is not completely understood and treatments are frequently ineffective. Chronic pain patients commonly develop emotional and cognitive comorbidities as anxiety, depression and executive dysfunction. However, chronic pain impact on decision-making remains relatively unexplored. The mesocorticolimbic dopaminergic pathway, classically associated with its role in reward/aversion, motivation and decision-making, has emerged as a fundamental player in chronic pain maintenance and, more interestingly, in chronic pain emergence.

In this work, we studied the impact of chronic pain on impulsive and goal-directed/habit-based decision-making and the underlying role of the mesocorticolimbic system. For that, adult male Wistar-Han rats were used. One month after a neuropathic lesion – spared nerve injury (SNI) – on the left (SNI-L) or right (SNI-R) hind paw, animals performed two independent studies: (i) the variable delay-to-signal (VDS) to evaluate impulsivity; and (ii) an operant conditioning task to study habit-based to goal-directed shifts. In general, SNI animals presented increased intolerance to longer delays and higher reliance on habits than Sham. These impairments were mild and dependent on lesion-side and, in the case of impulsive behavior, on trait impulsivity.

Depletion of dopaminergic neurons projecting to the left or right Nucleus Accumbens (NAc) mirrored the effect of the SNI lesions. More specifically, lesions on the right NAc affected goal-directed to habit-based transition, while left-sided lesions were associated with increased delay intolerance in the VDS. Subsequently, we measured DA levels by high performance liquid chromatography (HPLC) in the NAc and in other mesocorticolimbic regions (striatum, prefrontal cortex, orbitofrontal cortex and amygdala) to evaluate SNI impact. Surprisingly, the neuropathic lesion had no major impact on DA levels.

In conclusion, chronic pain impact in executive function is broader than previously thought affecting levels of inhibitory control and adaptive mechanisms of decision-making in addition to working memory and attention, which have been thoroughly investigated in this context. Such impacts are contingent not only with the characteristics of the lesion but also with individual traits. The NAc appears to be pivotal in the relation between chronic pain and the associated behavioral outcomes, which perfectly aligns with previous studies in experimental and clinical chronic pain as well as with its well-stablished role in decision-making. On-task recordings of NAc's activity in chronic pain conditions will be essential to clarify this hypothesis.

Keywords: Chronic pain, Cognition, Decision-making, Dopamine, Laterality

O impacto da dor crónica na tomada de decisão – o papel da via mesocorticolímbica

Resumo

A dor crónica (DC) afeta 20% dos adultos dos países desenvolvidos. Os doentes com DC desenvolvem regularmente comorbidades emocionais e cognitivas como ansiedade, depressão e disfunção executiva. No entanto, pouco se sabe sobre o impacto da DC na tomada de decisão. A via mesocorticolímbica, que tem um papel fundamental na recompensa/aversão, motivação e tomada de decisão, surgiu recentemente como uma peça fundamental na persistência e no surgimento da DC.

Neste trabalho estudámos o impacto da DC na tomada de decisão impulsiva e direcionada/habitual e o papel da via mesocorticolímbica nestas alterações. Para isso, foram utilizados ratos Wistar-Han machos adultos. Um mês após a lesão neuropática – *spared nerve injury* (SNI) – na pata esquerda (SNI-L) ou direita (SNI-R), os animais realizaram dois estudos independentes: (i) o teste *variable delay-to-signal*, para avaliar impulsividade; e (ii) um condicionamento operante para estudar decisões baseadas em objetivos/decisões em hábito. Em geral, os animais SNI apresentaram uma maior intolerância a tempos de espera longos e uma maior prevalência de decisões habituais que os Sham. Estes défices eram ligeiros e dependentes do lado da lesão e, no caso do comportamento impulsivo, da impulsividade inata. A depleção de neurónios dopaminérgicos que projetam para o núcleo *accumbens* (NAc) esquerdo e direito teve um impacto semelhante aos efeitos da lesão SNI. Mais especificamente, lesões no NAc direito afetaram a mudança de decisões dirigidas para habituais, enquanto que lesões no NAc esquerdo induziram, no VDS, uma maior intolerância aos tempos de espera longos. Subsequentemente, quantificámos os níveis de DA por cromatografia líquida de alta eficiência (HPLC) no NAc e em outras regiões mesocorticolímbicas (estriado, córtex prefrontal, córtex orbitofrontal e amígdala), de modo a avaliar o impacto do SNI. Surpreendentemente, a lesão neuropática não impactou os níveis de DA.

Em conclusão, o impacto da DC na função executiva é mais extenso do que inicialmente previsto, afetando os níveis de controlo inibitório e os mecanismos adaptativos de tomada de decisão, para além da memória de trabalho e atenção, domínios largamente investigados neste contexto. Tais impactos são contingentes, não só com as características da lesão, mas também com traços inatos. O Nac aparenta ser essencial na relação entre a DC e as suas consequências comportamentais, o que alinha perfeitamente com estudos de DC experimental e clínica, assim como com o seu bem estabelecido papel na tomada de decisão. Gravações da atividade do NAc durante a realização das tarefas, em condições de DC, serão essenciais para clarificar esta hipótese.

Palavras-chave: Cognição, Dopamina, Dor crónica, Lateralidade, Tomada de decisão

Index

List of abbreviations

#

2/3-Ch: Two/Three-chambers sociability test

$3s_f$: Final 3 seconds delay trials

$3s_i$: Initial 3 seconds delay trials

5-csrtt: 5-choice serial reaction time task

5-HIAA: 5-Hydroxyindoleacetic acid

5-HT: Serotonin

6/12s: 6/12 seconds delay trials

6-OHDA: 6-Hydroxydopamine

8-ARM: 8-arms radial maze

A

A: Adult

A-O: Action-outcome

ACC: Anterior cingulate cortex

ADHD: Attention deficit and hyperactive disorder

AMY: Amygdala

AS: Acidic saline

ASST: Attentional-set shifting task

B

BBE: Black box emergence task

BIS: Barret impulsivity scale

C

CCI: Chronic constriction injury

CD: Contingency degradation

CFA: Complete Freud's adjuvant

CFC: Contextual fear conditioning

CP: Chronic pain

CRF: Continuous reinforcement schedule

D

D/L: Dark/light test

d: days

D(ev): Devalue

DA: Dopamine

DAB: Diaminobenzidine tetrahydrochloride

DAT: Dopamine transporter

DBS: Deep brain stimulation

DD: Delay discounting

ddC: Dideoxynucleosides zalcitabine

DI: Discrimination index

DLS: Dorsolateral striatum

DMS: Dorsomedial striatum

DnMP: Delayed nonmatching-to-position task

DOPAC- 3,4-Dihydroxyphenylacetic acid

DR: Dorsal raphe nucleus

DSS: Dextran sulfate sodium

E

EEG: Electroencephalography

EMG: Electromyography

EPM: Elevated plus maze

EZM: Elevated zero maze

F

F: Female

FCS: Fetal calf serum

FR: Fixed ratio

FST: Forced swimming test

G

GAD: Glutamate decarboxylase

GPCRs: G protein-coupled receptors

H

HB: Holeboard test

HPA: Hypothalamic–pituitary–adrenal

HPLC- High performance liquid chromatography

HPLC-ED: High performance liquid chromatography with an electrochemical detector

HVA- Homovanillic acid

I

IGT: Iowa gambling task

ION: Infraorbital nerve chronic constriction injury

i.p.: Intraperitoneal

K

K/C: Kaolin/Carrageenan

L

L: Left

LC: Locus coeruleus

LDH: Lumbar disc herniation

LE: Long-Evans

LI: Laterality index

M

M: Male

MA: Monoarthritis

MAP2: Microtubule-associated protein 2

MB: Marble burying

MCL: Mesocorticolimbic system

MIA: Monosodium iodoacetate

mPFC: medial prefrontal cortex

MWM: Morris water maze

N

NA: Noradrenaline

NAc: Nucleus accumbens

NOR: Novel object recognition

NSF: Novelty suppressed feeding

NTG: Nitroglycerin

O

O: Old

OA: Osteoarthritis

OB: Operant box

OCD: Obsessive-compulsive disorder

OF: Open-field

OFC: Orbitofrontal cortex

OPR: Object place recognition

OPS- Object pattern separation

P

PaTRaT: Pawedness trait test

PBS: Phosphate buffer saline

PD: Parkinson's disease

PET: Positron emission tomography

PFA: Paraformaldehyde

PFC: Prefrontal cortex

PNL: Peripheral nerve ligation

PO: Post operation

POW: Post-operative week

PR: Prematurity rate

PSNL: Partial sciatic nerve ligation

PTX: Paclitaxel

R

R: Right

RGT: Rodent gambling task

RI: Random interval

RPL: Reversal preference learning

RR: Random ratio

S

s: Seconds

S-R: Stimulus-response

SA: Swiss Albino

SBB: Spontaneous burrowing behavior

S.c.: Subcutaneous

SC: Sucrose/Sugared food consumption

SCI: Spinal cord injury

SD: Sprague Dawley

SI: Social interaction

SN: Substantia nigra

SNC: Sciatic nerve crush

SNI: Spared nerve injury

SNI-L: Spared nerve injury in the left hind paw

SNI-R: Spared nerve injury in the right hind paw

SNL: Spared nerve ligation

SNRIs: Serotonin-noradrenalinereuptake inhibitors

SNT: Spinal nerve transection

SPB: Shock probe burying task

SPF: Specific pathogen free

SPT: Sucrose/Saccharine preference test

SRT – Social recognition test

SSRIs: Selective serotonin reuptake inhibitors

SST: Stop-signal task

ST: Splash test

STR: Striatum

STZ: Streptozotocin

T

TCOT: Tube co-occupancy test

TH: Tyrosine Hydroxylase

TIC: Trigeminal inflammatory compression

TNBS: Trinitrobenzene Sulfonic acid

TNT – Tibial nerve transection

TST: Tail suspension test

U

UPPS: Urgency, premeditation, perseverance and sensation-seeking

USVs: Ultrasonic vocalizations

V

V(al): Value

VDS: Variable delay-to-signal test

VF: Von Frey

VM: Ventral midbrain

VMT2: Vesicular monoamine transporter 2

VR – Variable ratio

VTA: Ventral tegmental area

VZV: Varicella zoster virus

W

W: Weeks

WH: Wistar-Han

WKY: Wistar Kyoto

WM: Working memory

Y

Y: Young

YA: Young adult

Z

ZDF – Zucker diabetic fatty

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FOREWORD

Foreword

Everyday each individual performs an unconceivable amount of conscious and unconscious decisions. Based on our memories and previous experiences, we make predictions and choose what we believe to be the best options. These options arise from a careful balance between reward value, negative or harmful consequences and time as well as effort needed (Fellows, 2016). Decision-making is a very important feature of human behavior and impairments on this function are related with pathologies such as gambling, addiction and obsessive-compulsive disorder (OCD). The process of decision-making, as well as the predictive scenarios associated with it, takes place essentially in frontal and limbic areas as the ventromedial prefrontal cortex (PFC) and the striatum (STR). Since these areas constitute part of the mesocorticolimbic system and taking into consideration its role on reward, learning and motivation, dopamine (DA), and to a lesser extent the other monoamines, has been considered a key molecular player in decision-making.

Imaging studies have systematically shown grey matter volume alterations in cortical and striatal areas of CP patients (see for instance - Barad et al., 2014; Krause et al., 2016; Niddam et al., 2017; Obermann et al., 2013; Schmidt-Wilcke et al., 2007), which appear to be reversed upon analgesic treatment (Seminowicz et al., 2011). Furthermore, functional synchrony between Nucleus Accumbens (NAc) and PFC predicts the transition from acute to chronic low back pain (Baliki et al., 2012). CP patients also present reduced levels of DA in several areas of the brain (Jääskeläinen et al., 2001; Wood et al., 2007a) and DA release in the basal ganglia, after a painful stimulus, is impaired in fibromyalgia patients (Wood et al., 2007b). However, very few have studied the impact of CP on decision-making.

This thesis assembles a set of studies aiming to clarify some of the issues raised above, namely the impact of CP on decision-making and the involvement of the mesocorticolimbic dopaminergic pathway on CP and decision-making. In chapter 1.1 a detailed review on CP-associated behavioral alterations in rodent models is presented with a detailed focus on executive function, namely on decision-making, attention and cognitive flexibility, which have been highly overlooked in the literature. Following this, we studied the impact of CP on goal-directed/habit-based and impulsive decision-making (Chapters 1.2 and 1.3, respectively). In the first work, we observed that rats with a chronic neuropathic lesion (spared nerve injury- SNI) presented impairments in reward devaluation and contingency degradation. In the second, published in Neurobiology of Pain, we showed that SNI rats were more intolerant to longer delays than control animals (Cunha et al., 2020a). Interestingly, these effects on habit and impulsive behavior were mostly observed in rats with left-sided SNI and, for the later, in animals with high trait impulsivity. To test if these lateralized effects could be related with behavioral lateralization – paw preference and dexterity

(pawedness) – we developed the Pawedness trait test (PaTRaT), published in Frontiers in Behavioral Neuroscience (Appendix A) (Cunha & Esteves et al., 2017). We showed that pawedness was not associated with animals' cognitive performance, particularly impulsivity and spatial and working memory (Appendix A). Also, SNI/CP had no impact on pawedness (Appendix B).

In section 2, we analyzed the potential role of dopamine as mediator between CP and executive dysfunction. Firstly, we reviewed the literature describing DA's role across executive function dimensions of attention, working memory and decision-making, as well as DA's role in CP and their potential relation (Chapter 2.1). With this in mind, we studied the lateralized impact of NAc's DA depletion (6-OHDA lesions) in the behaviors studied in chapters 1.2 and 1.3 (Chapter 2.2). In this study, published in Experimental Neurology, we showed that while left-sided lesions lead to increased delay intolerance, right-sided lesions induced impairments in the shift from goal-directed to habit-based decisions (Cunha et al., 2020b). Also, in a pilot study (Appendix C), we tested if lateralized antagonism of dopaminergic neurons that project to the NAC lead to the same effect on impulsive decision-making. A side-independent increase in delay tolerance upon D1 and D2 antagonism was observed. Trying to understand if alterations in DA or other monoamines could explain the deficits observed in CP rodent models, we evaluated mesocorticolimbic DA levels in CP conditions (Chapter 2.3). No major alterations were observed.

In the final chapter (Chapter 3), the above-mentioned results were discussed in an integrated manner at the light of the current literature.

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CHAPTER 1

Decision-making impairments in rodent models of chronic pain

CHAPTER 1.1

Chronic pain impact on rodents' behavioral repertoire

Submitted manuscript

Chronic pain impact on rodents' behavioral repertoire

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Abstract

Rodent models have been fundamental to understand chronic pain (CP) pathophysiology and to test for potential treatments. Pain assessment in CP models is most frequently based on the evaluation of allodynia or hyperalgesia. However, these correspond only to a part of CP-related problems which include ongoing pain, depression, anxiety, disrupted sleep and attentional deficits. A growing number of preclinical studies have been assessing these manifestations in CP rodent models. We reviewed and systematized this information by behavioral domain. Observational studies in ethologically relevant conditions, paradigms of anxiety- and depressive-like behavior as well as of memory and executive function were selected. A considerable number of studies reported deficits similar to those observed in CP patients. These behavioral alterations are informative regarding ongoing maladaptive plasticity in multiple brain regions and its use as pain proxies has the potential to greatly improve the predictive value of CP models. However, the inclusion of female and/or older rodents is rare which is in clear dissonance with the clinical representation of CP.

1. Introduction

Rodents are the most frequently used species in neuroscience (Bovenkerk and Kaldewaij, 2015; Ellenbroek and Youn, 2016; Roelfsema and Treue, 2014). General advantages include, among others, short reproductive cycles, docile behavior, and low husbandry requirements. The use of rodents in pain research, particularly mice and rats, increased substantially in the past decades when compared to other models – 1963-2007 (Mogil, 2009). Such propelled fundamental advances in pain neurobiology that were otherwise impossible (Mogil, 2009; Mogil et al., 2010a). The fundaments of pain circuitry were defined, including the existence of descending modulatory pathways and the involvement of higher brain centers, as well as the occurrence of plasticity and the definition of its major molecular players – e.g. (Almeida et al., 2006; Apkarian et al., 2009; Das, 2015; Hore and Denk, 2019; Sandkuhler, 2009). Despite these advances, translation in pain research is considered limited. Reasons are multifactorial and include underpowered studies, poor experimental designs and all sort of biases (sex is amongst the most debated) – see for instance (Andrews et al., 2016; Clark, 2016; King and Porreca, 2014; Mao, 2009; Mogil, 2009; Mogil and Chanda, 2005; Mogil et al., 2010a; Rice et al., 2008; Scholz and Yaksh, 2010). Specifically in chronic pain (CP) models (see table I for a detailed description), pain assessment is considered a major limitation –for detailed reviews on nociception tests see (Barrot, 2012; Cobos and Portillo-Salido, 2013; Deuis et al., 2017; Tappe-Theodor et al., 2019; Tappe-Theodor and Kuner, 2014; Turner et al., 2019). In general, pain assessment relies on sensorymotor responses elicited upon the application of nociceptive stimuli – reaction latencies, reaction thresholds or nocifensive behaviors (frequency or duration). However, these behaviors do not necessarily reflect the amount of pain perceived. More importantly, in clinical painful conditions, evoked pain represents only a part of the problem (e.g. Pfau et al., 2012). Many pain research laboratories have therefore broadened the behavioral characterization of CP models beyond the traditional tests – see table II for an overview. Such included spontaneous/voluntary behaviors like burrowing (e.g. Rutten et al., 2018; Rutten et al., 2014b; Shepherd et al., 2018), voluntary wheel running [(e.g. Cobos et al., 2012; Grace et al., 2017; Grace et al., 2014; Kandasamy et al., 2017; Pitzer et al., 2016b; Whitehead et al., 2017) – cf. (Mogil et al., 2010b; Pitzer et al., 2016a; Sheahan et al., 2017)], – weight bearing/gait [(e.g. Lolignier et al., 2011; Robinson et al., 2012; Tetreault et al., 2011) – cf. (Mogil et al., 2010b)] – and facial expressions/grimace scale [(e.g. Akintola et al., 2017; Klune et al., 2019; Langford et al., 2010; Leung et al., 2019; Nagakura et al., 2019; Sperry et al., 2018) – cf. (George et al., 2019)] – as well as abnormal postures, paw flinches and ultrasonic vocalizations (USVs) (e.g. Attal et al., 1990; Guimaraes et al., 2019; Kawasaki et al., 2008; Kurejova et al., 2010; Lee et al., 2012) (section 2.). In addition, paradigms of anxiety- and depressive-

like behaviors (section 3.) as well as of different aspects of memory and executive function (section 4.) have been increasingly used – see for review (Leite-Almeida et al., 2013b; Leite-Almeida et al., 2015; Liu and Chen, 2014; Moriarty et al., 2011; Yalcin et al., 2014) – and greatly contributed to reinforce face, construct and (eventually) predictive validity of CP models. These behavioral alterations reflect central maladaptive plasticity and might provide fundamental information to track disease progression as well as the effectiveness of analgesic therapy.

In the present review we systematize and discuss a comprehensive collection of experimental studies published until December 2019 on CP impact across the above-mentioned behavioral domains.

2. Spontaneous behavior

CP can have a substantial impact on life quality (Andrew et al., 2014; Breivik et al., 2006; Duenas et al., 2016; Mathias et al., 2018; Reid et al., 2011). Even though frequently underappreciated, CP patients rate difficulties in daily activities (household, physical, etc), social relations with family and friends, and sleep disturbances as highly important aspects affected by their condition (Turk et al., 2008). While parallels with animal models are difficult to establish, evaluation of rodents' impairments in equivalent dimensions has been increasingly used (Table II, Figure 1). Simple procedures, such as home-cage monitoring, allow to understand how rodents' daily activity (movement, rest, food and water intake) is affected under CP conditions. Furthermore, motor activity patterns can provide indirect information on the impact of this condition in sleep. A number of studies (see section 3.1. and Table III) have also used electroencephalography and electromyography (EEG/EMG) for direct quantification. Likewise, burrowing and digging behaviors have been assessed in CP models, particularly in the spontaneous burrowing behavior (SBB) test, in which the amount of gravel (or other substrate) displaced by the animal from a tube is quantified. SBB is considered to be associated with nest maintenance and to reflect well-being (Deacon, 2006a; Deacon et al., 2001). Finally, rodents also display social interactions (e.g. juvenile play, sexual-related and maternal behavior), which can be affected by CP. Paradigms of social behavior such as social interaction (SI) (File and Hyde, 1978) and the two/three chambers sociability test (2/3-Ch) (Moy et al., 2004; Nadler et al., 2004), are used to test rodents' interaction with familiar or unfamiliar conspecifics. USVs emission during social encounters or in resting conditions can also provide important information on rodents' well-being.

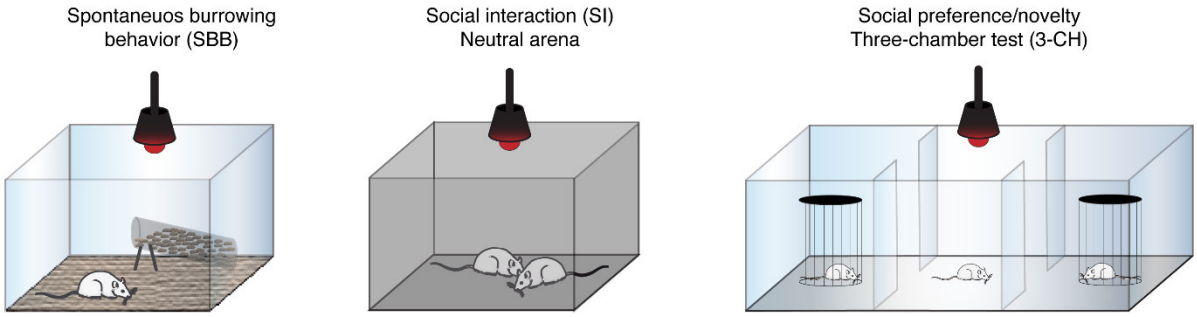

Figure 1. Ethologically relevant paradigms. A number of paradigms have been devised to assess behaviors that rodents manifest ubiquitously and voluntarily like burrowing and social interactivity. These activities are thought to reflect the general well-being of the animal.

2.1. Activity

In the last years there has been an emergence of automatized home-cages for rodent studies but continuous monitoring of home cage activity in CP models is still uncommon in the literature (Table III). Four studies reported no activity alterations in CP conditions (Parent et al., 2012; Pitzer et al., 2016a; Racz et al., 2015; Urban et al., 2011), while one reported decreased activity (Inglis et al., 2008). Also, using actigraphy, no alterations were observed in motor activity patterns in animals with chronic constriction injury (CCI) (Xu et al., 2018); interestingly, this study demonstrated that animals presented lower thresholds to innocuous mechanical stimulation (allodynia) when food was exclusively available during the resting period (day), suggesting that the disruption of resting/activity patterns can aggravate pain in rodents. On the other hand, the impact of pain on motor activity can be affected by housing conditions. Pitzer and colleagues (2016a) reported a decrease in voluntary wheel running in animals with spared nerve injury (SNI) who were group-housed, but not in isolated animals (Pitzer et al., 2016a), which may reflect isolation-induced stress. It is important to emphasize that activity and particularly voluntary wheel running, has been associated with better outcomes in CP models (Benson et al., 2015; Grace et al., 2016; Kami et al., 2018; Lima et al., 2017)).

Sleep seems not to be affected in neuropathy models (CCI) (Kontinen et al., 2003; Tokunaga et al., 2007) although, in chronic inflammatory pain models, decreased sleep efficacy, decreased percentage of slow wave and decreased paradoxical sleep were reported (Roizenblatt et al., 2010; Silva et al., 2008; Silva et al., 2011). Interestingly, Tokunaga and colleagues (2007), who found no alterations in sleep when CCI animals were in regular bedding (sawdust), reported increased sleep latency and time awake when sandpaper (aversive condition) was used as a bedding material (Tokunaga et al., 2007). Also, CCI rats with persistent or transient social impairments present impairments in sleep, which are not present in

CCI animals without social deficits (Monassi et al., 2003). Finally, Silva and colleagues (2011) observed a decrease in sleep efficacy and slow-wave sleep in the dark period that was more pronounced in osteoarthritis (OA) males than females, suggesting that sex might have a relevant role (Silva et al., 2011).

2.2. Burrowing

The SBB takes advantage of rodents' innate drive to burrow and can be used in multiple experimental conditions as a proxy of "well-being" (Deacon, 2006a, 2009; Jirkof, 2014). Decreased burrowing behavior has been systematically observed in multiple rat models of CP (Andrews et al., 2012; Guimaraes et al., 2019; Huang et al., 2013; Lau et al., 2013; Muralidharan et al., 2016; Rutten et al., 2018; Rutten et al., 2014a; Shepherd et al., 2018; Wodarski et al., 2016) up to 11 weeks after CP onset (Andrews et al., 2012). In mice, decreased burrowing has been observed in SNI but not in inflammatory pain (complete Freund's adjuvant - CFA) or migraine (nitroglycerin - NTG) conditions (Shepherd et al., 2018). Also, in a cross-center study using a unilateral intraplantar CFA injection, a decrease in burrowing was observed up to 10 days (peaking at 24h) although a high interlaboratory variability was reported (Wodarski et al., 2016). This consistent decrease in burrowing under CP conditions, aligned with its return to normal levels after analgesic treatment, has propelled the use of SBB as a pain-related readout. Interestingly, a number of studies failed to show a correlation between burrow and allodynia, suggesting that these manifestations reflect different dimensions (Lau et al., 2013; Muralidharan et al., 2016; Rutten et al., 2018). Indeed, it has been argued that burrowing levels are associated with anxiety-like behaviors (Jirkof, 2014) and, in this regard, the observed decrease in burrowing after CP induction is in general alignment with the results observed in this dimension in CP conditions (see section 3.1.; Table IV).

2.3. Ultrasonic vocalizations

Recording and analysis of USVs have been increasingly used in different experimental settings – see for instance (Burke et al., 2017; Chabout et al., 2012; Faure et al., 2017; Wright et al., 2010); see also for review (Brudzynski, 2013; Heckman et al., 2016; Johnson et al., 2015; Kisko et al., 2017; Simola and Granon, 2019). It is however controversial if CP affects USVs and in which conditions. An increase of spontaneous 22 KHz vocalizations (associated with aversive contexts; references above) and a decrease of 50 KHz vocalizations (associated with pleasant contexts; references above) was reported in rats with neuropathic pain (CCI; (Burgdorf et al., 2019; Ghoreishi-Haack et al., 2018)). However, other authors reported that spontaneous vocalizations are not detected in CFA- and Streptozotocin (STZ)-injected rats (Jourdan et al., 2002). Similarly, upon noxious stimulation both an increase (Burgdorf et al., 2019; Thompson et al., 2018a) or absence of vocalizations (Wallace et al., 2005) were reported. In social

conditions, an increase in 22 KHz vocalizations was observed in CP rats during non-agonistic interactions with conspecifics (Calvino et al., 1996), though other study found no difference in a similar setting (Jourdan et al., 2002). In mice, the only study on the matter reports results contrary to the studies in rats, presenting increased 50 KHz (and 37 KHz) USVs (Kurejova et al., 2010).

2.4. Social behavior

Paradigms of social behavior, particularly the SI, have been increasingly applied in CP models with several studies showing decreased sociability (Aissouni et al., 2017; Calvino et al., 1996; Gregoire et al., 2014; Hisaoka-Nakashima et al., 2019; Parent et al., 2012; Shang et al., 2015) and several others failing to observe this phenotype (Benbouzid et al., 2008; Gregoire et al., 2012; Jourdan et al., 2002; Liu et al., 2015b; Sheahan et al., 2017). No obvious experimental differences between studies namely species, strain, age, pain model or pain duration satisfactorily explains such discrepancy. Monassi and colleagues (2003) observed that CP affected social dominance but only in a subgroup of the animals (CCI model) (Monassi et al., 2003) indicating the occurrence of intergroup variability. Also, it has been suggested that decreased SI in rodents reflects anxiety- and/or depressive-like behaviors (File, 1980; File and Hyde, 1978; File and Seth, 2003), and indeed these phenotypes frequently manifest in CP models (see section 3. and Table IV). Interestingly, propinquity, i.e. appetence to maintain close proximity, which is normally high in dyads of familiar mice, is increased in unfamiliar dyads when both subjects are in pain (Tansley et al., 2019).

3. Emotional behavior

Depression is the most prevalent CP comorbidity, with afflicted patients presenting a 3-5 fold increase in the risk of developing depression in comparison with the general population (Bair et al., 2003). Not surprisingly, the majority of preclinical studies on CP comorbidities focuses on mood and related dimensions (Table IV). Since Hall's pioneer work in the early 1930's on rats' emotional behavior in an open-field (OF) (Hall, 1934a, b) that several other paradigms were designed to evaluate rodents' anxiety-like behavior (Table II; Figure 2). The most common are exploration-based paradigms like the OF, elevated-plus/zero maze (EPM/EZM; (Pellow et al., 1985)) and the dark/light (D/L; (Crawley, 1981)). In these paradigms, a conflict is established between the necessity to feel protected (OF – close to the wall/thigmotaxis; EPM – closed arms; D/L – enclosed dark area) and the drive to explore. Anxiety-like behavior manifests in an increased amount of time/distance travelled in the safe areas of the apparatus. Similarly, hyponeophagia – feeding inhibition in a novel environment – is an interesting proxy of anxiety, used in the novelty suppressed feeding (NSF) test (Soubrie et al., 1975). The marble burying (MB) test

(Broekkamp et al., 1986) is a paradigm based on defensive burying of aversive stimuli such as toxic food or dead conspecifics (Pinel and Treit, 1978), in which the burial of marbles is evaluated. It has been used as an anxiety paradigm, even though some argue that it is more suitable to assess obsessive-compulsive related behaviors (Harro, 2018). Interaction-based paradigms, namely SI, have increasingly been used in CP models (see Table III; section 2.4.); these behavioral displays have also been suggested to reflect anxiety-like behavior (File and Hyde, 1978). Finally, conditioned anxiety paradigms like the Geller–Seifter test, Vogel conflict test and conditioned fear, for instance, have almost no expression in preclinical pain research. For authoritative reviews on the topic consult (Cryan and Holmes, 2005; Cryan and Sweeney, 2011; Sousa et al., 2006).

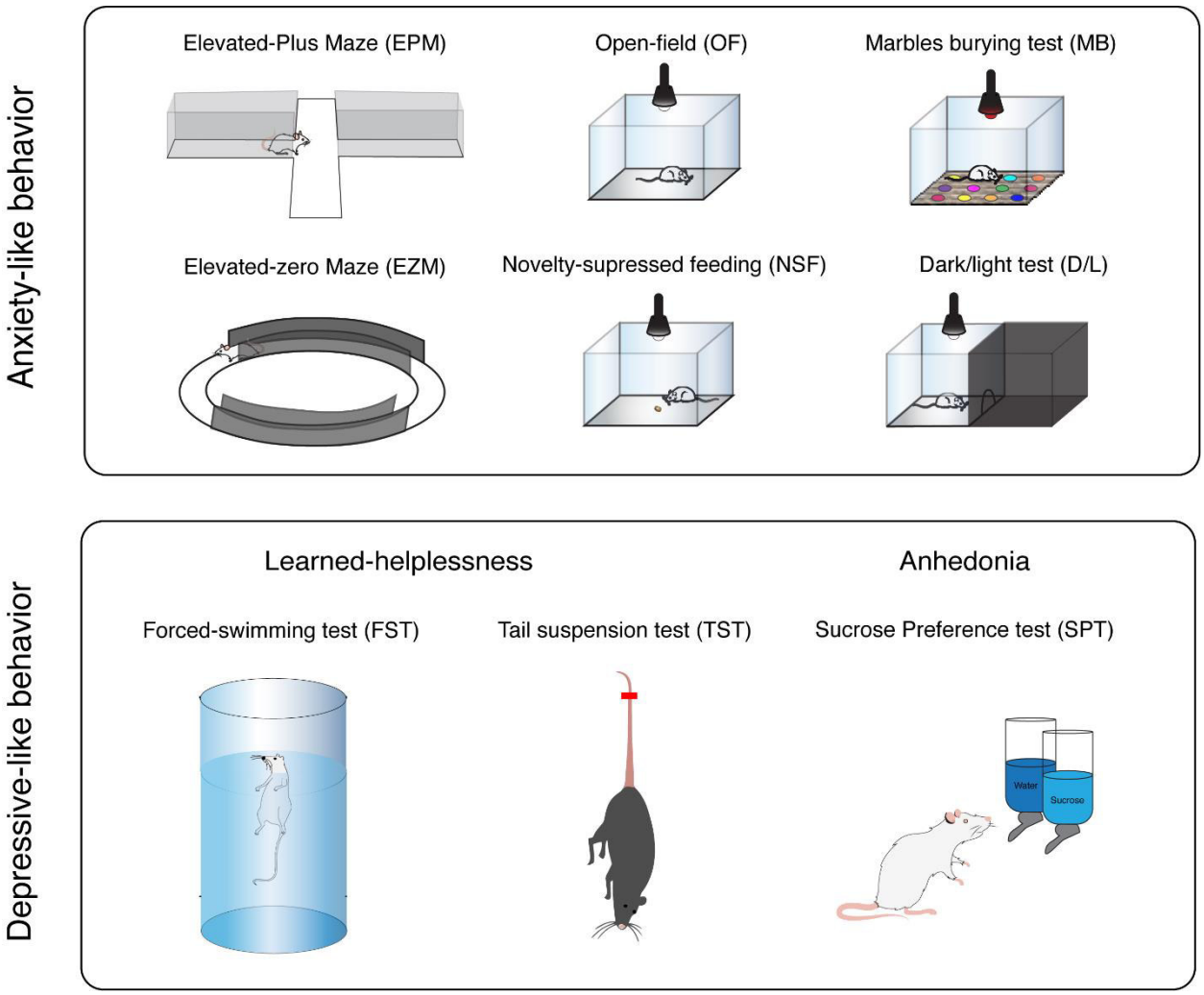

Figure 2. Main rodent paradigms of emotional behavioral paradigms. Exploration based as the open-field (OF), elevated-plus maze (EPM) elevated-zero maze (EZM) or the dark/light (D/L) but also hyponeophagia – novelty suppressed feeding (NSF) and defense-based paradigms – marble burying (MB) – have been used to assess anxiety-like behavior in rodent models of chronic pain. Depressive-like behaviors particularly learned helplessness and anhedonia have been tested in the forced swimming test (FST)/tail suspension test (TST) and sucrose preference test (SPT), respectively.

Concerning depressive-like behavior, two main dimensions are commonly assessed in rodents: helplessness and anhedonia. To investigate rodent helplessness, the forced swimming (FST) (Porsolt et al., 1977b) and tail suspension tests (TST) (Steru et al., 1985) are the most used paradigms. These tests rely on the principle that, when facing an inescapable aversive situation, rodents will initially attempt to escape and will then become immobile. Depressive-like behaviors manifests in decreased struggling and/or increased immobility periods. Anhedonia is normally assessed by the sucrose/saccharine preference test (SPT) (Willner et al., 1987), which evaluates rodents' preference for a sweet drink. Poor grooming, evaluated by the splash test (Kalueff and Tuohimaa, 2004), is also a reflection of depressive-like behavior. In this test, the time rodents spend grooming after a splash with sucrose is evaluated. Excessive grooming, similar to excessive digging, can also indicate the manifestation of compulsive behaviors.

3.1. Anxiety-like behavior

The EPM is the most commonly used paradigm to measure anxiety-like behaviors in CP models followed by the OF (Table IV). Regardless of the paradigm, the majority of studies reports an anxiety phenotype in CP conditions both in rats and mice, although some studies found no alterations (Table IV). Pain duration (Suzuki et al., 2007; Yalcin et al., 2011), lesion side (Guimaraes et al., 2019; Leite-Almeida et al., 2012) and age (Leite-Almeida et al., 2009) were found to be important for anxiety-like manifestations in CP models. Interestingly, some studies demonstrate anxiety-like behavior in specific paradigms – EPM but not D/L (Kontinen et al., 1999); EPM but not OF (Amorim et al., 2014); EZM but not OF (Shalini et al., 2017) – reinforcing the importance of the experimental conditions and the necessity to complement the analysis with other behavioral paradigms.

3.2. Depressive-like behavior

Depressive-like behaviors have also been extensively studied in CP rodent models (Table IV). Regarding learned helplessness, its manifestation is reported in the majority of studies. Again, a reduced number of studies reported no differences in this behavioral dimension (see table IV). Also, regarding anhedonia, the majority of studies demonstrate that CP animals present lower levels of sucrose/saccharin consumption (Table IV), though negative results were also reported (Andersen et al., 2009; Bravo et al., 2012; Gambeta et al., 2018; Gregoire et al., 2012; Maldonado-Bouchard et al., 2016; Racz et al., 2015; Urban et al., 2011; Wang et al., 2015d). Once more, no fundamental differences were found between studies presenting and those not presenting depressive-like manifestations. Finally, grooming in the splash test,

was used in 3 studies, using the cuff model, in mice; all reported decreased grooming in CP conditions (Barthas et al., 2015; Sellmeijer et al., 2018; Yalcin et al., 2011).

4. Cognition

The relation between CP and cognition is complex and remains less studied than depression and anxiety both in clinical and preclinical settings. Clinical studies have shown a negative impact of CP on memory (particularly working memory) and attention, but also in mental flexibility and decision-making – see for review (Bell et al., 2018; Berryman et al., 2014; Mazza et al., 2018; Moriarty et al., 2011). Cognitive function domains have been also assessed in rodent models of CP (Table II, Figure 3.). Amongst the most used paradigms to test memory in rodents is the Morris Water Maze (MWM) navigation task developed in 1981 by Richard G. Morris (Morris, 1984; Morris, 1981) as an alternative to the 8-arms radial maze (8-ARM (Olton and Samuelson, 1976)) and to the Barnes maze (Barnes, 1979). In the MWM, rodents have to find a hidden platform in a pool, based on spatial references. In alternative versions of the MWM protocol, the platform location changes after a certain number of trials allowing to evaluate short-term memory (Morris, 1984) – see for review (Schoenfeld et al., 2017; Vorhees and Williams, 2006). Other tasks, usually performed in Y- or T-mazes, take advantage of rodents' drive to explore previously unexplored areas, which result in a spontaneous alternation between the maze's arms; spontaneous alternation has been used as a proxy of short-term memory – see for review (Dudchenko, 2004; Kraeuter et al., 2019). Dimensions of both short- or long-term memory can also be evaluated in recognition tasks like the novel object recognition (NOR). This task takes advantage of rodents' preference for unexplored/new objects; after the initial period of exploration, retrieval can be evaluated 2 hours (h) (short-term) or 24h (long-term) later. Additional executive function dimensions, as inhibition/impulsivity, decision-making, attention, motivation and cognitive flexibility, have also been tested, particularly in the context of instrumental/operant conditioning paradigms (Table II) even though these tasks can be challenging to implement, requiring long periods of training and caloric restriction.

4.1. Memory

With few exceptions (Karl et al., 2019; Li et al., 2019b; Tyrtyshnaia et al., 2019), all studies assessing short-term memory recognition using the NOR conclude that CP animals spend a similar amount of time exploring novel and old objects or that its discrimination index (DI)/time exploring the new object was inferior to the controls (Table V), indicating a recall deficit. Data for object place recognition (OPR) and Y-maze are however scarcer and more conflicting (see table V for further details). SNI animals present impairments in reference short-term memory in the 8-ARM but it is not clear if this is also true in the

MWM, as impairments were only observed in right-lesioned animals (Leite-Almeida et al., 2009; Leite-Almeida et al., 2012). Concerning alternation tasks, the majority of the studies report impairments in both rats and mice across different CP models (Table V).

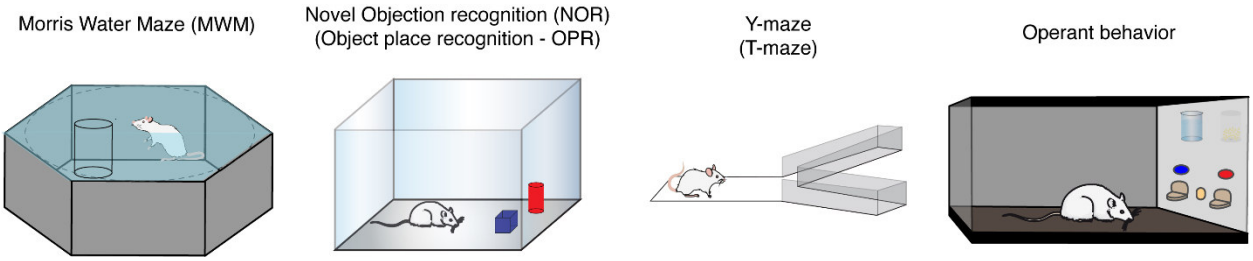

Figure 3. Common paradigms for assessment of cognitive behavior. Cognitive paradigms are diverse. In general, all require animals to perform a decision or a sequence of decisions to navigate in mazes (like the Morris water maze (MWM) and the Y-maze), explore objects (like in the novel object recognition (NOR)) or select options (as in operant behavior). In operant behavior, reinforcers are contingent with the decision. Different schedules and magnitudes of reinforcement can be combined in many different ways. For instance, to study risky decision-making, the animal is given the choice between small but certain or large but uncertain rewards.

Similar to short-term memory, in long-term memory as evaluated by NOR, CP animals have more difficulty distinguishing new and old objects, except in two studies in which differences were not found (Karl et al., 2019; Ren et al., 2011) (Table V). In rats, two studies reported a delayed manifestation in which DI significantly differs between CP and controls at 5-6 but not 2 weeks after surgery (Llorca-Torralba et al., 2019; Suto et al., 2014); in line with this observation, Ren and colleagues (2011) found no alterations 3 weeks after pain onset (Ren et al., 2011), raising the hypothesis of a delayed appearance of memory deficits in these animals. However, this was not evident in mice (Table V). Also, Liang and colleagues (2019) showed that, in a model of chemotherapy-induced neuropathic pain, impairments are present in male but not female mice (Liang et al., 2019). However, in a neuropathic model, no sex-related differences were observed (Martinez-Navarro et al., 2020). Similarly to NOR, long-term memory impairments in the MWM were reported in most studies – cf. however with (Karl et al., 2019; Leite-Almeida et al., 2009; Leite-Almeida et al., 2012; Moriarty et al., 2016). On the other hand, studies performed on the 8-ARM report no deficits on long-term memory under CP conditions (Fiore and Austin, 2018; Ren et al., 2011).

Regarding social memory and contextual fear memory, except for two works, (Palazzo et al., 2016) and (Guimaraes et al., 2019), all studies demonstrated the manifestation of deficits in CP models. Interestingly, Tajerian and colleagues (2015) reported that female, but not male, mice presented deficits

in contextual fear memory 5 weeks after a bone fracture though recovering 4 weeks later (Tajerian et al., 2015).

4.2. Decision-making

Studies on the impact of CP in decision-making and related motivation, attention and cognitive flexibility are relatively scarce in CP experimental models. One of the most studied dimensions in CP patients is attention (Moriarty et al., 2011). In rodents, the 5-choice serial reaction time task (5-csrtt) has been used to study this dimension, as well as impulsivity. In this task, performed in an operant box with 5 apertures, animals have to perform nosepokes in the illuminated aperture in order to obtain a sugared reward. The duration of the light signal is decreased across sessions thereby increasing the attentional load of the task. Using this tasks, Higgins and colleagues (2015) observed an increased number of errors (selection of incorrect apertures) indicating increased attentional deficits in CP (Higgins et al., 2015). In the delayed nonmatching-to-position task (DnMP), a working memory task with a high requirement of sustained attention, similar deficits were observed (Cain et al., 1997; Cardoso-Cruz et al., 2019; Lindner et al., 1999). Regarding risk taking and impulsive decision-making studies, increased bias to risk (Ji et al., 2010; Pais-Vieira et al., 2012; Pais-Vieira et al., 2009) as well as increased delay intolerance in the variable delay-to-signal test (Cunha et al., 2020; Leite-Almeida et al., 2012) were reported. Differences in delay intolerance were however dependent on the lesion side and on trait impulsivity prior to pain onset, which adds an additional level of complexity (Cunha et al., 2020). No differences were however observed in action impulsivity (Cunha et al., 2020; Higgins et al., 2015; Kniffin et al., 2015).

Regarding cognitive flexibility, it was shown that when compared with control rats, SNI animals presented impairments in the reversal phases of the attentional-set shifting task (ASST) but not in the intra- and extradimensional shifts (Leite-Almeida et al., 2012; Leite-Almeida et al., 2014); in other words, animals in CP conditions required more trials to recognize that a previously irrelevant stimulus became essential to retrieve a sugared reward although they performed similarly to controls if an entirely new set of cues from the same dimension (odor to odor; interdimensional shift) or even from a different dimension (odor to texture; extradimensional shift) was introduced. Such suggests that while flexibility in the ASST is impaired, CP impact on learning is relatively limited. Similarly, while a reasonable number of studies failed to observe alterations in the classical MWM, a cognitive flexibility assay in which the target platform is relocated from its previous position, revealed CP-related impairments (Leite-Almeida et al., 2009; Moriarty et al., 2016). In the same line, after extinction in a variable ratio probabilistic task, spared nerve ligation (SNL) rats preferred the familiar, although less profitable, option (Cowen et al., 2018). In contrast, SNI did not affect the mice performance of a reversal place learning (RPL) (Albuquerque et al., 2013). It

should be noted however that RPL's construct – water access location is altered after a 7 days preference learning protocol – is very different from the previous paradigms. Reliance on habit-based as opposed to goal-directed decision-making can also be indicative of reduced behavioral flexibility as they are more rigid and reflexive (Dolan and Dayan, 2013). Goal-direct strategies on the other hand are more adaptive and essential in constantly changing environments. In rodents, devaluation protocols can be used to assess habit-based to goal-direct transitions. Essentially, animals that have been trained to press a lever to obtain a reward receive an excessive amount of the same reward prior to the instrumental behavior session. Mor and colleagues (2017) demonstrated that CCI animals maintained a high level of lever pressing after contingency devaluation, but only when an acute stressor preceded the test (Mor et al., 2017). Altogether, this set of data indicates that CP is associated with cognitive rigidity and less efficient adaptation when new strategies are more advantageous. A potential problem of instrumental behavior like the one described above is that is highly dependent on animals' motivation. Surprisingly, using the progressive ratio (PR), a task in which the effort required to obtain a reward is systematically increased, only one study in mice, found deficits in motivation associated with CP (La Porta et al., 2016) – cf. (Cowen et al., 2018; Higgins et al., 2015; Okun et al., 2016). Such is in apparent conflict with other behavioral dimensions, namely those associated with depression, in which a significative number of studies reported reduced motivation to escape from stressful conditions (helplessness; Table IV) and reduced preference for palatable drinks/food (anhedonia; Table IV). However, it is important to note that animals performing PR, in opposition to the ones tested in the SPT or similar tasks, are normally under prolonged caloric restriction regimens which might act as an incentive. Nevertheless, these results exclude a potentially confounding factor in instrumental behavior.

5. Conclusion

Increasingly, studies using CP models extended their phenotypical characterization beyond sensory manifestations that have traditionally been used to assess pain. Particularly anxiety- and depressive-like behaviors but also memory deficits, are being thoroughly investigated. While these efforts might increase face, construct and (eventually) predictive validity of CP models, the characteristics of the models and experimental subjects employed in most studies are in frank dissonance with the clinical reality. For instance, epidemiological studies systematically indicate that musculoskeletal pain (e.g. low back pain, neck pain, arthritis) is the most frequent CP complaint (Elliott et al., 1999) whereas in preclinical studies neuropathic pain models clearly predominate. In addition, most of these neuropathic pain models were traumatic, while postherpetic neuralgia and diabetic peripheral neuropathy represent the majority of neuropathic CP cases (van Hecke et al., 2014; van Hecke et al., 2013). Furthermore, assessment in CP

models frequently takes place early after pain onset while in human patients, pain can last for months or years. Regarding the characteristics of the experimental subjects, only an insignificant number of studies used females despite being well-stablished that CP is more prevalent in women and that women are more prone to suffer multiple pain conditions (Breivik et al., 2006; Chenaf et al., 2018). For instance, in the case of depressive-like behaviors, more than 91% of the studies exclusively used male rodents (42/46 in anhedonia and 107/117 in learned-helplessness), although women are 1.7 times more prone to develop depression (Albert, 2015). In fact, for both dimensions, only 6 studies were conducted using both females and males (Garg et al., 2017; Liang et al., 2019; Nagakura et al., 2009; Nishinaka et al., 2015; Pitzer et al., 2019; Racz et al., 2015). This is a recurrent issue in basic research. 15 years-ago, Mogil and Chanda (2005) reported that from all animal studies published in PAIN (1996-2005), 79% exclusively used male non-human mammals (Mogil and Chanda, 2005). Fortunately, the problem has been recognized and NIH, for instance, strongly encourages the inclusion of both sexes in preclinical research (https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable). Similarly, while recurrently reported that CP is more frequent in older individuals – e.g. (Chenaf et al., 2018; Mills et al., 2019) – very few studies were performed in old animals (Albuquerque et al., 2013; D'Aniello et al., 2017; Gai et al., 2014; Kamal et al., 2000; Karl et al., 2019; Karl et al., 2017; Leite-Almeida et al., 2009; Liu et al., 2015a; Moriarty et al., 2016; Tyrtyshnaia et al., 2019). Despite these discrepancies, most studies report similar comorbidities to those observed in human patients. In nearly all studies, an increase in anxiety- and depressive-like behaviors is observed in different CP models and behavioral paradigms. The same appears to be true for memory, particularly in NOR and MWM paradigms. It is important to note that there are also studies reporting no impairments or that associate the manifestation of behavioral deficits with specific experimental conditions like age (Leite-Almeida et al., 2009), lesion location (Cunha et al., 2020; Guimaraes et al., 2019; Leite-Almeida et al., 2012; Leite-Almeida et al., 2014), time after the lesion (Llorca-Torralba et al., 2019; Sellmeijer et al., 2018; Suto et al., 2014; Suzuki et al., 2007; Yalcin et al., 2011) and housing conditions (Norman et al., 2010). Furthermore, it has been shown that even in relatively homogenous rodent populations, CP impact can differ substantially across individuals – e.g. anhedonia (Fang et al., 2018; Hakim et al., 2020; Li et al., 2018; Xie et al., 2017; Yang et al., 2019a). Keay's laboratory, for instance, observed that some neuropathic animals (CCI model) presented stable impairments in social and sleep behaviors (30%), while in others these were transient (25%) or even absent (45%) (Monassi et al., 2003); the proportion of affected animals was stable across studies and was associated with specific morphological, endocrine, inflammatory and genetic outcomes (Austin et al., 2015a; Austin et al., 2015b; Austin et al., 2010; Castorina et al., 2019; Kalman and Keay, 2014; Kilburn-

Watt et al., 2010; Mor et al., 2011; Mor et al., 2010; Mor et al., 2015; Mor and Keay, 2013). Also, we recently reported that impulsivity is affected by CP in rats that at the baseline are high- but not low-impulsive (Cunha et al., 2020) further stressing the importance of considering individual differences when assessing CP impacts on behavior. Additionally, the presence of emotional and cognitive impairments after neuropathic lesions has been observed in animals with low or absent signs of allodynia, raising the hypothesis that, though related, sensory and behavioral deficits can manifest independently (Dimitrov et al., 2014; Guimaraes et al., 2019; Liu et al., 2015b; Ren et al., 2011). It cannot be excluded, however, that these animals are in pain though not presenting allodynia. In either way, while the behavioral manifestations described throughout this review paper are fundamental aspects of CP pathophysiology and associated maladaptive central plasticity, they should not be regarded as universal pain proxies.

Table I. Rodent models of chronic pain

Model	Description	Associated CP condition	References
Neuropathic			
Chronic Constriction Injury (CCI)	Loose ligation of the sciatic nerve	Peripheral traumatic neuropathy	(Bennett and Xie, 1988)
Cuff model	Implantation of a cuff around the sciatic nerve	Peripheral traumatic neuropathy	(Benbouzid et al., 2008)
HIV gp120	Intra-peritoneal injection of ddC (dideoxycytidine) + perineural injection of HIV gp120	HIV - associated neuropathy	(Wallace et al., 2007b)
Infraorbital Nerve CCI (ION)	Loose ligation of the infraorbital nerve	Trigeminal neuralgia	(Vos et al., 1994)
Partial Sciatic Nerve Ligation (PSNL)	Ligation of approximately half of the sciatic nerve	Peripheral traumatic neuropathy	(Seltzer et al., 1990)
Paclitaxel (PTX)	Intra-peritoneal or intravenous injection of PTX	Chemotherapy-induced neuropathy	(Polomano et al., 2001)
Spinal Cord Injury (SCI)	Traumatic lesions in the spinal cord (e.g. compression, contusion)	Central neuropathic pain	(Dietz and Schwab, 2017; Nakae et al., 2011)[1]
Spared Nerve Injury (SNI)	Ligation and axotomy of 2 of the 3 terminals of sciatic nerve	Peripheral traumatic neuropathy	(Decosterd and Woolf, 2000)
Spinal Nerve Ligation (SNL)	Ligation of the L5 spinal nerve	Peripheral traumatic neuropathy	(Kim and Chung, 1992)
Spinal Nerve Transection (SNT)	Ligation and transaction of the L5 spinal nerve	Peripheral traumatic neuropathy	(Hasnie et al., 2007a)
Stavudine (d4t)	Intravenous injection of d4t	HIV - associated neuropathy	(Joseph et al., 2004)
Streptozotocin (STZ)	Intravenous or intra-peritoneal injection of STZ	Diabetic neuropathy	(Kamei et al., 1991)
Trigeminal Inflammatory Compression (TIC)	Insertion of chronic gut suture between the infraorbital nerve and the maxillary bone	Trigeminal neuralgia	(Ma et al., 2012)
Tibial Nerve Transection (TNT)	Ligation and axotomy of tibial nerve	Peripheral traumatic neuropathy	(Lee et al., 2000)
Varicella Zoster Virus (VZV)	Subcutaneous injection of VZV	Postherpetic neuralgia	(Dalziel et al., 2004)
Inflammatory			
Carrageenan	Intra-articular injection of carrageenan	Monoarthritis	(Hansra et al., 2000)
Complete Freund's Adjuvant intra articular (CFA)	Intra-articular injection of CFA	Monoarthritis	(Donaldson et al., 1993)
Complete Freund's Adjuvant intra plantar (CFA)	Intra-plantar injection of CFA	Polyarthritis	(Larson et al., 1986)
Dextran Sulfate Sodium (DSS)	Ingestion of water with DSS	Colitis	(Okayasu et al., 1990)
Kaolin/Carragenaan (K/C)	Intra-articular injection of a K/C mixture	monoarthritis	(Sluka and Westlund, 1993)
Monosodium Iodoacetate (MIA)	Intra-articular injection of MIA	Monoarthritis	(Marker and Pomonis, 2012)
Reserpine	Intravenous, intra-peritoneal or sub-cutaneous injection of Reserpine	Fibromyalgia	(Nagakura et al., 2009)
Trinitrobenzene Sulfonic acid (TNBS)	Intra-colonic injection of TNBS	Colitis	(Morris et al., 1989)
Zymosan	Intra-colonic injection of Zymosan	Colitis	(Coutinho et al., 1996)
Others			
Acidic Saline (AS)	Intra-muscular injections of AS	Widespread muscle-induced CP	(Sluka et al., 2001)
Dental pulp injury (DPI)	Dental pulp mechanical exposure	Pulpitis	(Gibbs et al., 2013)
Fracture	Bone fracture and stabilization	Skeletal Pain	(Koewler et al., 2007)
Lumbar Disc Herniation (LDH)	Autologous nucleus pulposus implants (L5)	Radicular pain in lumbar disc herniation	(Takebayashi et al., 2001)
Nitroglycerin (NTG)	Intravenous, intra-peritoneal or sub-cutaneous injection of NTG	Migraine	(Iversen and Olesen, 1996)

Table I. A summary of the most common strategies to model chronic pain in rodents is presented.

CP – Chronic Pain; [1]spinal cord injury models vary substantially regarding injury nature and location – consult references within for more details.

Table II. Behavioral paradigms applied in the context of chronic pain

Paradigm	Construct	References
Anxiety-like behavior		
Dark/light (D/L)	Exploration-based paradigm in an arena with a dark (D) and an illuminated area (L); increased D/L ratios associated with anxiety-like behavior; dependent of novelty and motor performance	(Crawley and Goodwin, 1980); (Belovicova et al., 2017; Bourin and Hascoet, 2003; Cryan and Holmes, 2005; Cryan and Sweeney, 2011; Harro, 2018)
Elevated plus maze (EPM)	Exploration-based paradigm in a cross shaped maze with alternating closed (walled) and open arms elevated from the floor; increased open/close arms ratio associated with anxiety-like behavior; dependent of novelty and motor performance	(Pellow et al., 1985); (Belovicova et al., 2017; Castanheira et al., 2018; Cryan and Holmes, 2005; Cryan and Sweeney, 2011; Harro, 2018; Lister, 1990)
Elevated zero maze (EZM)	Similar to the EPM; open and closed area alternate in a circular structure elevated from the floor	(Shepherd et al., 1994); (Cryan and Holmes, 2005; Cryan and Sweeney, 2011; Harro, 2018)
Open field (OF)	Exploration-based paradigm in a squared-shaped arena; conflict between safety (periphery/thigmotaxia) and exploration (center) is evaluated; decreased center/periphery time/distance associated with anxiety-like behavior; dependent of novelty and motor performance	(Hall and Ballechey, 1932); (Belovicova et al., 2017; Castanheira et al., 2018; Cryan and Holmes, 2005; Denenberg, 1969; Harro, 2018; Prut and Belzung, 2003)
Hole board test (HB)	The modified HB test is an exploration-based paradigm; activity in an arena containing a holed board (e.g. entries and time spent on the center of the board; holes visited) is evaluated; reduced exploration reflects anxiety-like behavior	(Boissier and Simon, 1962); (Cryan and Sweeney, 2011; Harro, 2018)
Marble burying (MB)	Defense-based paradigm; relies on rodents' natural burying behavior; marble burying associated with anxiety-like behavior; also used as a measure of compulsive behavior	(Broekkamp et al., 1986); (Cryan and Holmes, 2005; Cryan and Sweeney, 2011; Deacon, 2006b; Harro, 2018)
Novelty suppressed feeding (NSF)	Hyponeophagia; relies on the conflict between hunger and safety; anxiety-like associated with increased latencies to eat in a novel environment; dependent of novelty and motor performance; requires food restriction	(Soubrie et al., 1975); (Belovicova et al., 2017; Cryan and Holmes, 2005; Cryan and Sweeney, 2011)

(Continues)

Table II. Behavioral paradigms applied in the context of chronic pain (continued)

Paradigm	Construct	References
Depression-like behavior		
Forced swimming test (FST)	Evaluates rodents' response to an inescapable aversive scenario (drowning); depressive-like behavior is associated with increased immobility (helplessness); potentially stressful	(Porsolt et al., 1977a); (Belovicova et al., 2017; Cryan and Holmes, 2005; Ferreira et al., 2018; Planchez et al., 2019; Slattery and Cryan, 2014)
Splash test (ST)	Evaluates rodents' self-care; depressive-like behavior is associated with decreased grooming after a splash of a sucrose solution in animals' coat; excessive grooming can reflect compulsive behaviors; dependent of motor coordination	(Ducottet et al., 2003); (Planchez et al., 2019)
Sucrose preference test (SPT) Sweet food consumption	Evaluates rodents' interest in a palatable drink (1-2% sucrose solution) or food; decreased preference for sweetened drinks/food reflects anhedonia; can be repeated frequently, being often used in longitudinal studies; requires food and water restriction	(Willner et al., 1987); (Belovicova et al., 2017; Cryan and Holmes, 2005; Ferreira et al., 2018; Planchez et al., 2019; Slattery and Cryan, 2014)
Tail suspension (TST)	Evaluates rodents' escape-oriented movements in an inescapable stressful situation (the animal is suspended by the tail); depressive-like behavior is associated with increased immobility (helplessness); potentially stressful; not suitable for adult rats	(Steru et al., 1985); (Belovicova et al., 2017; Cryan and Holmes, 2005; Cryan et al., 2005; Ferreira et al., 2018; Planchez et al., 2019; Slattery and Cryan, 2014)
Well-being		
Spontaneous burrowing behavior (SBB)	Relies on rodents' natural drive to burrow; decreased burrowing reflects behavioral dysfunction; also associated with anxiety-like behavior and pain; SBB depends on strain and environmental conditions (material, type of tube, light conditions,)	(Deacon et al., 2001); (Deacon, 2006a, 2009; Jirkof, 2014)
Social behavior		
Social interaction test (SI)	Evaluates interactions between familiar or unfamiliar conspecifics in a neutral area; sniffing, chasing or stand on top/bottom among others can be used as parameters	(File and Hyde, 1978); (Castanheira et al., 2018; Cryan and Sweeney, 2011)
Three-chamber test (3-CH)	Evaluates rodents' preference to interact with 2 conspecifics placed in separated chambers of the maze; in normal conditions animals have a preference toward the unfamiliar animal;	(Moy et al., 2004); (Arakawa and Iguchi, 2018)
Tube co-occupancy test (TCOT)	Measures propinquity, i.e. the tendency of rodents to maintain close physical proximity; propinquity is higher among cage-mates	(Tuttle et al., 2017)
Cognition		
5-choice serial-reaction time task (5-csrtt)	Operant paradigms in which the animal is given the choice to nose poke in 1 out of 5 apertures; evaluates attention (responses in the illuminated aperture) and impulsivity (responses prior to the light signal); requires food restriction	(Carli et al., 1983); (Bari et al., 2008; Callahan and Terry, 2015; Dalley et al., 2011; Dalley and Robbins, 2017; Dalley and Roiser, 2012)

(Continues)

Table II. Behavioral paradigms applied in the context of chronic pain (continued)

Paradigm	Construct	References
8-Arms radial maze (8-ARM)	Evaluates reference memory (identification of the baited arm in relation to external cues) and working memory (alternation between baited arms); requires food restriction	(Olton and Samuelson, 1976); (Vorhees and Williams, 2014)
Y-maze/T-maze	Relies on animals' spontaneous alternation between visited and unvisited arms; evaluates short term memory	(Roberts et al., 1962); (Deacon and Rawlins, 2006; Kraeuter et al., 2019; Lalonde, 2002; Tanila, 2018)
Attentional set-shifting task (ASST)	The animal is presented with a set of relevant and irrelevant cues signaling the presence of a reward; evaluates associative learning, shifting of perceptual attentional setting and cognitive flexibility; analogue of the human Wisconsin Card Sorting task; requires food restriction	(Birrell and Brown, 2000); (Brown and Tait, 2016; Tait et al., 2018)
Contextual fear conditioning (CFC)	A mild shock (unconditioned stimulus) is given in a specific context or paired with a conditioning stimulus (CS; light, tone); subsequent exposure the context or CS should evoke freezing; evaluates contextual memory; stressful	(Dexter and Merrill, 1969); (Arakawa and Iguchi, 2018)
Delayed nonmatching-to-position task (DnMP)	Operant behavior in which the tested animal has to press the lever not previously shown in order to obtain a reward; evaluates attention and working memory; requires food restriction	(Dunnett, 1985)
Goal-directed vs habit-based	Evaluates action-outcome (goal-directed) and stimulus-response (habit-based) associations in instrumental behavior; habit-based associations are maintained after reward devaluation (satiation or aversion) or contingency degradation; requires food restriction	(Adams and Dickinson, 1981); (Balleine and O'Doherty, 2010)
Stop-signal task (SST)	Evaluates inhibitory control/impulsivity by assessing time taken to inhibit an already initiated response; requires food restriction	(Eagle and Robbins, 2003); (Dalley et al., 2011; Dalley and Robbins, 2017; Dalley and Roiser, 2012)
Morris water maze (MWM)	Time/distance swam to find a platform slightly submerged is measured in a sequence of trials; spatial clues are used to assist navigation; evaluates long term spatial memory; recurrent modification of platform location is used to evaluate short-term memory	(Morris, 1984); (Tanila, 2018; Vorhees and Williams, 2006, 2014)
Novel object recognition (NOR)	Time spent investigating a familiar *vs* a novel object is measured; evaluates short- and long-term memory in recall sessions 2 or 24h after the initial exposure, respectively; alternatively, in the Object Place Recognition (OPR) task time spent investigating old vs new object location is compared; dependent of motor performance	(Ennaceur and Delacour, 1988); (Tanila, 2018)
Progressive-ratio (PR)	Requirements for reward delivery are increased systematically (number of lever presses) up to breaking point (response ceases); evaluates motivation and/or reinforcement efficacy; requires food restriction	(Hodos, 1961); (Stafford et al., 1998)
Rodent Gambling Task (RGT)	The animal is given a choice between high probability/low reward and low probability/high reward options; evaluates risk-based decision-making; analogue of the human Iowa Gambling task; requires food restriction	(van den Bos et al., 2006); (Yates, 2019)
Reversal Preference Learning (RPL)	The animal associates water availability with one box' corner; after a few days, the water access is shifted to another corner; evaluates associative learning and cognitive flexibility	(Krackow et al., 2010)
Variable-delay to signal (VDS)	The animal is trained to wait for a signal (light) and then to nose poke an aperture in order to obtain a reward; large delays increases premature response rate; evaluates delay tolerance/impulsivity; requires food restriction	(Leite-Almeida et al., 2013a); (Soares et al., 2018)

Table II. A summary of paradigms and respective construct used in chronic pain models is presented. References provided refer to the original description of the paradigm; relevant reviews on the paradigms, technical descriptors or conceptual frames are also provided when available.

Table III. Spontaneous behaviors in chronic pain models

	Species	Strain	Sex	Age	Model	POW	Main readouts	References
Activity/Exploration								
Actigraphy	Mice	C57BL7/6J	M	YA	CCI	1-2	No alterations observed	(Xu et al., 2018)
Home cage monitoring	Rat	SD	M	YA	CFA[1]	4	No alterations observed in home cage activity	(Parent et al., 2012)
Home cage monitoring	Mice	Balb/c; C57BL/6	M	YA	CFA[2]; CCI; SNI	1-3	No alterations observed in home cage activity and in the food and water intake	(Racz et al., 2015; Urban et al., 2011)
Home cage monitoring	Mice	C57BL/6	M	A	OA[3]	8	OA animals spend more time immobile; no alterations were observed in grooming	(Inglis et al., 2008)
Home cage monitoring	Mice	C57BL/6N	M	YA	SNI	1-12	Grouped (but not isolated) SNI animals present a significant decrease in voluntary wheel running; SNI animals present a reduction in climbing; no major alterations were observed in home cage locomotion (frequency and distance)	(Pitzer et al., 2016a)
Burrowing								
SBB	Rat	SD	M	YA	SNI	1-3	SNI animals burrow less gravel; no correlation with allodynia	(Lau et al., 2013)
SBB	Rat	SD	M	YA	SNI	7	Left (but not right) lesioned SNI animals burrow less gravel	(Guimaraes et al., 2019)
SBB	Rat	SD	M	A	CFA[2]	1-2	CFA injected animals burrow less gravel	(Andrews et al., 2012)
SBB	Rat	SD	M	YA	CCI; CFA[2]	1-2	CCI and CFA injected rats burrow less gravel; no correlation with allodynia	(Muralidharan et al., 2016)
SBB	Rat	SD	M	Y	CFA[1]	2	MA (CFA) animals burrow less gravel	(Rutten et al., 2014a)
SBB	Rat	SD; Wistar	M	YA	CCI; STZ[4]	1-4	CCI and diabetic (STZ) animals burrow less gravel and sand; no correlation with allodynia	(Rutten et al., 2018)
SBB	Rat	SD; Wistar	M	YA	CFA[2]	1-2	Cross-center evaluation; overall decrease in spontaneous burrowing; high inter-laboratory variability	(Wodarski et al., 2016)
SBB	Rat	SD; WH	M	Y	TNT, PSNL, SNT	2-11	TNT, PSNL and SNT animals burrow less gravel	(Andrews et al., 2012)
SBB	Rat	Wistar	M	A	Stavudine d4T[5]	3	HIV (Stavudine) animals burrow less gravel	(Huang et al., 2013)
SBB	Rat	ZDF	M	YA; A	-	-	ZDF rats burrow less sand	(Rutten et al., 2018)
SBB	Mice	C57BL7/6J; FVB/NJ	M; F	YA	SNI, CFA[2], NTG[4]	1-4	SNI animals present decreased burrowing up to 1w in daily SBB tests and up to 4w in intercalated 5d tests; there is a correlation between corncob burrowed and allodynia at day 6; no alterations were observed in CFA or migraine (NTG) models	(Shepherd et al., 2018)
Sleep								
EEG/EMG	Rat	SD	M	YA	CCI	2-21	No alterations observed	(Kontinen et al., 2003)
EEG/EMG	Rat	SD	M	YA	CCI	1	No alterations observed when using sawdust bedding; with sandpaper bedding, CCI animals present increased latency to sleep and time awake	(Tokunaga et al., 2007)

(Continues)

Table III. Spontaneous behaviors in chronic pain models (continued)

	Species	Strain	Sex	Age	Model	POW	Main readouts	References
EEG/EMG	Rat	SD	M	YA	CCI	1	CCI animals with changes in SI present increased time awake and decrease percentage of slow wave sleep during the light period after CCI; no alterations were observed in CCI animals without alterations in SI	(Monassi et al., 2003)
EEG/EMG	Rat	Wistar	M; F	YA	MIA[1]	1-4	OA (MIA injected) animals present decreased sleep efficacy, decreased percentage of slow wave sleep, and decreased paradoxical sleep (percentage and number of episodes)	(Silva et al., 2008; Silva et al., 2011)
EEG/EMG	Rat	Wistar	F	Y	CFA[2]	13	CFA animals presented longer sleep latency, reduced paradoxical sleep latency and sleep efficiency	(Roizenblatt et al., 2010)
Ultrasonic vocalizations								
Spontaneous	Rat	SD	M	A	CCI	3	CCI animals show decreased 50 KHz USVs and increased 20 KHz USVs during non-stimulation periods	(Ghoreishi-Haack et al., 2018)
Spontaneous and in social exploration	Rat	SD	M	YA	CFA[6]/STZ[4]	3	USVs not detected when CFA or STZ diabetic rats are isolated; During social interaction no alterations were observed	(Jourdan et al., 2002)
Social exploration	Rat	SD	M	YA	CFA[6]	3	CFA increases 22-28 KHz vocalizations	(Calvino et al., 1996)
Hedonic, neutral and aversive stimuli	Rat	SD	M	A	CCI	2-3	CCI animals emit less USVs under hedonic conditions and more under aversive conditions	(Burgdorf et al., 2019)
After noxious stimuli	Rat	SD	M	A	SNL	1-5	SNL animals emit more USVs from 2 to 5w PO	(Thompson et al., 2018a)
After noxious stimuli	Rat	Wistar	M	YA	PSNL	1	USVs not detected	(Wallace et al., 2005)
Spontaneous	Mice	C57BL/6	M; F	A	SNI	1-6	SNI increases the number of vocalizations at 50Hz 2,3 and 4w PO, and at 37 KHz 2 and 3w PO	(Kurejova et al.)
Social behavior								
SI	Rat	SD	M	YA	CFA[6]	3	CFA animals present decreased exploration time and increased immobility	(Calvino et al., 1996)
SI	Rat	SD	M	YA	CFA[6]/STZ[5]	3	No alterations observed	(Jourdan et al., 2002)
SI	Rat	SD	M	Y	CFA[2]	4	CFA animals spend less time interacting	(Parent et al., 2012)
SI	Rat	SD	M	Y	CCI	2-3	No alterations observed	(Gregoire et al., 2012)
SI	Rat	SD	M	Y	CFA[1]	2-3	CFA animals spend less time interacting	(Aissouni et al., 2017; Gregoire et al., 2014)
SI (resident-intruder)	Rat	SD	M	YA	CCI	1-2	Some animals present no alterations after CCI (no-effect group) while others present a decrease in dominant behavior and an increase in non-social behaviors, which is maintained along the days (persistent changes group) or only present in the first days after CCI (recovery group)	(Monassi et al., 2003)
SI (home cage)	Mice	C57BL/6	M	YA	Cuff	1	No alterations observed	(Benbouzid et al., 2008)

(Continues)

Table III. Spontaneous behaviors in chronic pain models (continued)

	Species	Strain	Sex	Age	Model	POW	Main readouts	References
SI	Mice	C57BL/6	M	YA	DPI	1-2	DPI animals present decreased social exploration 14d PO	(Shang et al., 2015)
SI	Mice	C57BL/6; FVB/NJNju	M	YA	CFA[2]	1-2	No alterations observed	(Liu et al., 2015b)
SI	Mice	ddY	M	Y	PSNL	2-10	PSNL animals present decreased sociability time 6 and 8w PO	(Hisaoka-Nakashima et al., 2019)
2-Ch	Mice	C57BL/6	M	YA	SNI	8-14	No alterations observed	(Sheahan et al., 2017)
TCOT	Mice	CD-1	M; F	YA	SNI	1	Propinquity higher in dyads of unfamiliar SNI animals	(Tansley et al., 2019)

Table III. A detailed overview of the studies assessing spontaneous behaviors in chronic pain (>1 POW) models and major outcomes is provided.

2-Ch – 2 Chamber social interaction test; A – Adults[a]; CCI – Chronic Constriction Injury ; CFA – Complete Freund's Adjuvant; d-days; DPI- Dental Pulp Injury; EEG – Electroencephalogram; EMG – Electromyogram; F – Female; M – Male; MA- Monoarthritis; MIA- Monosodium Iodoacetate; NTG – Nitroglycerin; OA- Osteoarthritis; PO- Post Operation; POW – Post-Operative Week; PSNL – Partial Sciatic Nerve Ligation; SBB – Spontaneous Burrowing Behavior; SD – Sprague Dawley; SI – Social Interaction; SNI – Spared Nerve Injury ; SNT – Spinal Nerve Transection; STZ – Streptozotocin; TCOT – Tube Co-Occupancy Test; TNT – Tibial Nerve Transection; USVs- Ultrasonic Vocalizations; Y – Young[a]; YA – Young Adult[a]; w- weeks; WH- Wistar-Han; ZDF – Zucker Diabetic Fatty.

[1]intra-articular; [2]Intra-plantar; [3]transection of the medial meniscotibial ligament; [4]Intra-peritoneal; [5]Intravenous; [6]Base of the tail. [a]Rats: Y (<7w or 200g); YA (7-11w or 200-350g); A (11-36w or 350-450g); Mice: Y (<7w or 18g (F) /23g (F)); YA (7-11w or 18-20g(F)/23-25g(M)); A (11-20w or 20-25g(F)/25-33g(M)).

Table IV. Emotional behavior in models of chronic pain

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
Anxiety-like behavior								
BBE; NSF	Mice	C57BL/6	M	YA	SNI	2	SNI present increased latency to exit the BBE and to feed in the NSF	(Mutso et al., 2012)
D/L	Rat	Hannover-Wistar	M	A	SNI	2	No alterations observed	(Chen et al., 2019)
D/L	Rat	SD	M	A	SNI	5	Left-lesioned SNI spend less time in the light side	(Guimaraes et al., 2019)
D/L	Mice	CD-1	M	Y	SNI	4	SNI spend less time and enter less in the light side	(Palazzo et al., 2016)
D/L	Mice	Swiss	M	YA	CFA[1]	2	MA (CFA) animals spend less time in the light side	(Omorogbe et al., 2018)
D/L	Mice	Swiss	M	O	PSNL	4	No alterations observed	(Gai et al., 2014)
D/L; EPM	Rat	SD	M	Y	SNL	1-2	SNL spend less time and enter less in the open arms; results are independent of allodynia	(Kontinen et al., 1999)
D/L; EPM	Rat	Wistar	M	YA	CFA[1]	1-2	MA (CFA) animals enter less in the open arms and spend less time in the open arms and light side 10d PO; MA animals cross less to the light side 7 and 10d PO	(do Nascimento and Leite-Panissi, 2014)
D/L; EPM	Rat	Wistar	M	YA	ION	2	ION allodynic rats spend less time in open arms and in the light side; no alterations were observed in non-allodynic animals	(Gambeta et al., 2018)
D/L; EPM	Mice	C57Bl/6	M	Y	CFA[1]; PSNL	1-4	MA (CFA) and PSNL mice spend less time in the light side and in the open arms, except at 1w PO	(Martinez-Navarro et al., 2020; Matsuzawa-Yanagida et al., 2008; Narita et al., 2006a; Narita et al., 2006b)
D/L; EPM; HB	Mice	C57BL/6	M; F	YA	SNI	4-6	SNI animals spend less time in the open arms, in the light side and in the center of HB	(Sieberg et al., 2018)
D/L; EPM; HB; OF	Mice	C57BL/6J(6N)	M; F	A	CFA[1]; SNI	1-12	No major alterations observed	(Pitzer et al., 2019)
D/L; EPM; OF	Rat	SD	M	Y	CFA[2]	4	CFA increases anxiety-like behavior in all tests	(Parent et al., 2012)
D/L; EPM; OF	Mice	C57BL/6	M	YA	SNL	1-8	SNL animals present anxiety-like behavior 4 and 8w PO	(Suzuki et al., 2007)
D/L; EPM; OF	Mice	C57BL/6	M	A	Zymosan[3]	1-4	Colitis' (Zymosan') animals spend less time in the open arms, the center and the light side	(Zhang et al., 2014a)
D/L; EPM; OF	Mice	C57BL/6J	M	YA; O	CCI; SNI	1-3	No alterations observed	(Karl et al., 2019; Karl et al., 2017)
D/L; EZM	Mice	C57BL/6J	M	YA	ION	1-4	ION animals spend less time in the light side	(Shalini et al., 2017)
D/L; EZM	Mice	C57BL/6	M; F	A	PSNL	4-5	No alterations observed	(Racz et al., 2015)
D/L; NSF	Mice	C57BL/10	M	A	Cuff	2-21	Cuffed animals spend less time in the light side at 8w PO and show an increased latency to feed at 8,11 and 16w PO	(Sellmeijer et al., 2018)
D/L; NSF	Mice	C57BL/6	M	Y	CCI	1-3	No alterations observed	(Zhao et al., 2015)
D/L; NSF	Mice	C57BL/6	M	YA	Cuff	2-8	Cuffed animals spend less time in the light side 4,6 and 8w PO and present increased latency to feed 7 and 8w PO	(Yalcin et al., 2011)
D/L; OF	Mice	Balb/c	F	YA	SCI	1-8	No alterations observed	(Boadas-Vaello et al., 2018)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
D/L; OF	Mice	C57BL/6	M	A	TIC	1-8	At 8w, TIC animals spend less time in the light	(Lyons et al., 2015)
EPM	Rat	Crl:CD	M	YA	CCI	4-5	CCI decreases the time, number of entries in open arms and the distance travelled 5w PO	(Murasawa et al., 2020)
EPM	Rat	LE	M	YA	SNI	24	SNI animals spend less time in the open arms	(Low et al., 2012)
EPM	Rat	SD	M	YA	Carrageenan[1]	4	No alterations observed	(Gabriel et al., 2010)
EPM	Rat	SD	M	A	CCI	2-6	CCI animals spend less time and enter less in the open arms	(Alba-Delgado et al., 2018)
EPM	Rat	SD	M	Y	CCI	1-3	CCI animals spend less time in the open arms	(Li et al., 2014)
EPM	Rat	SD	M	Y	CFA[1]	2	MA (CFA) animals spend less time and enter less in the open arms	(Aissouni et al., 2017)
EPM	Rat	SD	M	YA; A	DSS[4]; TNBS	2-3	Colitis (DSS) and TNBS animals spend less time in the open arms	(Chen et al., 2015; Huang et al., 2015)
EPM	Rat	SD	M	YA; A	SNL	4	SNL spend less time in the open arms	(Brunori et al., 2018; Ji et al., 2018; Ji et al., 2017)
EPM	Rat	Wistar	M	YA	CCI	1-8	High and low anxiety-like rats decrease the number of entries in the open arms after CCI	(Roeska et al., 2009)
EPM	Rat	Wistar	M	YA	CCI; PNL	3-4	CCI spend less time in the open arms; No alterations were observed in PNL	(Caspani et al., 2014; Filho et al., 2016; Roeska et al., 2008)
EPM	Rat	Wistar	M	YA	SNI	4	Left (but not right) lesioned SNI animals spend less time in the open arms	(Leite-Almeida et al., 2012)
EPM	Rat	Wistar	M	YA; A; O	SNI	4	YA and O SNI spend less time in the open arms; no alterations observed in A	(Leite-Almeida et al., 2009)
EPM	Rat	WKY	M	-	SNI	2	No alterations observed	(Yang et al., 2019b)
EPM	Rat	Wistar	M; F	Y	CFA[1]	3-6	MA (CFA) animals spend less time in the open arms; MA F spend more time in open arms than MA M	(Lima et al., 2014)
EPM	Rat	SD	F	YA	SCI	4-12	SCI animals spend less time and enter less in the open arms 6 and 12w PO	(Baastrup et al., 2011)
EPM	Rat	Wistar	F	Y	CFA[2]	12	Neonatal CFA leads to decreased entries in the open arms	(Roizenblatt et al., 2010)
EPM	Mice	C57BL/6	M	YA	MIA[2]	4	MIA injected animals spend less time in the open arms	(Negrete et al., 2017)
EPM	Mice	C57BL/6	M	A	PSNL	1-4	PSNL animals spend less time in the open arms 28d PO	(Sawada et al., 2014)
EPM	Mice	C57BL/6	M	YA	Zymosan[3]	1	Colitis (Zymosan) animals spend less time in the open arms	(Wang et al., 2019c)
EPM	Mice	C57BL/6J	M	YA	CCI	4	CCI animals spend less time and enter less in the open arms	(Ferreira-Chamorro et al., 2018)
EPM	Mice	CD-1	M	YA	PSNL	1-8	PSNL animals spend less time and enter less in the open arms at 12, 26, 33 and 47d PO, but not at 54d; It is correlated with allodynia	(Gonzalez-Sepulveda et al., 2016)
EPM	Mice	SA	M	YA	PSNL	1-3	PSNL animals spend less time in the open arms	(La Porta et al., 2016)
EPM	Mice	C57BL/6	M; F	YA	PTX[5]	3	PTX decreases the number of entries in open arms only in males	(Liang et al., 2019)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
EPM; OF	Rat	LE	M	Y	SNI	1-25	SNI rats exit less from the closed arms in the EPM at 20 and 25w PO and spend less time in the center of OF 9w PO	(Seminowicz et al., 2009)
EPM; OF	Rat	SD	M	A	AS[6]	2-3	AS injected animals spend less time in the open arms and in the center	(Liu et al., 2014)
EPM; OF	Rat	SD	M	A	CCI	1-3	CCI animals spend less time and enter less in the open arms; no alterations were observed in the OF	(Li et al., 2019a)
EPM; OF	Rat	SD	M	Y	CCI	2-3	CCI animals spend less time and enter less in the center of OF; no alterations were observed in the EPM	(Gregoire et al., 2012)
EPM; OF	Rat	SD	M	YA	CCI	1-2	CCI animals spend less time in the open arms and in the center	(Zhang et al., 2014b)
EPM; OF	Rat	SD	M	YA	CFA[2]	1	CFA injected animals spend less time in the open arms and in the center	(Wang et al., 2015b)
EPM; OF	Rat	SD	M	YA	ION	2	ION animals spend less time and enter less in the open arms and spend less time in the center	(Wang et al., 2015c)
EPM; OF	Rat	SD	M	YA	PSNL	4	PSNL animals spend less time in the open arms and in the center	(Wang et al., 2015d)
EPM; OF	Rat	SD	M	Y	SNI	3-10	SNI animals spend less time in the open arms and in the center	(Gong et al., 2018)
EPM; OF	Rat	SD	M	YA; A	SNI	4	SNI animals spend less time in the open arms and in the center	(Satyanarayanan et al., 2019; Zhang et al., 2017b)
EPM; OF	Rat	SD	M	Y	SNL	1-2	SNL animals spend less time in the open arms and in the center	(Jiang et al., 2014)
EPM; OF	Rat	Wistar	M	Y	CCI	2	No alterations observed	(Seno et al., 2018)
EPM; OF	Rat	Wistar	M	YA	K/C[1]	5-6	MA (K/C) animals spend less time in the open arms	(Amorim et al., 2014)
EPM; OF	Rat	Wistar	M	YA	SNI	7	No alterations observed	(Gonçalves et al., 2008)
EPM; OF	Rat	Wistar	M	YA	SNI	4-8	At 4w PO, SNI rats enter less in the center area; at 8w PO, SNI animals enter less in the open arms and center and spend less time in the open arms	(Sang et al., 2018)
EPM; OF	Rat	SD	F	YA	SNI	2-19	No alterations observed	(Hubbard et al., 2015)
EPM; OF	Mice	C57BL/6	M	YA	CFA[2]	1-3	CFA injected mice spend less time in the open arms and in the center; CFA injected animals enter less in the open arms	(Sun et al., 2016; Tian et al., 2017; Wang et al., 2015a; Yue et al., 2018)
EPM; OF	Mice	C57BL/6	M	YA	PSNL	1-3	PSNL animals enter more in the open arms 2w PO	(Hasnie et al., 2007b)
EPM; OF	Mice	C57BL/6	M	YA; A	SNI	3-7	SNI animals spend less time in the open arms and in the center	(Descalzi et al., 2017; Zhao et al., 2018; Zhou et al., 2019)
EPM; OF	Mice	C57BL/6	M	YA	Zymosan[3]	1-2	Colitis (Zymosan) animals spend less time in the open arms and in the center	(Chun et al., 2018)
EPM; OF	Mice	C57BL/6J	M	A	CCI	6	CCI mice spend less time in the open arms and in the center	(Li et al., 2019d)
EPM; OF	Mice	C57BL/6J	M	A	PSNL	4	PSNL animals spend less time in the open arms and in the center	(Wang et al., 2017)
EPM; OF	Mice	C57BL/6; FVB/NJNju	M	YA	CFA[2]	1-2	CFA injected animals from both strains enter less in the open arms	(Liu et al., 2015b)

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Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
EPM; OF	Mice	ChAt-Cre	M	A	CFA[2]	4	CFA injected animals spend less time in the open arms and in the center	(Jiang et al., 2018b)
EPM; OF	Mice	C57BL/6J	M; F	YA	DSS[4]	1	M and F colitis (DSS) mice spend less time in the open arms and in the center	(Nyuyki et al., 2018)
EPM; OF	Mice	C57BL/6	F	O	CFA[2]	2	CFA injected animals spend less time in the open arms and in the center and enter less in the open arms	(Liu et al., 2015a)
EPM, OF; NSF	Rat	Wistar	M	A	SNI	3	SNI animals spend less time in the open arms and in the center; increased latency to feed in NSF	(De Gregorio et al., 2019)
EPM; MB	Mice	C57BL/6	M	YA	Cuff	4	Cuffed animals spend less time in the open arms and burrow more marbles	(Benbouzid et al., 2008)
EPM; MB	Mice	CD1	M	A	SNI	2	SNI mice choose less the open arms and dig more and burrow more marbles	(Palazzo et al., 2016; Palazzo et al., 2015)
EZM	Rat	SD	M	YA	CFA[1]	1-4	MA (CFA) animals spend less time in the open arms at 4w	(Borges et al., 2017; Borges et al., 2014; Borges et al., 2015)
EZM	Rat	SD	M	YA	SNL	2	SNL animals spend less time in the open arms	(Shao et al., 2015)
EZM	Rat	SD	M	YA	CCI	1-4	CCI rats spend less time in the open arms	(Alba-Delgado et al., 2013; Llorca-Torralba et al., 2018)
EZM	Mice	C57BL/6	M	A	Cuff	5	Cuffed animals spend less time in the open arms	(Dimitrov et al., 2014)
EZM	Mice	Swiss	M	YA	PSNL	2	PSNL animals spend less time in the open arms	(Martinez-Navarro et al., 2019)
EZM; OF	Rat	LE	M	A	CCI	2-6	At 5-6 w PO, CCI animals spend less time in the open arms and in the center, and enter less in the open arms	(Llorca-Torralba et al., 2019)
EZM; OF	Rat	SD	M	YA	CCI	2-4	CCI animals spend more time in the open arms and in the center	(Alba-Delgado et al., 2016)
EZM; OF	Rat	SD	M	YA	CFA[2]	4	CFA injected rats present reduced time/travelled distance in the open arms and in the center	(Du et al., 2017)
EZM; OF	Rat	SD	M	YA	CFA[2]	1-4	CFA injected animals present reduced traveled distance in the open arms of the EZM at 4w and less time and traveled distance in the center of OF at 3 and 4w PO	(Wu et al., 2017)
EZM; OF	Mice	Balb/c; C57BL/6	M	YA	CCI; CFA[2]; SNI	1-5	Balb/c SNI and CFA- injected mice spend more time in the center; no effects on CCI mice	(Urban et al., 2011)
EZM; OF	Mice	C57BL/6	M	A	Fracture	7-9	Fractured animals spend less time in the open arms	(Tajerian et al., 2014)
EZM; OF	Mice	C57BL/6J	M	A	Fracture	4	No alterations observed	(Li et al., 2019b)
MB	Rat	SD	M	YA	CFA[1]	2-4	MA (CFA) bury more marbles	(Borges et al., 2014)

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Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
MB	Mice	C57BL/6	M	A	CCI	1-2	CCI animals bury less marbles and spend less time digging 6d PO	(Wilkerson et al., 2018)
MB	Mice	C57BL/6	M	YA	Cuff	2-6	Cuffed animals bury more marbles at 4w and 6w PO	(Benbouzid et al., 2008; Yalcin et al., 2011)
MB	Mice	CD-1	M	A	CCI	3	CCI decreases the latency to dig and increases the digging and the marble burying	(Medeiros et al., 2019)
MB	Mice	CD-1	M	YA	PSNL	2-6	PSNL animals bury more marbles	(Gonzalez-Sepulveda et al., 2016)
MB	Mice	CD-1	M	O	SNI	52	SNI animals dig more marbles	(D'Aniello et al., 2017)
MB	Mice	Balb/c	F	A	STZ[3]	4-6	No alterations observed	(Aguilar-Avila et al., 2019)
MB	Mice	CD-1	M	A	SNI	4	SNI animals bury more marbles and perform more digging events	(Guida et al., 2015)
NSF	Rat	Wistar	M	YA	CCI	2-4	CCI rats present increased latency to feed	(Jiang et al., 2019)
NSF	Mice	C57BL/6	M	YA; A	Cuff	5-8	Cuffed animals present increased latency to feed	(Barthas et al., 2017; Barthas et al., 2015; Cardenas et al., 2019)
NSF	Mice	C57BL/6	F	YA	SNI	6	SNI increases latency to feed	(Vichaya et al., 2019)
OF	Rat	B&K	M	YA	PSNL; SNT; VZV[2]	2	SNT animals spend less time and enter less in the center; VZV injected animals enter less in the center; Number of entries for both models correlate with mechanical thresholds; no effects on PSNL	(Hasnie et al., 2007a)
OF	Rat	SD	M	A	CCI	2	CCI enter less in the center	(Missig et al., 2017)
OF	Rat	SD	M	Y	CFA[1]	2-3	MA (CFA) animals spend less time and enter less in the center	(Gregoire et al., 2012)
OF	Rat	SD	M	YA	SCI	6	No alterations observed	(Galan-Arriero et al., 2014)
OF	Rat	SD	M	YA	SNI; SNL	2	No alterations observed	(Chung et al., 2017; Pan et al., 2018)
OF	Rat	SD	M	YA; Y	SNI	3	SNI animals spend less time in the center	(Avila-Martin et al., 2014; Galan-Arriero et al., 2015; You et al., 2018)

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Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
OF	Rat	Wistar	M	YA	ddC/ HIV-gp120[3]	1-3	HIV animals spend less time and enter less in the center	(Wallace et al., 2007a; Wallace et al., 2007b; Wallace et al., 2008)
OF	Mice	C57BL/6	M	A	CCI	8	No alterations observed	(Barcelon et al., 2019)
OF	Mice	C57BL/6	-	Y	CCI	1-12	No alterations observed	(Dellarole et al., 2014)
OF	Mice	C57BL/6	M	A	PSNL	5	PSNL mice spend less time in the center	(Dimitrov et al., 2014)
OF	Mice	C57BL/6J	M	YA	CCI	6	No alterations observed	(Wang et al., 2019b)
OF	Mice	C57BL/6J	M	YA	SNI	2-3	No alterations observed	(Sheahan et al., 2017)
OF	Mice	CD-1	M	YA	SNI	24	SNI animals spend less time in the center	(Tajerian et al., 2013)
OF	Mice	Swiss	M	A	PSNL	4	No alterations observed	(Birmann et al., 2019)
OF; SPB	Rat	SD	M	A	SCI	1-3	No alterations observed	(Maldonado-Bouchard et al., 2016)
Anhedonia								
SPT	Rat	Hannover-Wistar	M	A	SNI	1	SNI animals present increased preference to sucrose	(Chen et al., 2018)
SPT	Rat	SD	-	YA	SNT	2	SNT animals have a decreased preference for sucrose	(Zong et al., 2018)
SPT	Rat	SD	M	YA	SNI	1-3	70% of SNI animals have a decreased preference for sucrose	(Li et al., 2018)
SPT	Rat	SD	M	Y; YA; A	SNI	1-11	SNI animals present a decreased preference to sucrose	(Fang et al., 2019b; Goffer et al., 2013; Gong et al., 2018; Pan et al., 2018; Satyanarayanan et al., 2019; Thompson et al., 2018b; Xu et al., 2017; Zhang et al., 2019)
SPT	Rat	SD	M	A	SNI	1-3	56% of SNI rats have a decreased preference for sucrose	(Yang et al., 2019a)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
SPT	Rat	SD	M	YA	STZ[5]	1-6	Diabetic (STZ) animals present a decreased preference for sucrose after 2w	(Guan et al., 2019)
SPT	Rat	SD	M	Y	CCI	2-3	No alterations observed	(Gregoire et al., 2012)
SPT	Rat	SD	M	YA	SNI	1-8	SNI animals have a decreased preference for sucrose 2 and 8w PO	(Wang et al., 2011)
SPT	Rat	SD	M	YA	SNL	1-5	SNL animals have a decreased preference for sucrose from 2-5w	(Hu et al., 2017; Ji et al., 2017)
SPT	Rat	SD	M	A	SCI	1-3	No alterations observed	(Maldonado-Bouchard et al., 2016)
SPT	Rat	SD	M	YA	SNI	2-3	40% of SNI animals have a decreased preference for sucrose	(Fang et al., 2018; Xie et al., 2017)
SPT	Rat	SD	M	YA	CFA[2]	2	CFA injected animals have a decrease preference for sucrose	(Zhang et al., 2016)
SPT	Rat	SD	M	Y	CFA[1]	2-3	MA (CFA) have a decreased preference for sucrose	(Gregoire et al., 2014)
SPT	Rat	SD	M	YA	DSS[4]	2-3	Colitis (DSS) animals have a decreased preference for sucrose	(Chen et al., 2015)
SPT	Rat	SD	M	YA	PSNL	4	No alterations observed	(Urban et al., 2011; Wang et al., 2015d)
SPT	Rat	SD	M	Y	CCI	1-2	40% of CCI rats present decreased sucrose consumption after lesion; no alterations in preference	(Hakim et al., 2020)
SPT	Rat	SD	M	YA	CFA[1]; SNI	1-2	MA (CFA) rats have a decreased preference for sucrose 7d PO and SNI animals 7 and 14d PO	(Su et al., 2015)
SPT	Rat	Wistar	M	YA	SNI	3-6	SNI animals have a decrease preference for sucrose at 6w PO	(Fang et al., 2019a)
SPT	Rat	Wistar	M	YA	K/C[1]	4	MA (K/C) induces a decreased preference for sucrose	(Amorim et al., 2014)
SPT	Rat	Wistar	M	YA	ION	2-6	No alterations observed	(Gambeta et al., 2018)
SPT	Rat	Wistar	M	Y	CCI	4	CCI animals have a decreased preference for sucrose	(Li et al., 2017)
SPT	Mice	C57BL/6	M	YA	SNI	6-7	SNI animals have a decreased preference for sucrose	(Descalzi et al., 2017; Zhou et al., 2019)
SPT	Mice	C57BL/6	M	Y	MIA[1]	1-4	MA (MIA) animals have a decreased preference for sucrose 10, 25 and 30d PO	(Negrete et al., 2017)
SPT	Mice	C57BL/6	M	YA	CFA[2]; SNI	3-6	No alterations observed	(Urban et al., 2011)
SPT	Mice	C57BL/6	M	A	SCI	12	SCI mice have a decreased preference for sucrose	(Wu et al., 2014b)
SPT	Mice	C57BL/6J	M	A	CCI	6	CCI decreases sucrose preference	(Li et al., 2019d)
SPT	Mice	ICR	M	A	SNL	2	SNL animals present decreased preference for sucrose	(Wu et al., 2018)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
SPT	Mice	SA	M	YA	PSNL	1-5	PSNL animals have a decreased preference for sucrose	(La Porta et al., 2016; Martinez-Navarro et al., 2019)
SPT	Mice	C57BL/6	M; F	A	PSNL	6	No alterations observed	(Racz et al., 2015)
SPT	Mice	C57BL/6	-	Y	CCI	1-12	CCI animals have a decreased preference for sucrose from 4w to 10w PO	(Dellarole et al., 2014)
SPT	Mice	C57BL/6	F	YA	CFA[2]	4	CFA injected animals have a decreased preference for saccharin	(Refsgaard et al., 2016)
SC	Rat	SD	M	A	AS[6]	2-3	AS injected rats consume less sucrose 15 and 17d PO	(Liu et al., 2014)
SC	Rat	Wistar	M	A	LDH	2-6	LDH animals consume less sucrose	(Cai et al., 2019)
SC	Rat	SD	M	YA	CCI	1-2	No alterations observed in cereal consumption	(Bravo et al., 2012)
Learned-helplessness								
FST	Rat	Crl:CD	M	YA	CCI	6-8	No alterations observed	(Murasawa et al., 2020)
FST	Rat	SD	M	Y; YA; A	SNL	4	SNL animals spend more time immobile	(Chung et al., 2017; Hu et al., 2010; Ji et al., 2018; Thompson et al., 2018a)
FST	Rat	SD	M	YA; A	CCI	1-6	CCI animals spend more time immobile	(Alba-Delgado et al., 2018; Li et al., 2019a; Li et al., 2019c)
FST	Rat	SD	M	YA	SNI	5	No alterations observed	(Guimaraes et al., 2019)
FST	Rat	SD	M	A	Reserpine[5]	1-3	Reserpine increases the immobility time after 3d PO	(Fusco et al., 2019; Siemian et al., 2019)
FST	Rat	SD	M	YA	CCI	2-3	CCI rats spend more time immobile; Immobility time is correlated with thermal hyperalgesia	(Fukuhara et al., 2012)
FST	Rat	SD	M	YA	CCI, CFA[1]	1-4	CCI and CFA injected animals spend more time immobile and less time climbing 4w PO	(Alba-Delgado et al., 2013; Borges et al., 2014)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
FST	Rat	SD	M	YA; A	SNI	1-4	SNI animals spend more time immobile	(Goffer et al., 2013; Gui et al., 2016; Pan et al., 2018; Satyanarayanan et al., 2019; Wang et al., 2011; Xu et al., 2017)
FST	Rat	SD	M	YA	SNL	1-5	SNL increase immobility at 3 and 5w and decrease climbing at 2-5w PO	(Hu et al., 2017)
FST	Rat	SD	M	A	SCI	3	No alterations observed	(Maldonado-Bouchard et al., 2016)
FST	Rat	SD	M	YA	SNI	2-3	40% of SNI animals spend more time immobile	(Xie et al., 2017)
FST	Rat	SD	M	YA	CFA[1]	1-2	MA (CFA) animals spend more time immobile	(Zhang et al., 2016)
FST	Rat	SD	M	YA	SNI, CFA[2]	1-2	SNI and CFA injected rats spend more time immobile	(Le et al., 2014)
FST	Rat	SD; Wistar	M	Y; YA; A	CCI	1-5	CCI animals spend more time immobile	(Caspani et al., 2014; Hu et al., 2009, 2016; Ishikawa et al., 2014; Jiang et al., 2019; Li et al., 2014; Li et al., 2017; Murad and Ayuob, 2015; Seno et al., 2018; Yasuda et al., 2014; Zhu et al., 2014)
FST	Rat	SD	M	YA	DSS[4]	2-3	Colitis (DSS) animals spend more time immobile	(Chen et al., 2015)
FST	Rat	SD	M	YA	PSNL	4	No alterations observed	(Wang et al., 2015d)
FST	Rat	SD	M	A	AS[6]	3	AS injected animals spend more time immobile	(Liu et al., 2014)
FST	Rat	Wistar	M	A	SNI	3	No alterations observed	(De Gregorio et al., 2019)
FST	Rat	Wistar	M	YA	SNI	3-6	SNI rats spend more time immobile at 6w PO	(Fang et al., 2019a)
FST	Rat	Wistar	M	A	LDH	2-6	LDH animals spend more time immobile	(Cai et al., 2019)
FST	Rat	Wistar	M	YA	CFA[1]	1-2	MA (CFA) rats spend more time immobile; Immobility time is correlated with thermal hyperalgesia	(Kim et al., 2012)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
FST	Rat	Wistar	M	YA	K/C[1]	4	MA (K/C) spend more time immobile and less time climbing and swimming	(Amorim et al., 2014)
FST	Rat	Wistar	M	YA	Reserpine[5]	1	Reserpine injected animals spend more time immobile	(Arora et al., 2011)
FST	Rat	Wistar	M	YA	SNI	7	SNI animals spend less time swimming	(Gonçalves et al., 2008)
FST	Rat	Wistar	M	YA; A; O	SNI	4	A SNI animals spend more time immobile; no alterations on YA and O SNI rats	(Leite-Almeida et al., 2009)
FST	Rat	Wistar	M	YA	STZ[7]	7	Diabetic (STZ) animals spend more time immobile	(Amorim et al., 2017)
FST	Rat	Wistar	M	YA	ION	2-7	No alterations observed	(Gambeta et al., 2018)
FST	Rat	Wistar	M	YA	CFA[2]	1	CFA injected rats spend more time immobile	(Hamann et al., 2016)
FST	Rat	Wistar	M	Y	STZ[7]	2-4	Diabetic (STZ) animals are more times immobile	(Redivo et al., 2016)
FST	Rat	Wistar	M	YA	SNI	1-2	SNI animals spend more time immobile	(de Souza et al., 2017; Piccinelli et al., 2015)
FST	Rat	Wistar; WKY	M	YA	CFA[1]	1	WKY MA (CFA) animals spend more time immobile; no effects on Wistar animals	(Wang et al., 2012)
FST	Rat	WKY	M	-	SNI	2	SNI animals spend more time immobile	(Yang et al., 2019b)
FST	Rat	SD	M; F	YA	Reserpine[5]	1-2	Reserpine injected animals present increase immobility time in the FST	(Nagakura et al., 2009)
FST	Rat	Wistar	M; F	YA	CCI	1-4	CCI animals spend more time immobile	(Garg et al., 2017)
FST	Mice	Balb/c; C57BL/6	M	YA	CFA[2]; SNI	1-5	No alterations observed	(Urban et al., 2011)
FST	Mice	C57BL/10	M	A	Cuff	7-21	Cuffed animals spend more time immobile at 7,14 and 17w PO	(Sellmeijer et al., 2018)
FST	Mice	C57BL/6	M	YA	Cuff	6-9	Cuffed animals spend more time immobile 8 and 9w PO	(Yalcin et al., 2011)
FST	Mice	C57BL/6	M	A	SNI	1	Isolated SNI mice spend more time immobile; no alterations on group-housed SNI animals	(Norman et al., 2010)
FST	Mice	C57BL/6; ICR	M	YA; A	SNL	1-8	SNL mice spend more time immobile 2, 4 and 8w PO	(Suzuki et al., 2007; Wu et al., 2018)
FST	Mice	C57BL/6	M	YA	SNI	7	SNI animals spend more time immobile	(Descalzi et al., 2017)
FST	Mice	C57BL/6	M	A	Cuff	8	Cuffed animals spend more time immobile	(Barthas et al., 2015)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
FST	Mice	C57BL/6	M	A	Cuff; PSNL	6	PNL and Cuffed animals spend more time immobile	(Dimitrov et al., 2014)
FST	Mice	C57BL/6	M	A	SNI	1	SNI animals spend more time immobile	(Zhou et al., 2015)
FST	Mice	C57BL/6	M	YA; Y	CCI	1-3	No alterations observed	(Karl et al., 2019; Zhao et al., 2015)
FST	Mice	C57BL/6J	M	A	Cuff	7-21	Cuffed animals spend more time immobile 7, 14 and 17w PO	(Sellmeijer et al., 2018)
FST	Mice	C57BL/6J	M	YA	CFA[2]	1-3	CFA injected animals spend more time immobile	(Wang et al., 2019a)
FST	Mice	C57BL/6J	M	A	CCI	6	CCI animals spend more time immobile	(Li et al., 2019d)
FST	Mice	C57BL/6J	M	A	PSNL	2-3	PSNL spend more time immobile	(Martinez-Navarro et al., 2020)
FST	Mice	C57BL/6J	M	YA; O	SNI	-	No alterations observed	(Karl et al., 2017)
FST	Mice	C57BL/6J	M	A	SNI	1	SNI animals spend more time immobile	(Laumet et al., 2017)
FST	Mice	C57BL/6J	M	YA	ION	1-5	No alterations observed	(Shalini et al., 2017)
FST	Mice	C57BL/6J	M	A	PSNL	4	PSNL animals spend more time immobile	(Wang et al., 2017)
FST	Mice	ddY; C3H	M	Y	PSNL	2-10	PSNL increase immobility time only at 8w PO	(Hisaoka-Nakashima et al., 2019)
FST	Mice	SA	M	YA	PSNL	1-3	PSNL animals spend more time immobile 3w PO	(La Porta et al., 2016)
FST	Mice	Swiss	M	A	Reserpine[3]	1	Reserpine increases immobility time	(Sousa et al., 2018)
FST	Mice	Swiss	M	YA	MIA[1]	3-5	MIA injected animals spend more time immobile	(Carcole et al., 2019)
FST	Mice	Swiss	M	A	PSNL	4	PSNL animals spend more time immobile	(Birmann et al., 2019)
FST	Mice	ddY	M; F	YA	PSNL	3	No alterations observed	(Nishinaka et al., 2015)
FST	Mice	C57BL/6	M; F	YA	PTX[5]	3	PTX increases immobility time both in males and females	(Liang et al., 2019)
FST	Mice	C57BL/6J(6N)	M; F	A	CFA[1]; SNI	1-12	MA(CFA) M present higher immobility time at 1w PO and M SNI animals at 4w PO; no alterations on F	(Pitzer et al., 2019)
FST	Mice	Balb/c	F	A	STZ[5]	4-6	No alterations observed	(Aguilar-Avila et al., 2019)
FST	Mice	Balb/c	F	YA	SCI	8	SCI animals with extensive (but not mild) lesions spend more time immobile	(Boadas-Vaello et al., 2018)
FST	Mice	C57BL/6	F	YA	SNI	6	SNI animals spend more time immobile	(Vichaya et al., 2019)
FST	Mice	C57BL/6	F	-	SNI	8	No alterations observed	(Terzi et al., 2014)

(Continues)

Table IV. Emotional behavior in models of chronic pain (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
FST; TST	Rat	SD	M	YA	SNI	4	SNI animals spend more time immobile	(Zhang et al., 2019)
FST; TST	Rat	SD; WKY	M	YA	CCI	1	WKY CCI animals spend more time immobile; no effects on SD animals	(Zeng et al., 2008)
FST; TST	Rat	SD	-	YA	SNT	2	SNT animals spend more time immobile	(Zong et al., 2018)
FST; TST	Mice	C57BL/6	M	A	CCI	8	CCI animals spend more time immobile	(Barcelon et al., 2019)
FST; TST	Mice	C57BL/6	M	YA	SNI	3	SNI animals spend more time immobile	(Zhao et al., 2017)
FST; TST	Mice	C57BL/6J	M	YA	CCI	4-5	CCI animals spend more time immobile	(Diaz et al., 2019; Wang et al., 2019b)
FST; TST	Mice	CD-1	M	A	CCI	2-3	CCI animals spend more time immobile	(Medeiros et al., 2019)
FST; TST	Mice	CD-1	M	Y	SNI	4	SNI animal spend more time immobile	(Palazzo et al., 2016)
FST; TST	Mice	ICR	M	Y	CCI	1-6	CCI animals spend more time immobile 2, 4 and 6w PO	(Jiang et al., 2018a)
FST; TST	Mice	SA	M	O	PSNL	4	PSNL animals spend more time immobile	(Gai et al., 2014)
FST; TST	Mice	Swiss	M	A	SNC	2	SNC animals spend more time immobile	(Nascimento et al., 2015)
TST	Mice	C57BL/6	M	YA	Cuff	3-6	No alterations observed	(Benbouzid et al., 2008)
TST	Mice	C57BL/6	M	YA	PSNL	1-4	No alterations observed	(Hasnie et al., 2007b)
TST	Mice	C57BL/6	M	A	SCI	10	SCI animal spend more time immobile	(Wu et al., 2014b)
TST	Mice	C57BL/6J	M	YA	CCI	4	CCI animals spend more time immobile	(Ferreira-Chamorro et al., 2018)
TST	Mice	C57BL/6J	M	YA	CFA[1]; CCI	2-4	CCI animal spend more time immobile; no effects on MA (CFA) mice	(Polo et al., 2019)
TST	Mice	C57BL/6J	M	YA	PSNL	2	PSNL animals spend more time immobile	(Bura et al., 2018)
TST	Mice	C57BL/6J	M	YA	SNI	6	SNI animals spend more time immobile	(Zhou et al., 2019)
TST	Mice	CD1	M	A	SNI	2	SNI animal spend more time immobile	(Palazzo et al., 2015)
TST	Mice	CD-1	M	YA	SNI	4-8	SNI animals spend more time immobile when compared to environmental enriched Sham, but not impoverished Sham	(Vachon et al., 2013)
TST	Mice	CD-1	M	YA	PSNL	3-9	PSNL animals spend more time immobile	(Gonzalez-Sepulveda et al., 2016)
TST	Mice	CD-1	M	O	SNI	52	SNI animals spend more time immobile	(D'Aniello et al., 2017)
TST	Mice	CD-1	M	A	SNI	4	SNI animals spend more time immobile	(D'Aniello et al., 2017)
TST	Mice	SA	M	YA	CFA[1]	2	CFA animal spend more time immobile	(Omorogbe et al., 2018)
Self-care								
ST	Mice	C57BL/6	M	YA	Cuff	2-8	Cuffed animals spend less time grooming 6 and 8w PO	(Yalcin et al., 2011)
ST	Mice	C57BL/10	M	A	Cuff	9-16	Cuffed animals spend less time grooming 9, 12 and 14w PO	(Sellmeijer et al., 2018)
ST	Mice	C57BL/6	M	A	Cuff	7	Cuffed animals spend less time grooming	(Barthas et al., 2015)

Table IV. A detailed overview of the studies assessing anxiety- and depressive-like behaviors in chronic pain (>1POW) models and major outcomes is provided.

A-adult[a]; AS- Acidic Saline; BBE- Black Box Emergence task; CCI – Chronic Constriction Injury; CFA – Complete Freund´s Adjuvant; d-days; ddC – Dideoxynucleosides zalcitabine; D/L- Dark/Light; DSS- Dextran Sulfate Sodium; EPM – Elevated Plus Maze; EZM – Elevated Zero Maze; F – Female; FST – Forced Swimming Test; HB- Holeboard; ION – Infraorbital Nerve chronic constriction injury; K/C – Kaolin/Carragenaan; LDH- Lumbar Disc Herniation; LE – Long-Evans; M – Male; MA – Monoarthritis; MB – Marble Burying; MIA- Monosodium Iodoacetate; NSF- Novelty Supressed Feeding; O- Old[a]; OF- Open Field; PO- Post operation; POW – Post-Operative Week; PSNL – Partial Sciatic Nerve Ligation; PTX-Paclitaxel; SA – Swiss Albino; SC- Sucrose/Sugared food consumption; SCI- Spinal Cord Injury; SD- Sprague Dawley; SNC – Sciatic Nerve Crush; SNI – Spared Nerve Injury; SNL – Spinal Nerve Ligation; SNT- Spinal Nerve Transection; SPB – Shock probe Burying task; SPT – Sucrose Preference Test; ST – Splash Test; STZ – Streptozotocin; TIC - Trigeminal Inflammatory Compression; TNBS-Trinitrobenzene Sulfonic acid ; TST – Tail Suspension Test; VZV-Varicella Zoster Virus; w-weeks; WKY – Wistar Kyoto; Y-young[a]; YA-young adult[a].

[1]Intra-articular; [2]Intra-plantar; [3]Intra-colonic; [4]Oral; [5]Intravenous; [6]Intra-muscular; [7]Intra-peritoneal [a]Rats: Y (<7w or 200g); YA (7-11w or 200-350g); A (11-36w or 350-450g); O (>36w or 450g); Mice: Y (<7w or 18g (F) /23g (F)); YA (7-11w or 18-20g(F)/23-25g(M)); A (11-20w or 20-25g(F)/25-33g(M)); O (>20w or >25g(F)/33g(M)).

Table V. Cognitive alterations chronic pain models

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
Short term memory (recognition)								
NOR	Rat	SD	M	Y; YA	SNI	1-3	DI is decreased in SNI animals	(Ren et al., 2011; Wang et al., 2013)
NOR	Rat	SD	M	YA	SCI	9	DI is decreased in SCI animals	(Wu et al., 2014a)
NOR	Rat	SD	M	YA	SNI	2	SNI rats present reduced novel object exploration; DI does not correlate with allodynia	(Gui et al., 2016)
NOR	Rat	SD	M	YA	SNI	2	SNI animals spend the same time exploring the familiar and the novel object	(You et al., 2018)
NOR	Rat	SD	M	O	SNL	3	SNL animals present reduced novel object exploration	(Moriarty et al., 2016)
NOR	Rat	WH	M	Y	SNI	2	SNI animals present reduced novel object exploration	(Abdulmajeed et al., 2016)
NOR[1]	Rat	LE	M	A	SNI	25	SNI animals present reduced novel object exploration	(Low et al., 2012)
NOR	Mice	CD1	M	A	SNI	16	SNI animals do not have preference for the novel object	(Gregoire et al., 2017)
NOR	Mice	CD1	M	A	SNI	52	DI is decreased in SNI animals	(D'Aniello et al., 2017; Guida et al., 2015)
NOR	Mice	CD1	M	Y	SNI	4	SNI animals present reduced novel object exploration	(Palazzo et al., 2016; Palazzo et al., 2015)
NOR	Mice	C57	M	YA	SNI	3	DI is decreased in SNI animals	(Ren et al., 2011)
NOR	Mice	C57BL/6	M	YA	SNI	-	SNI animals present reduced novel object exploration	(Gui et al., 2016)
NOR	Mice	C57BL/6	M	Y	MIA[2]	4	DI is decreased in MA (MIA) animals	(Negrete et al., 2017)
NOR	Mice	C57BL/6J	M	A	Cuff	7	Cuffed mice do not have preference for the novel object	(Dimitrov et al., 2014)
NOR	Mice	C57BL/6	M	O	CCI	2	No alterations observed	(Tyrtyshnaia et al., 2019)
NOR	Mice	C57BL/6	M	A	SNI	4	DI is decreased in SNI animals	(Boccella et al., 2019)
NOR	Mice	C57BL/6	M	YA	CCI	3-4	No alterations observed	(Karl et al., 2019)
NOR	Mice	SA	M	YA	PSNL	1-3	PSNL animals present reduced novel object exploration	(La Porta et al., 2016)
NOR/OPS	Rat	LE	M	A	CCI	2-6	DI is decreased in CCI animals 5-6w PO	(Llorca-Torralba et al., 2019)
NOR/OPR	Mice	C57BL/6J	M	YA	Fracture	4	No alterations observed	(Li et al., 2019b)
NOR/OPR	Mice	C57BL/6J	M; F	A	Fracture	9	In the NOR, fractured animals (M and F) do not have preference for the novel object; No alterations were observed in the OPR	(Sahbaie et al., 2018)
OPR	Mice	C57BL/6	M	Y	CFA[3]	2	CFA injected mice present reduced novel place exploration	(Zheng et al., 2017)
Y-maze	Rat	SD	M	Y	CCI	2-3	No alterations observed	(Gregoire et al., 2012)
Y-maze	Rat	SD	M	Y	SNL	1	SNL animals present reduced novel arm exploration	(Morel et al., 2013)
Y-maze	Rat	SD	M	Y	CFA[2]	2-3	MA (CFA) animals present reduced novel arm exploration	(Gregoire et al., 2014)

(Continues)

Table V. Cognitive alterations chronic pain models (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
Short term memory (spatial reference)								
8-ARM	Rat	SD	M	YA	SNI	2-6	SNI animals present increased number of errors along the days	(Ren et al., 2011)
8-ARM	Mice	C57	M	YA	SNI	2-3	SNI animals present increased number of errors along the days	(Ren et al., 2011)
MWM	Rat	WH	M	YA; A; O	SNI	4	No alterations observed	(Leite-Almeida et al., 2009)
MWM	Rat	WH	M	YA	SNI	4	Right-lesioned SNI take more distance to reach the platform than Sham and left-lesioned SNI	(Leite-Almeida et al., 2012)
Short term memory (alternation)								
DSAT	Rat	SD	M	YA	SNI	1-3	SNI animals present decreased % of correct responses	(Cardoso-Cruz et al., 2014; Cardoso-Cruz et al., 2013a)
DSAT	Rat	SD	M	YA	CFA[2]	1-2	No alterations observed after 1 POW	(Cardoso-Cruz et al., 2013b)
T-maze	Rat	SD	M	YA	SNI	1-3	SNI animals present decreased % of correct responses	(Cardoso-Cruz et al., 2018; Cardoso-Cruz et al., 2013a)
Y-maze	Rat	WH	M	Y	SNL	2	No alterations observed	(Abdulmajeed et al., 2016)
Y-maze	Mice	CD-1	M	YA	SNI	4	SNI animals present decreased % of spontaneous alternation	(Palazzo et al., 2016; Palazzo et al., 2015)
Y-maze	Mice	CD-1	M	A	CCI	2-3	No alterations observed	(Medeiros et al., 2019)
Y-maze	Mice	C57BL/6	M	YA	SCI	9	SCI animals present decreased % of spontaneous alternation	(Wu et al., 2014b)
Y-maze	Mice	C57BL/6	M	A; O	CCI	2-4	CCI animals present decreased % of spontaneous alternation	(Tyrtyshnaia et al., 2019; Tyrtyshnaia et al., 2017)
Y-maze	Mice	C57BL/6	M	A	Cuff	7	Cuffed animals present decreased number of alternations	(Dimitrov et al., 2014)
Long term memory (recognition)								
NOR	Rat	SD	M	YA	SNI	3	No alterations observed	(Ren et al., 2011)
NOR	Rat	SD	M	YA	SNL	2-6	DI is decreased in SNL animals at 6w	(Suto et al., 2014)
NOR[5]	Rat	LE	M	A	SNI	25	SNI animals present reduced novel object exploration	(Low et al., 2012)
NOR	Rat	LE	M	A	CCI	2-6	DI is decreased in CCI animals 5-6w PO	(Llorca-Torralba et al., 2019)
NOR	Mice	CD-1	M	A	CCI	3	DI is decreased in CCI animals	(Medeiros et al., 2019)

(Continues)

Table V. Cognitive alterations chronic pain models (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
NOR	Mice	C57BL/6	M	O	CCI	2	CCI animals present increased exploration of the familiar object and decreased DI	(Tyrtyshnaia et al., 2019)
NOR	Mice	C57BL/6	M	YA	SCI	11	SCI animals present reduced novel object exploration	(Wu et al., 2014b)
NOR	Mice	C57BL/6	M	YA	CCI	4	CCI animals present reduced novel object exploration	(Wang et al., 2019b)
NOR	Mice	C57BL/6	M	YA	CCI	3-4	No alterations observed	(Karl et al., 2019)
NOR	Mice	SA	M	YA	MIA[2]	2	DI is decreased in MA (MIA) animals	(Carcole et al., 2019)
NOR	Mice	C57BL/6	M; F	YA	PTX[4]	3	M PTX (but not F) animals present reduced novel object exploration	(Liang et al., 2019)
NOR	Mice	C57BL/6	M; F	A	PSNL	3	DI is decreased in PSNL animals	(Martinez-Navarro et al., 2020)
NOR/OPR	Mice	C57BL/6	M	A	Fracture	7	In the NOR, fractured animals do not have a preference for the novel object; No alterations were observed in the OPR	(Tajerian et al., 2014)
Long term memory (spatial reference)								
8-ARM	Rat	SD	M	YA	SNI	2-6	No alterations observed	(Ren et al., 2011)
8-ARM	Rat	SD	M	YA	CCI	1-2	No alterations observed	(Fiore and Austin, 2018)
MWM	Rat	SD	M	-	STZ[4]	6	Diabetic (STZ) animals have more cumulative errors	(Lindner et al., 2006)
MWM	Rat	SD	M	Y	SNL	4	SNL animals take more time to reach the platform and cross less the platform location in the probe trial	(Hu et al., 2010)
MWM	Rat	SD	M	YA	SCI	8	SCI animals take more time to reach the platform, spend less time in the platforms' quadrant in the probe trial and use less spatial strategies	(Wu et al., 2014a)
MWM	Rat	SD	M	Y	ION	2- 8	ION animals take more time to reach the platform at 4, 5 and 8w PO and spend less time in the platforms' quadrant in the probe trial at 5 and 8w PO	(Wu et al., 2015; Zhang et al., 2017a)
MWM	Rat	SD	M	O	SNL	6-7	No alterations observed	(Moriarty et al., 2016)
MWM	Rat	Wistar	M	YA	CCI	1-2	CCI animals take more time and distance to reach the platform, spend less time in the platforms' quadrant in the probe trial and use less spatial strategies	(Saffarpour et al., 2017)
MWM	Rat	Wistar	M	YA	STZ[5]	11	Diabetic (STZ) animals take more time and distance to reach the platform	(Biessels et al., 1996; Biessels et al., 1998)
MWM	Rat	WH	M	YA; O	STZ[5]	8	Diabetic (STZ) animals take more time and distance to reach the platform (only on testing days 6-8 in O animals); no effects in YA animals	(Kamal et al., 2000)
MWM	Rat	WH	M	YA; A; O	SNI	4	No alterations observed	(Leite-Almeida et al., 2009; Leite-Almeida et al., 2012)

(Continues)

Table V. Cognitive alterations chronic pain models (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
MWM	Mice	C57BL/6	M	A	SNI	4	SNI animals take more time to reach the platform at testing days 2 and 3 and spend less time in the platform quadrant in the probe trial	(Boccella et al., 2019)
MWM	Mice	C57BL/6	M	YA	CCI	4-5	CCI animals spend less time in the platforms' quadrant in the probe trial	(Wang et al., 2019b)
MWM	Mice	C57BL/6	M	YA	CCI	3-4	No alterations observed	(Karl et al., 2019)
MWM	Mice	C57BL/6	M	YA	SCI	9	SCI animals take more time to reach the platform, spend less time in the platforms' quadrant in the probe trial and use less spatial strategies	(Wu et al., 2014b)
MWM	Mice	SA	-	YA	STZ[4]	8	Diabetic animals (STZ) take more time to reach the platform and spend less time in the platforms' quadrant in the probe trial	(Patel and Udayabanu, 2013)
Social memory								
3-Ch	Mice	CD-1	M	Y	SNI	4	No alterations observed	(Palazzo et al., 2016)
SRT	Rat	SD	M	YA	CCI	2-3	CCI animals have a decreased social recognition score	(Gregoire et al., 2012)
SRT	Rat	SD	M	Y	CFA[2]	2-3	MA (CFA) have a decreased social recognition score	(Gregoire et al., 2014)
SRT	Mice	C57BL/6	M	A	Fracture	7	Fractured animals do not have a preference for the new mice	(Tajerian et al., 2014)
Contextual fear memory								
CFC	Rat	LE	M	A	CCI	3-7	CCI animals freeze more during conditioning and after context alteration 5-6w PO	(Llorca-Torralba et al., 2019)
CFC	Rat	SD	M	YA	SNI	4-5	No alterations observed	(Guimaraes et al., 2019)
CFC	Mice	C57BL/6	M	YA	SNI	1-2	SNI mice do not extinguish contextual fear; no differences observed in cue acquisition/extinction	(Mutso et al., 2012)
CFC	Mice	C57BL/6	M	YA	Cuff	2-8	Cuffed animals present Increased fear response	(Cardenas et al., 2019)
CFC	Mice	C57BL/6	M; F	A	Fracture	5-9	F fractured animals show deficits in context (but not cue) fear memory at 5w but not 9w PO; no impairments in M animals	(Tajerian et al., 2015)
Attention								
5-CSRTT	Rat	SD	M	A	SNI	1-12	SNI animals perform a decreased number of correct responses after lesion; No differences in comparison with Sham	(Higgins et al., 2015)
DnMP	Rat	Lewis	M	YA	CFA[2]	3	MA (CFA) animals perform a decreased number of correct responses; Correct responses decline as the delay duration increases; All animals improve with time	(Cain et al., 1997; Lindner et al., 1999)
DnMP	Rat	SD	M	YA	SNI	4	SNI animals perform a decreased % of correct responses at 3 and 6s delays	(Cardoso-Cruz et al., 2019)

(Continues)

Table V. Cognitive alterations chronic pain models (continued)

Paradigm	Species	Strain	Sex	Age	Model	POW	Main readouts	References
Risk decision-making								
Gambling	Rat	SD	M	YA	K/C[2]; CFA[2]	3-8	MA (K/C and CFA) animals show a preference for the high-risk (high but less probable) lever; animals persist on their preferred lever irrespective of having received or not received a reward in the preceding visit to that lever	(Ji et al., 2010; Pais-Vieira et al., 2009)
Gambling	Rat	SD	M	YA	CFA[2]	1	MA (CFA) animals prefer the high-risk (high but less probable) lever	(Pais-Vieira et al., 2012)
Impulsive decision-making								
5-CSRTT	Rat	SD	M	A	SNI	1-2	No alterations observed	(Higgins et al., 2015)
SST	Rat	SD	M	YA	ION	4	No alterations observed	(Kniffin et al., 2015)
VDS	Rat	WH	M	YA	SNI	6	No alterations observed; when baseline impulsivity is considered, high (but not low)-impulsive SNI rats perform more premature responses	(Cunha et al., 2020)
VDS	Rat	WH	M	YA	SNI	4	Right-lesioned SNI animals present higher intolerance for longer delays than the left-lesioned SNI and Sham rats	(Leite-Almeida et al., 2012)
Habit-based/goal-directed decision-making								
Devaluation	Rat	SD	M	YA	CCI	2	No alterations observed	(Mor et al., 2017)
Cognitive flexibility								
ASST	Rat	WH	M	YA	SNI	4	Right-lesioned SNI animals need more trials to complete the task in all the reversal phases than left-lesioned SNI and Sham	(Leite-Almeida et al., 2012; Leite-Almeida et al., 2014)
MWM	Rat	WH	M	YA; A; O	SNI	4	Adult SNI animals spend more time in the old vs new quadrant; No effects on YA and A rats	(Leite-Almeida et al., 2009)
MWM	Rat	SD	M	O	SNL	6-7	SNL animals spend more time in the old vs new quadrant	(Moriarty et al., 2016)
RPL	Mice	C57BL/6	F	YA; O	SNI	8	No alterations observed	(Albuquerque et al., 2013)
VR	Rat	SD	M	YA	SNL	3-8	SNL animals have preference for familiar associations	(Cowen et al., 2018)
Motivation								
PR	Rat	SD	M	YA	SNL	3-8	No alterations observed	(Cowen et al., 2018)
PR	Rat	SD	M	YA	SNL	3-17	No alterations observed	(Okun et al., 2016)
PR	Rat	SD	M	A	SNI	1-12	No alterations observed	(Higgins et al., 2015)
PR	Mice	SA	M	A	PSNL	1-3	PSNL animals present a decreased breakpoint	(La Porta et al., 2016)
FR	Rat	Fisher 344	M	YA	SNL	2	No alterations observed	(Ewan and Martin, 2014)

Table V. A detailed overview of the studies assessing cognitive behaviors in chronic pain (>1POW) models and major outcomes is provided.

3-Ch – 3 Chamber task; 5-CSRTT-5-Choice Serial Reaction Time Task; 8-ARM – 8-Arm Radial Maze; A-Adult[a]; ASST- Attentional Set-Shifting Task; CCI- Chronic Constriction Injury; CFA- Complete Freund's Adjuvant; CFC- Contextual Fear Conditioning; DnMP – Delayed Nonmatching-to-Position task; DSAT – Delayed Spatial Alternation Task; DI – Discrimination Index; F – Female; FR – Fixed Ratio; ION – Infraorbital Nerve Chronic constriction injury; K/C – Kaolin/Carragenaan LE – Long Evans; M – Male; MA- Monoarthritis; MIA- Monosodium Iodoacetate; MWM – Morris Water Maze; NOR – Novel Object Recognition; O- Old[a]; OPR – Object Place Recognition; OPS- Object Pattern Separation; PO- Post Operation; POW – Post-Operative Week; PR – Progress Ratio; PSNL- Partial Sciatic Nerve Ligation; PTX- Paclitaxel; RPL – Reversal Preference Learning: s – Seconds; SA – Swiss Albino; SCI – Spinal Cord Injury; SD – Sprague Dawley; SNI – Spared Nerve Injury; SNL – Spinal Nerve Ligation; SST – Stop Signal Task; SRT – Social Recognition Test; STZ – Streptozotocin; VDS- Variable Delay-to-Signal test; VR – Variable Ratio; w-weeks; WH – Wistar Han; Y-Young[a]; YA-Young Adult[a].

[1] non-selective, non-sustained attention task is a modification of the NOR; [2]Intra-articular; [3]Intra-plantar; [4]Intra-peritoneal; [5]Intra-venous [a]Rats: Y (<7w or 200g); YA (7-11w or 200-350g); A (11-36w or 350-450g); O (>36w or 450g); Mice: Y (<7w or 18g (F) /23g (F)); YA (7-11w or 18-20g(F)/23-25g(M)); A (11-20w or 20-25g(F)/25-33g(M)); O (>20w or >25g(F)/33g(M)).

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CHAPTER 1.2

Lateralized effects of chronic pain in the transition from habit to goal-directed decision-making

Manuscript in preparation

Lateralized effects of chronic pain in the transition from habit to goal-directed decision-making

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Abstract

Goal-directed and habit-based are equally important decision-making strategies. Goal-direct actions are flexible but computationally demanding while habit-based strategies are more rigid but also more efficient. In changing environments, the transition between these strategies is a fundamental adaptive trait. While chronic pain is frequently comorbid with executive function deficits, few is known about its impact on goal-direct and habitual decisions.

To test this, male rats with a spared nerve injury in the left (SNI-L) or right hind paw (SNI-R) and Sham controls performed an operant task in which an action (pressing a lever) was contingent with an outcome (sucrose pellets or 20% sucrose). Two devaluation tests, in which the animals had previous access for 1 hour to the usual reward (devalued condition) or other (valued condition), were performed in an early and in a late phase of the training. A second test – contingency degradation (CD) – was also performed. In this case, for half of the animals the reward was delivered independently of lever presses.

Lever press activity evolved equally between groups. However, SNI-L maintained the habitual behavior both in the late devaluation and CD test. In a second experiment SNI-L correctly identified value and devalued levers when they were presented simultaneously. However, they again failed to reduce lever press in CD. Dorsomedial striatum neurons, an area involved in goal-directed behavior, were less complex in SNI-L. Chronic stress has been associated with striatal maladaptive plasticity and concomitantly with a bias toward habits. However, in our experimental conditions, no differences were observed in cortisone levels.

1. Introduction

Chronic pain is defined as pain that persist for more than the expected healing time, which is usually considered 3 months. It affects 19% of industrialized countries' adults and is the main cause of long-term disability in these countries (Blyth et al., 2001; Breivik et al., 2006; Kennedy et al., 2014). Chronic pain patients frequently experience attentional deficits and executive dysfunction impairments (Bair and Robinson, 2003; Berryman et al., 2014; Moriarty et al., 2011); similar observations have been reported in preclinical experimental settings (Leite-Almeida et al., 2015; Liu and Chen, 2014; Moriarty et al., 2011). The ability to shift from goal-directed to habit-based decision-making (and vice-versa) is key in the adaptation to changing environments and has received limited attention in the context of chronic pain.

Goal-directed actions (A) are performed based on the knowledge of the outcome (O) and adapted to it (A-O). Changes in the motivation towards the outcome or in its value lead to alterations in action performance (Balleine and Dickinson, 1998; Dickinson, 1985; Dolan and Dayan, 2013; Wood and Rünger, 2015). With the constant repetition of the same A-O, the action becomes less goal-directed and more automatized. In these cases, the habitual response (R) is immediately elicited by the stimuli (S) (S-R), independently of the value of the reward (Balleine and Dickinson, 1998; Dickinson, 1985; Dolan and Dayan, 2013; Wood and Rünger, 2015). Habitual responses are more rapid and efficient but also more rigid. On the other hand, goal-directed strategies are more demanding but highly adaptive.

Lesion and inhibition' studies on rodents reveal an independent involvement of dorsomedial striatum (DMS) and dorsolateral striatum (DLS) on goal-direct and habitual actions, respectively (Balleine et al., 2009; Yin et al., 2009, 2005, 2004). The shift between both types of actions encompasses, in that way, a gradient shift of activation from DMS to DLS (or vice-versa) and of the cortical-striatal circuitries which encompass these regions. In humans, imaging studies reveal similar patterns in the caudate and putamen and the associate frontal areas (Tricomi et al., 2009; Valentin et al., 2007). It is known that factors such as distraction, poor control and reduced decision time favor a reliance on habitual strategies (Wood and Rünger, 2015). In the same way, it is well described that stress (Dias-Ferreira et al., 2009; Schwabe and Wolf, 2010) and addiction (Everitt, 2014; Voon et al., 2015) induce a decrease in goal-directed behaviors and consequently a prevalence of habitual choices. Considering the high percentage of chronic pain patients with depression (Bair and Robinson, 2003) and addictive behaviors (Vowles et al., 2015), as well as the well-established morphofunctional impact of chronic pain in the above-mentioned areas (Borsook et al., 2010; Seminowicz and Moayedi, 2017), we addressed this question using a devaluation paradigm in a traumatic neuropathy model (spared nerve injury; SNI) in the rat. We observed that both Sham

controls and SNI learned the task equally. However, animals with a spared nerve injury in the left hind paw (SNI-L) presented deficits in the transition from habit to goal-directed behavior.

2. Methods

2.1 Animals

A total of 107 Wistar-Han male rats (Charles River, Spain) were used in this work, divided by four independent experiments, as described in table I. Animals were kept in groups of 2 or 3 with food (4RF21; Mucelona SRL, Italy) and water *ad libitum*, except during the behavior, in which food consumption was restricted to 1 hour (h)/day and water was taken at least 1h before the test. Animals weight and well-being was checked frequently and efforts were made to minimize their suffering. Cages were kept in a room with controlled light (12h light/dark cycle), temperature (21±1°C) and humidity (50-60%). All procedures were made in accordance with the guidelines of the European Communities Council Directive 2010/63/EU and approved by the Portuguese National Authority for Animal Health (Direção Geral de Alimentação e Veterinária – DGAV).

2.2 Spared Nerve Injury (SNI)

SNI was performed in 2 months old rats. For that, animals were anesthetized with a mixture of Dorbene® (medetomidine hydrochloride; Laboratorios SYVA, Spain) and Imalgene® (Ketamine; MERIAL, France) (1:1.5 (1 ml/kg), injected intraperitoneal (i.p.)) (Esteves et al., 2019). SNI was then performed in the left (SNI-L) or right (SNI-R) hind paw as described before (Decosterd and Woolf, 2000). Briefly, the three branches of the sciatic nerve were exposed, and the tibial and peroneal nerves were individually ligated (4-0 Silk suture thread, FST, Germany) and distally sectioned while the sural nerve was spared (Figure 1A). Great care was taken to not damage the spared nerve. In Sham animals, the three branches were exposed (half in the left and half in the right hind paw) but left intact. Then, muscle and skin were sutured in two layers and the anesthesia reverted by Antisedan® (80 µl; Atipamezole; ORION Corporation, Finland) subcutaneous (s.c.) injection. Animals were left to recover in their cages and were monitored in the following days for open wounds and signs of inflammation. No major problems were found. Behavior was performed 4 weeks after the surgery.

2.3 Mechanical Allodynia

Mechanical allodynia was measured by the up-and-down method with Von Frey (VF) monofilaments (Chaplan et al., 1994). Eight monofilaments (0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0, 15.0 g) were used. Each

test started with stimulation of the hind paw with the central monofilament (2.0 g). If the animal withdrew or licked the paw after stimulation, an inferior monofilament was applied, if not, a superior one was used. The test ended when the animal responded to the 0.4 g monofilament, do not responded to the 15 g monofilament or after a total of 6 measures around threshold first crossing. 50% threshold was calculated using the formula:

50% g threshold $=\frac{10^{(X_f+k\delta)}}{10000}$,

in which Xf is the value (in log units) of the last monofilament used, k is the tabular value for the pattern of positive/negative responses (Dixon, 1980) and δ is the mean difference between stimuli (in this case 0.224).

2.4 Goal-directed/Habit-based decision-making

The decision-making behavior was done in a skinner box (30.5 cm L × 24.1 cm W × 21.0 cm H, ENV-467, MedAssociates, USA) placed within a sound attenuating box. During the test only one lever (side randomized across experimental groups) was available and reinforcement – a sucrose pellet (dustless precision pellets ® 45 mg, PHYMEP, France) or 0.1 ml of 20% sucrose (Laborsprint, Portugal) – was delivered into a food magazine localized between the two levers (Figure 2A).

After two days of habituation to the box and the reward, the rats were trained to perform an action (lever press) contingent with an outcome (reinforcement). Each session ended after 30 reinforcements or 30 minutes, whichever came first. The action/outcome ratio was sequentially increased across sessions from a continuous reinforcement schedule (CRF) up to a random ratio of 20 lever presses/outcome (RR20) (Figure 2B). An early (de)valuation session was performed at this stage in two sequential days. In this case, 1 hour before the test, animals were given free access to the usual reward (devaluation) or to a different reward (valuation). Animals were then placed in the operant box and lever presses were quantified during 5 minutes in extinction (i.e. the lever was presented but no reward was delivered) (Figure 3A). On the second day, the reinforcement available was the opposite of the 1st day. In the following 6 sessions, animals were trained in a RR20 schedule and finally a late devaluation test (equal to the early test) was performed (Figure 2B). In the second group of animals (Table I) a contingency degradation (CD) was performed. For that, animals performed 2 more RR20 sessions after late (de)valuation. In the next day, half of the animals continued to perform RR20 (non-degraded group) while for the other half the reward was delivered independently of lever presses (CD group) (Figure 3A).

In a following experiment (Table I), training was done with two levers (one at a time) contingent with the two outcomes. As before, an early and a late (de)valuation was performed but, in this case, during the 5 minutes in extinction both levers were available (Figure 4A). For the CD each animal degraded one lever (and one reward) and continued doing RR20 in the other (Figure 4A).
Number of lever presses and session time were recorded in an attached computer by MED-IV software (MedAssociates).

2.5 Neuronal reconstruction

To analyze neurons morphology, a Golgi-Cox staining was performed as described previously (Alves et al., 2017). At the end of the behavioral experiment, anesthetized (Pentobarbital; Ceva Sante Animale, France; i.p.) animals were perfused transcardially with 0.9% Saline and the brains removed. Brains were then immersed in a Golgi-Cox solution (1:1 solution of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with 5% potassium chromate) for 14 days, in the end of which they were transferred to a 30% sucrose solution and sectioned coronally in a vibrating microtome (VT1000S, Leica, Germany). 3D reconstruction of striatal neurons was done under the microscope (BX51, Olympus, USA) with Neurolucida software (MBF Bioscience, USA) by a blind investigator. A minimum of 8 and a maximum of 11 neurons (half in the left and half in the right) were studied for each area, for each animal (N= 4 Sham, 5 SNI-L, 4 SNI-R).

2.6 Corticosterone measurement and dexamethasone suppression test

For corticosterone measurements, blood was collected by vein puncture 3 times in basal conditions (8am, 4pm and 7pm) at non-consecutive days and plus a 4th time after a dexamethasone injection (s.c. 30 μg/Kg; 8pm). Blood was centrifuged at 13 000 rpm for 10 minutes and serum was collected to a separate tube. Serum levels of corticosterone were measured by radioimmunoassay (RIA; Biomedicals, USA).

2.7 Statistical analysis

Graphs were done in GraphPad PRISM 8.0 software (GraphPad software, Inc., USA) and statistical analysis performed in JASP 0.9.2 software (JASP Team (2019), Netherlands). For each test, animals considered extreme outliers according to Tukey's criterion (higher than Q3 + 3(Q3 – Q1), being Q1 and Q3, the 1st and 3rd quartiles, respectively) were excluded. Repeated-measures ANOVA were used to analyze the learning in the operant behavior, the neurons' complexity in sholl analyzes and the corticosterone

levels along the day. One-way ANOVA weas used to study the mechanical allodynia, the levels of corticosterone after dexamethasone injection and neurons' length and paired t-test to compare the valued and the devalued conditions in the operant task. One-tail independent t-tests (1 lever paradigm) and paired t-tests (2 levers paradigm) were used to compare the degraded and the non-degraded lever in the second day of CD. Tukey was used as post-hoc test and Cohens' d (*d*) and eta squared (η^2) were used as effect size measures in t-tests and ANOVA's. Data were considered significant if $p<0.05$. All results are represented as mean ± SEM.

3. Results

We studied the impact of lateralized neuropathic chronic pain on goal directed and habitual decision-making. As expected, SNI induced a marked allodynia in response to VF monofilaments ($F_{(2, 80)}=150.047$, $p<0.001$, $\eta^2=0.790$; Sham vs SNI-L: t= -15.634, $p<0.001$, d=-5.048; Sham vs SNI-R: t= -14.382, $p<0.001$, d=-3.896; conjugated results from all the animals that performed behavior). Importantly, no significant differences in allodynia were found between SNI-L and SNI-R (t=-1.264, p=0.420, d=-0.296) (Figure 1B).

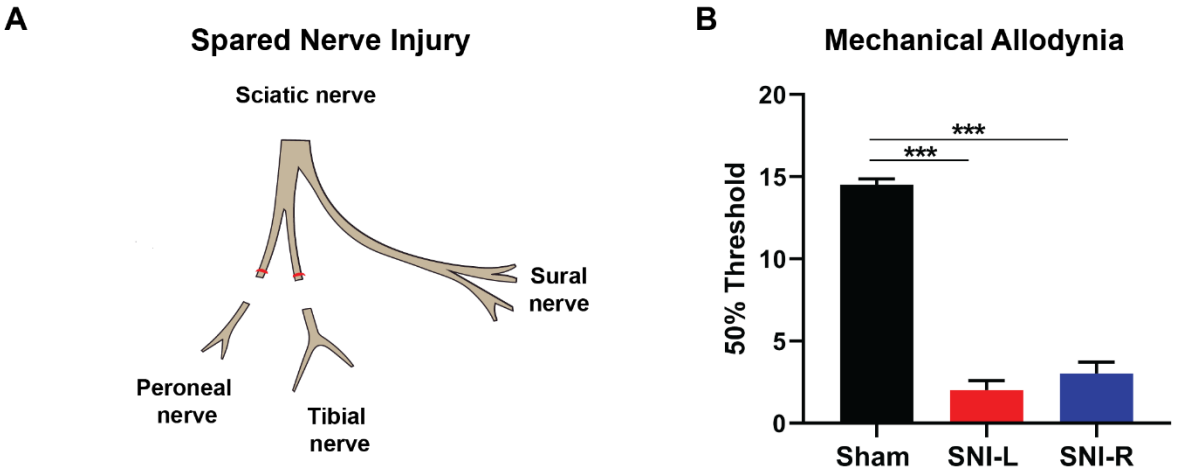

Figure 1. Neuropathy model. For the spared nerve injury (SNI) model, two terminals of the sciatic nerve were ligated and distally cut in the left (SNI-L) or right hind paw (SNI-R) **(A).** SNI-L and SNI-R lead to increased allodynia in response to Von Frey monofilaments in all the animals that perform behavior tests. However, no differences were found between them **(B)**. Results present as mean ± SEM. *** p< 0.001.

Two individual sets of animals performed the operant task with only one lever, and consequently one reward, available (Table I). Animals from both sets perform similarly so results are presented here as one. Furthermore, another group of animals performed 2 sessions per day, one for each lever/reward. All

animals earn the task and progressively increased the number of lever presses per minute across sessions, for both rewards (Figure 2).

For the 1 lever experiment, no statistically significant differences were found between the experimental groups working for sucrose pellets (Group: $F_{(2, 25)}$=0.548, p=0.585, η^2=0.042; Group*Time: $F_{(24,300)}$=1.028, p=0.429, η^2=0.011) (Figure 2C) and for liquid sucrose (Group: $F_{(2, 26)}$=0.082, p=0.922, η^2=0.006; Group*Time: $F_{(24,312)}$=0.372, p=0.997, η^2=0.006) (Figure 2E). In the same way, no differences were found in the 2 levers experiment for sucrose pellets (Group: $F_{(2, 21)}$=2.606, p=0.097, η^2=0.199; Group*Time: $F_{(28, 294)}$=1.403, p=0.090,η^2=0.016) (Figure 2D) and liquid sucrose (Group: $F_{(2, 20)}$=1.218, p=0.317, η^2=0.109; Group*Time: $F_{(28, 280)}$=0.952, p=0.539,η^2=0.013) (Figure 2F).

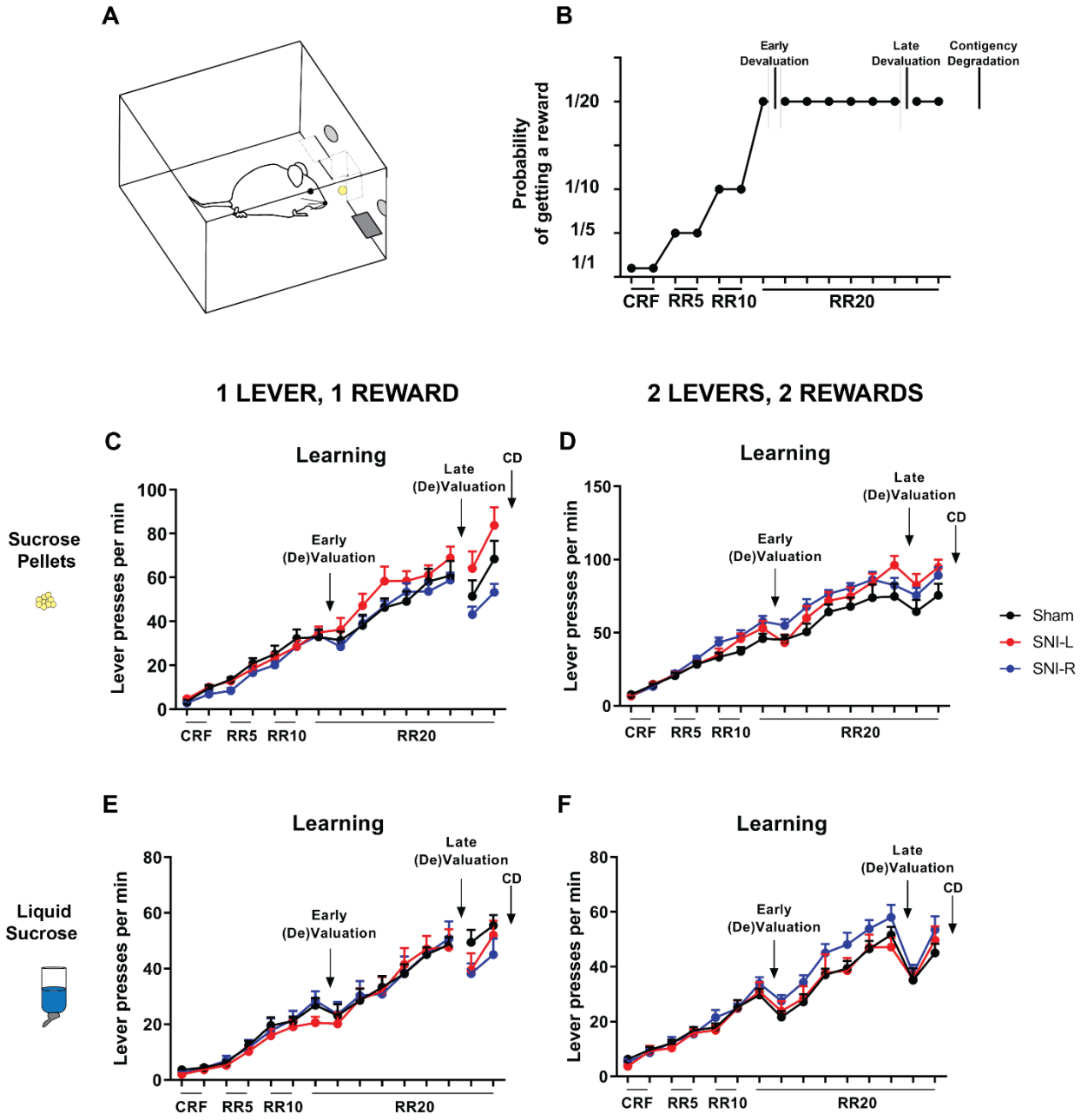

Figure 2. Learning. The goal-directed/habit task was performed in an operant box with a left and a right lever with a reward delivery system in the middle **(A)**. Along the days, the ratio action-outcome increased from a continuous reinforcement schedule (CRF) to a random-ratio (RR) 20 and animals had to press more times the lever to receive the same amount of reward. Two (de)valuation tests were performed, an early and a late test, followed by a contingency degradation **(B)**. SNI did not impact the number of lever presses along the sessions in the one **(C, E)** and two levers **(D, F)** experiment, for sucrose pellets **(C, D)** and 20% sucrose **(E, F)** rewards. On the one lever design only a group of animals performed the extra RR20 sessions before the contingency degradation (CD) **(C, E)**. Results present as mean ± SEM.

To test goal-directed decision/ habitual response, a devaluation test was performed in 2 consecutive days before and after overtraining. In the early (de)valuation all groups from the 1 lever paradigm show a tendency to press less the devalued lever than the valued (Sham: $t_{(16)} = 1.671$, p=0.114, d=0.405; SNI-L: $t_{(17)} = 1.982$, p=0.064, d=0.467; SNI-R: $t_{(16)} = 2.088$, p=0.053, d=0.506) (Figure 3B). When the number of lever presses is normalized for the previous RR20 session, we observe that all groups distinguish the value from the devalue lever (Sham: $t_{(18)} = 2.209$, p=0.040, d=0.507; SNI-L: $t_{(18)} = 2.244$, p=0.038, d=0.515; SNI-R: $t_{(19)} = 2.080$, p=0.051, d=0.465) (Figure 3C). In the late (de)valuation test, both Sham and SNI-R are capable of distinguish the value and the devalue conditions but not SNI-L (Sham: $t_{(17)} = 2.809$, p=0.012, d=0.662; SNI-L: $t_{(16)} = 2.056$, p=0.057, d=0.499; SNI-R: $t_{(16)} = 3.034$, p=0.008, d=0.736; Normalized- Sham: $t_{(16)} = 2.818$, p=0.012, d=0.684; SNI-L: $t_{(15)} = 1.321$, p=0.206, d=0.737; SNI-R: $t_{(15)} = 2.949$, p=0.010, d=0.465) (Figure 3D-E). Importantly, the consumption of either reward during the (de)valuation was not different between the groups (Pellets: $F_{(2, 56)}$=0.400, p=0.672, η^2=0.014; Sucrose: $F_{(2, 56)}$=0.717, p=0.493, η^2=0.025). On the CD, Sham and SNI-R press less the degraded lever than de non-degraded (Sham: $t_{(10)} = 1.775$, p=0.053, d=1.025; SNI-R: $t_{(10)} = 2.769$, p=0.010, d=1.599), while SNI-L do not ($t_{(10)} = -0.058$, p=0.523, d=-0.033) (Figure 3F).

In the 2 levers paradigm, SNI-L appear to be unable to distinguish the value and devalue lever in the early test (Sham: $t_{(6)} = 2.999$, p=0.024, d=1.134; SNI-L: $t_{(5)} = 2.111$, p=0.088, d=0.862; SNI-R: $t_{(6)} = 2.615$, p=0.040, d=0.988; Normalized- Sham: $t_{(6)} = 4.167$, p=0.006, d=1.575; SNI-L: $t_{(6)} = 1.884$, p=0.712, d=0.737; SNI-R: $t_{(7)} = 2.918$, p=0.022, d=1.032) (Figure 4B-C) but not in the late test (Sham: $t_{(7)} = 3.570$, p=0.009, d=1.262; SNI-L: $t_{(6)} = 2.823$, p=0.030, d=1.067; SNI-R: $t_{(6)} = 4.861$, p=0.003, d=1.837; Normalized- Sham: $t_{(7)} = 3.826$, p=0.006, d=1.353; SNI-L: $t_{(7)} = 3.459$, p=0.712, d=1.223; SNI-R: $t_{(7)} = 5.316$, p=0.001, d=1.879) (Figure 4D-E). No statistically significant differences were found in the consumption of pellets ($F_{(2, 21)}$=0.318, p=0.731, η^2=0.029) and sucrose ($F_{(2, 20)}$=0.637, p=0.539, η^2=0.060) during the devaluation. SNI-L animals present a deficit in the CD, not pressing the degraded lever less than the non-degraded ($t_{(7)} = 1.182$, p=0.862, d=-0.418), while SNI-R and Sham degraded the contingency (Sham: $t_{(7)} = -1.950$, p=0.046, d=-0.689; SNI-R: $t_{(6)} = -2.150$, p=0.038, d=-0.813) (Figure 4F).

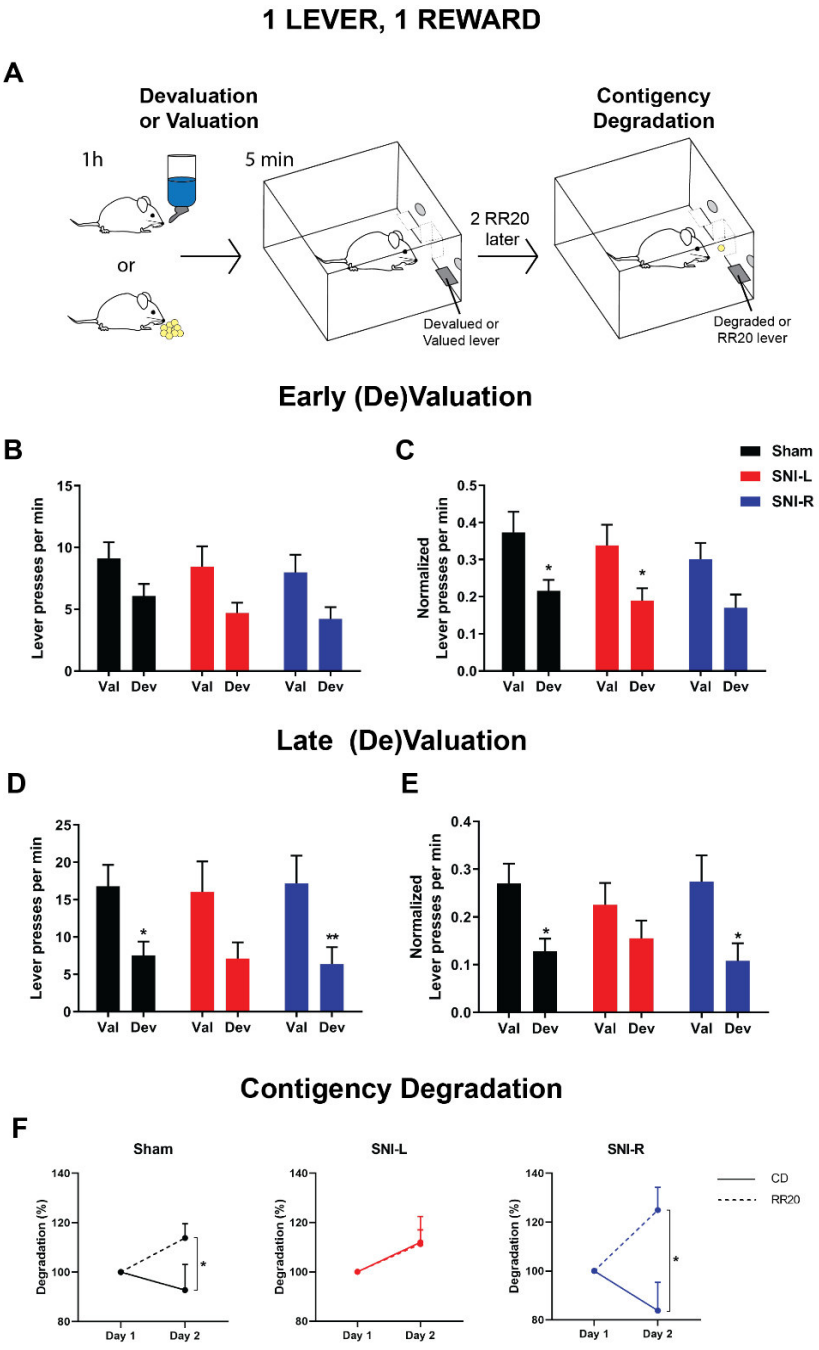

Figure 3. Impact of SNI on the habit behavior in the 1 lever experiment. In the early and late (de)valuation tests, animals were placed for 5min in extinction in the operant box after 1h consuming one of the rewards (devalued reward). Lever presses for the devalued and the valued reward were compared to test goal-directed behavior. During the contingency degradation, for half the animals the reward was delivered independently of lever presses (CD) while the other half remain in RR20 **(A)**. In the early test, all groups show a tendency to devaluate the reward **(B)**, which becomes significant after normalization for the previous RR20 **(C)**. In the late test, the same is true for Sham and SNI-R, but SNI-L do not distinguish between valued and devalued levers **(D, E)**. Similarly, while Sham and SNI-R decrease the number of lever presses from the first to the second day of CD, SNI-L increase **(F)**. Results present as mean ± SEM. * p< 0.05, ** p< 0.01. Dev- devalued; L- left; R- right; RR- random ratio; SNI- spared nerve injury; Val- valued

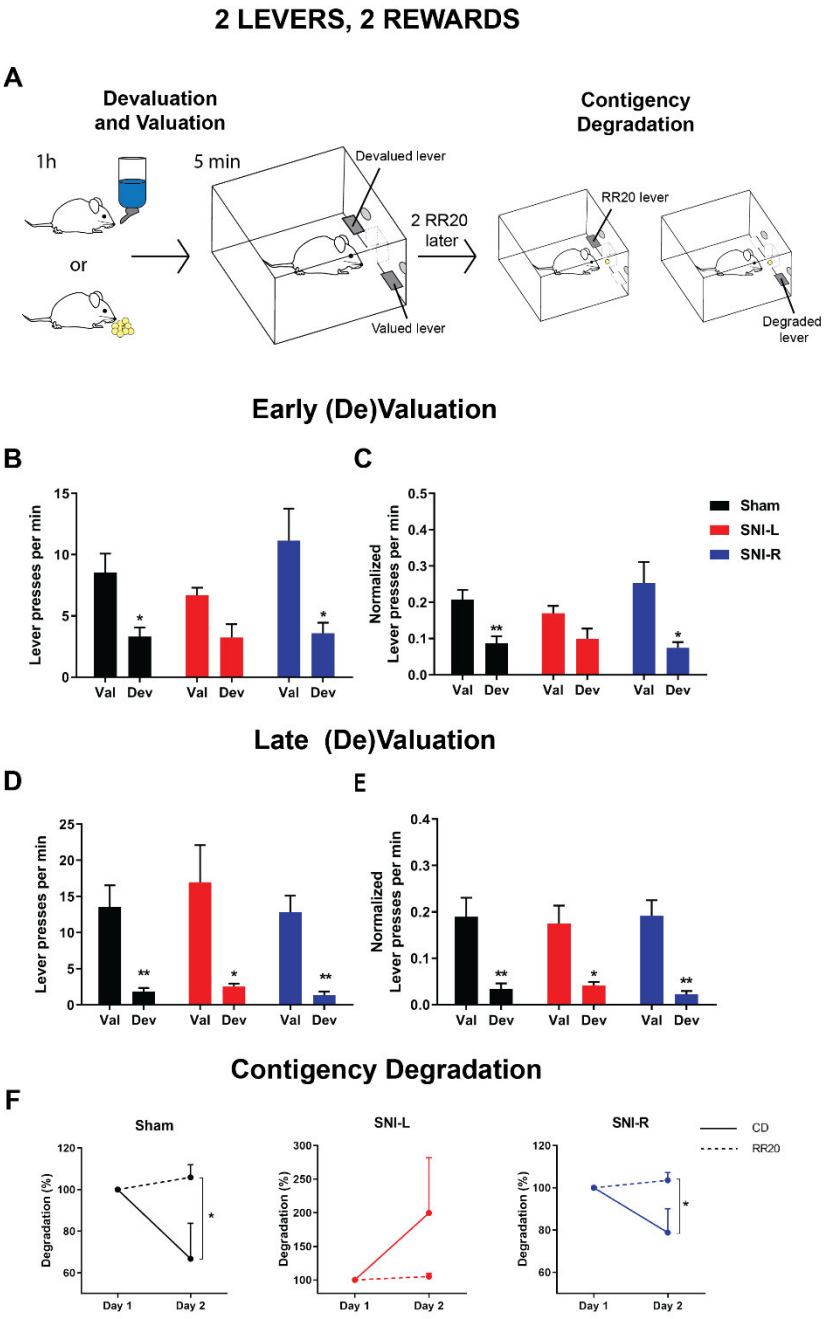

Figure 4. Impact of SNI on the habit behavior in the 2 levers experiment. In the early and late (de)valuation tests, animals were placed for 5min in extinction in the operant box with both levers available, after 1h consuming one of the rewards (devalued reward). Lever presses for the devalued and the valued reward were compared to test goal-directed behavior. During the contingency degradation, one of the rewards was delivered independently of lever presses (CD) while the other remained in RR20 **(A)**. In the early test, SNI-R and Sham devaluate the reward but not SNI-L **(B, C)**. In the late test, all groups distinguish between valued and devalued levers **(D, E)**. As for the 1 lever experiment, while Sham and SNI-R decrease the number of lever presses from the first to the second day of CD, SNI-L increase **(F)**. Results present as mean ± SEM. * p< 0.05, ** p< 0.01. Dev- devalued; L- left; R- right; RR- random ratio; SNI- spared nerve injury; Val- valued

Striatal neurons from some of the animals which perform the operant task with 2 levers were reconstructed later. No differences between groups were found in the length of DMS ($F_{(2,10)}$=2.003, p=0.186, η^2=0.286) and DLS neurons ($F_{(2,10)}$=0.955, p=0.417, η^2=0.160) (Figure 5A-B).

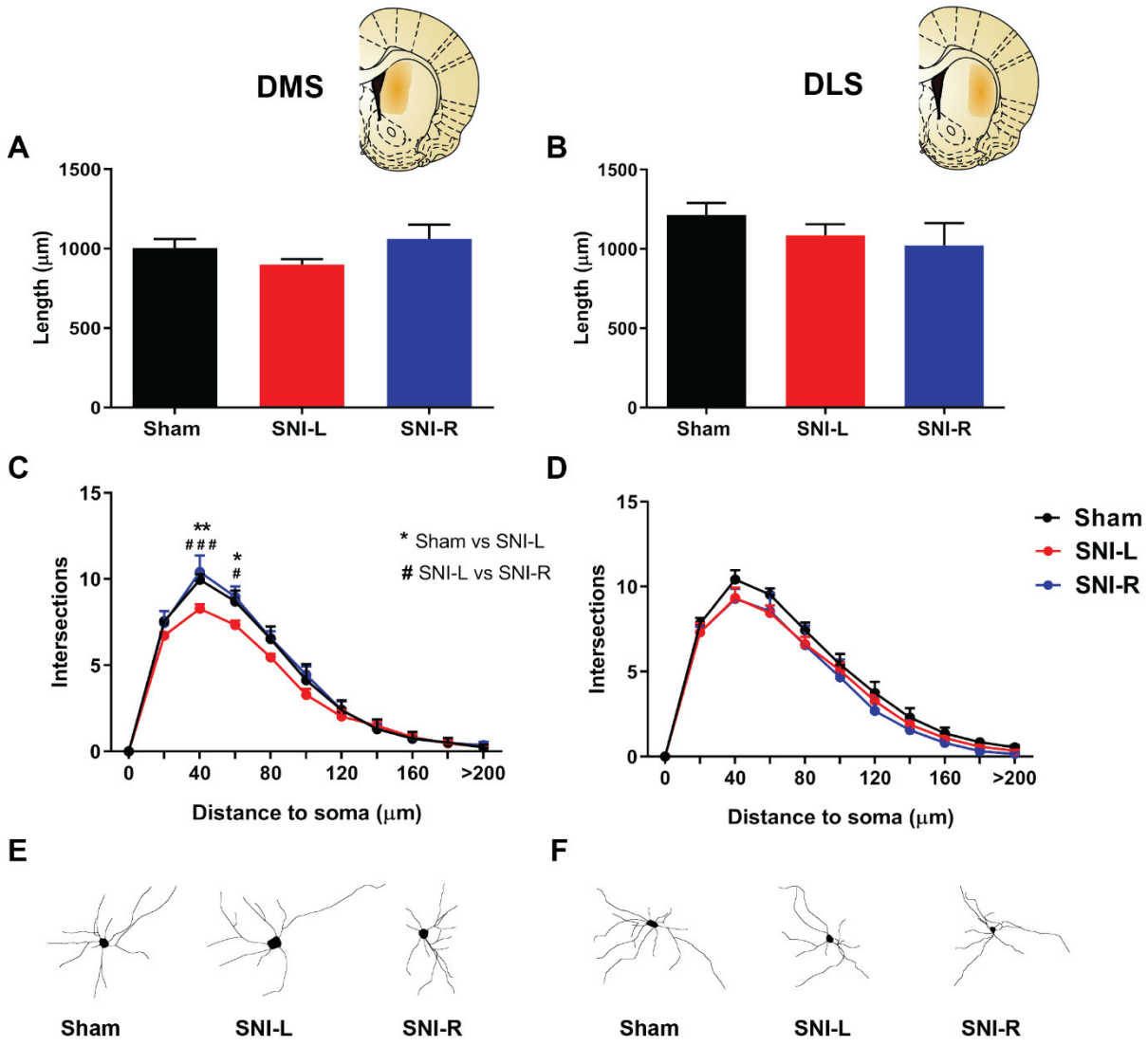

Figure 5. 3-D reconstruction of striatal neurons. Neurons from the dorsal medial (DMS) and lateral (DLS) striatum were reconstructed after Golgi staining. Results reveal no differences between the groups in the length of the neurons in both areas **(A, B)**. Neurons complexity is not affected on the DLS by the neuropathic lesion **(D, F)**, but on DMS, neurons from SNI-L animals are less complex **(C, E)**. Results present as mean ± SEM. * p< 0.05, ** p< 0.01 SNI-L vs Sham # p< 0.05, ### p< 0.001 SNI-L vs SNI-R. DLS- dorsolateral striatum; DMS- dorsomedial striatum; L- left; R- right; SNI- spared nerve injury

Similarly, no differences were found in neurons' complexity in DLS (Group: $F_{(2,\ 10)}$=1.100, p=0.370, η^2=0.180; Group*Distance: $F_{(20,\ 100)}$=0.298, p=0.998, η^2=0.002). A significant difference in the Group and distance to soma interaction was observed in the DMS neurons (Group: $F_{(2,\ 10)}$=1.562, p=0.257, η^2=0.238; Group*Distance: $F_{(20,100)}$=0.077, p=0.010, η^2=0.009). Posterior comparisons revealed that DMS neurons

from SNI-L were less complex than Sham and SNI-R neurons at the most proximal regions (40µm- Sham vs SNI-L: p=0.001; SNI-L vs SNI-R: p<0.001; 60µm- Sham vs SNI-L: p=0.042; SNI-L vs SNI-R: p=0.012) (Figure 5C-F).

Finally, we show, in a separated group of animals, that no alterations in the levels of corticosterone along the day were found in SNI-L and SNI-R animals 1 month after the lesion (Group: $F_{(2,21)}$=2.247, p=0.131, η^2=0.176; Group*Time: $F_{(4,42)}$=0.513, p=0.726, η^2=0.011) (Figure 6A). Also, SNI-L and SNI-R did not present any impairment in the inhibition of hypothalamic–pituitary–adrenal (HPA) axis after a dexamethasone injection ($F_{(2,21)}$=0.097, p=0.908, η^2=0.009) (Figure 6B).

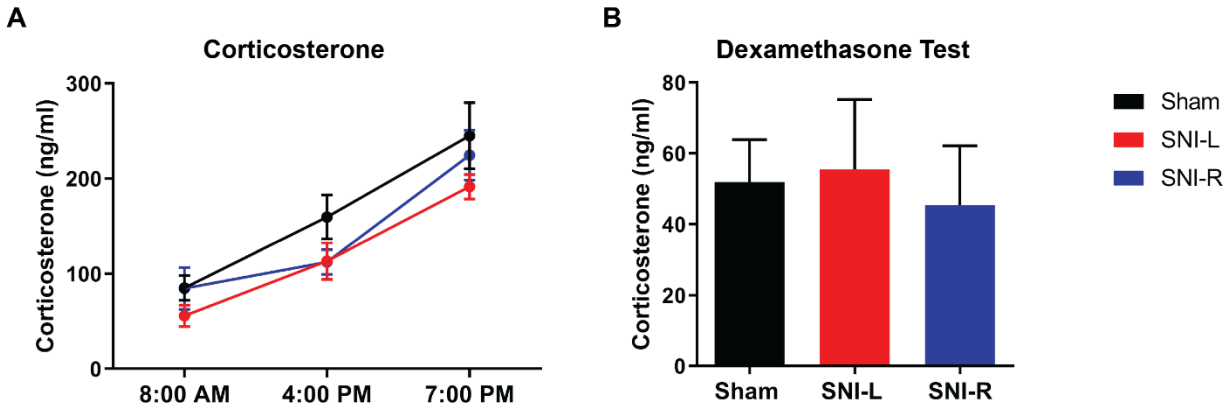

Figure 6. Impact of SNI on corticosterone. Corticosterone levels were measured in the blood serum by radioimmunoassay at 3 different timepoints **(A)** and after a dexamethasone injection **(B)**. No differences between the groups are found in none of the timepoints **(A, B)**, discarding an interference of stress in the behavioral results. Results present as mean ± SEM. L- left; R- right; SNI- spared nerve injury

4. Discussion

In this work we studied the impact of CP on the transition from habit-based to goal-directed decision-making. We showed that SNI-L (but not SNI-R) animals maintained their habitual behavior upon reward devaluation and CD indicating an impairment in the shift from habit to goal-directed behavior. Importantly, when both valued and devalued options were presented simultaneously in the 2 levers protocol, SNI-L distinguished the 2 conditions. Such indicates that the high lever pressing levels in the devalued 1 lever protocol was not a result from a recognition problem but from the maintenance of the habitual behavior. Mor and colleagues (2017) have previously investigated this with a 2 levers' operant task similar to ours. They show that SNI-R animals devaluate the reward as well as controls, which is concordant with our results (Mor et al., 2017). Previous rodent studies have also shown that neuropathic chronic pain leads to impairments in cognitive flexibility in the reversal of Morris Water Maze (MWM) and Attentional Set

Shifting Task (ASST) (Leite-Almeida et al., 2014, 2012, 2009; Moriarty et al., 2016). Even though these are reported on older or right-lesioned rats, we could not exclude similar mechanisms for the impact of chronic pain on behavioral flexibility and the prevalence of habitual behaviors described here. Interestingly, in a recent report, we observed that SNI-R and Sham correct their behavior from the first to the second test, in an impulsivity operant task, reflecting a learning/re-test effect. But the same was not true for SNI-L, which can again reflect a decrease capacity to shift behavior (A.M. Cunha et al., 2020a).

Other lateralized impacts of chronic pain on emotional and cognitive comorbidities have been previously observed (Leite-Almeida et al., 2012, 2014). We hypothesize that these differences arouse from different impacts of left and right peripheral lesions in the brain, as already observed for c-fos expression (Leite-Almeida et al., 2014). Rats which rely more on habitual behaviors present shorter and less complex neurons in the DMS and longer and more complex neurons on the DLS (Dias-Ferreira et al., 2009). Not surprisingly, our results show that DMS neurons of SNI-L animals are less complex than the ones from Sham and SNI-R. However, more studies are needed to determine if this is a direct consequence of the neuropathic lesion and why only affects SNI-L animals. Also to take into consideration it is the fact that goal-directed and habitual strategies appear to be lateralized in the brain. In recent work we demonstrated that DA depletion in the right (but not left) Nucleus Accumbens negatively affects CD (A.M. Cunha et al., 2020b). Furthermore, DA content in the left DMS and sensitivity to devaluation are negatively associated and in the right DLS the inverse is observed (Gemikonakli et al., 2019). In humans, evidences of striatal lateralization in related behaviors have also been reported (Porat et al., 2014; Szatkowska et al., 2011).

SNI-L, and not SNI-R, present an anxious-like phenotype when compared with Sham (Leite-Almeida et al., 2012). It is known that stress, by itself or in chronic pain conditions, induces a higher reliance on habit-based strategies (Dias-Ferreira et al., 2009; Mor et al., 2017). However, we show that SNI-L, 1 month after lesion, do not present any impairment in the release of corticosterone neither in the control of HPA axis. Still, we cannot exclude the possibility of stress-related alterations in the acute phase of the lesion, which impact brain structure and behavior in a later timepoint.

In conclusion, with this work we show that SNI-L present an impairment in the transition from habit to goal-direct decisions. Higher reliance on habitual-responses in chronic pain status as well as alterations in the limbic system can give important clues about the lack of motivation and the prevalence of addictive behaviors on chronic pain patients.

Table I. Animals distribution according to the 4 experiments performed

Experiment	Group number (Sham/SNI-L/SNI-R)
• Goal-directed/Habit-based decision-making (devaluation) - 1 lever	23 (7/8/8)
• Goal-directed/Habit-based decision-making (devaluation and contingency degradation) - 1 lever	36 (12/12/12)
• Goal-directed/Habit-based decision-making - 2 levers • Golgi-Cox staining and morphological reconstruction of neurons	24 (8/8/8)
• Corticosterone quantification • Dexamethasone suppression test	24 (8/8/8)

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Authors Contributions

SB and HLA design the experiment. AMC, SB, MRG, OFXA and HLA performed the experiences. AMC did the statistical analysis. AMC and HLA wrote the first version of the manuscript. All authors discussed and interpreted the data and revised and approved the final version of the manuscript.

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CHAPTER 1.3

High trait impulsivity potentiates the effect of chronic pain on impulsive behavior

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Original Research

High trait impulsivity potentiates the effects of chronic pain on impulsive behavior

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ABSTRACT

Preclinical studies on impulsive decision-making in chronic pain conditions are sparse and often contradictory. Outbred rat populations are heterogeneous regarding trait impulsivity manifestations and therefore we hypothesized that chronic pain-related alterations depend on individual traits. To test this hypothesis, we used male Wistar-Han rats in two independent experiments. Firstly, we tested the impact of spared nerve injury (SNI) in impulsive behavior evaluated by the variable delay-to-signal test (VDS). In the second experiment, SNI impact on impulsivity was again tested, but in groups previously categorized as high (HI) and low (LI) trait impulsivity in the VDS.

Results showed that in an heterogenous population SNI-related impact on motor impulsivity and delay intolerance cannot be detected. However, when baseline impulsivity was considered, HI showed a significantly higher delay intolerance than the respective controls more prevalent in left-lesioned animals and appearing to result from a response correction on prematurity from VDS I to VDS II, which was present in Sham and right-lesioned animals.

In conclusion, baseline differences should be more often considered when analyzing chronic pain impact. While this study pertained to impulsive behavior, other reports indicate that this can be generalized to other behavioral dimensions and that trait differences can influence not only the manifestation of comorbid behaviors but also pain itself in a complex and not totally understood manner.

1. Introduction

Impulsivity is defined as a predisposition toward rapid and unplanned actions without regard to their negative consequences. It is classically divided in two main dimensions: motor and choice impulsivity, the former related with the incapacity to suppress actions and the latter reflecting delay intolerance, characterized, for instance, as a preference for small but immediate instead of larger but delayed rewards (Bari and Robbins, 2013; Dalley et al., 2011). Impulsivity can be advantageous in competitive environments, where riskier and quicker actions can result in positive outcomes. However, impulsivity can be maladaptive, manifesting in conditions such as attention deficit and hyperactivity disorder (ADHD), addiction and substance abuse (Dalley and Robbins, 2017; Patros et al., 2016; Smith et al., 2014) and even in neurodegenerative disorders (Gleichgerrcht et al., 2010). On the other hand, evidence suggests that trait impulsivity is a predisposing factor for the development of maladaptive behaviors – see for instance (de Wit, 2009).

Around 10% of chronic pain patients develop addictive behaviors and are highly predisposed to opioid misuse (Vowles et al., 2015). However, little is known about the relationship between impulsivity and chronic pain. Some studies indicate that chronic pain patients and healthy subjects present similar scores in the Barret Impulsivity Scale (BIS) (Berger et al., 2014; Margari et al., 2014). Similarly, no differences were observed in the Go/No-Go and Stroop tasks (Glass et al., 2011; Jongsma et al., 2011; Pidal-Miranda et al., 2019; Veldhuijzen et al., 2012). On the other hand, chronic pain patients with comorbid opioid misuse score higher on BIS than chronic pain patients without this problem (Margari et al., 2014; Marino et al., 2013; Tompkins et al., 2016). Additionally, urgency and sensation-seeking dimensions of the urgency, premeditation, perseverance and sensation-seeking (UPPS) impulsivity scale can predict this misuse (Vest et al., 2016), suggesting an importance of trait impulsivity on analgesic-related addiction.

In rodent models of chronic pain, data is scarce and conflicting –

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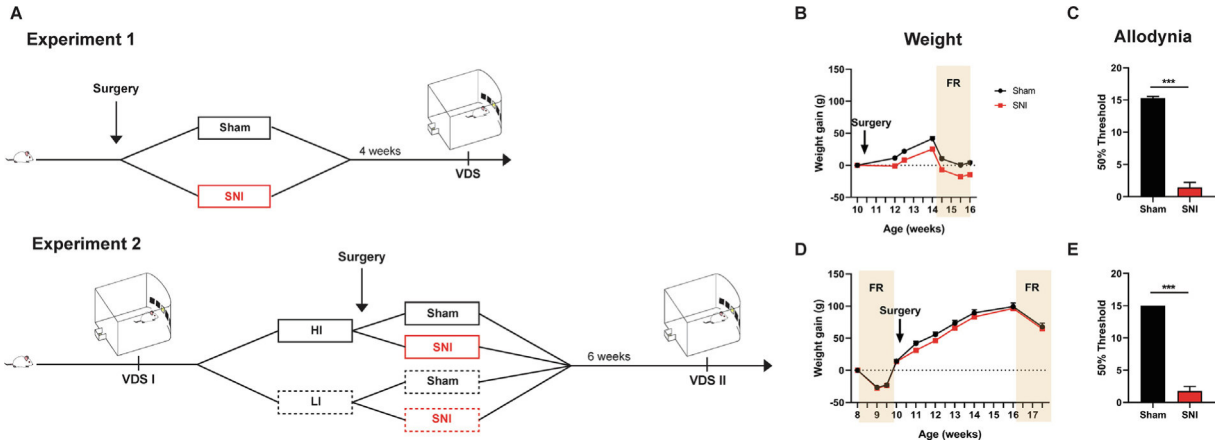

Fig. 1. Experimental Design. Experimental design for experiments 1 and 2 (A). Weight evolution of Sham and SNI was similar in experiment 1 (B) and 2 (D); average difference between groups was at its maximum < 4% of controls' weight. SNI animals present increased allodynia to the Von Frey monofilaments in comparison to controls in experiment 1 (C) and 2 (E). Data presented as mean ± SEM. *** $p < 0.001$. FR – Food restriction; HI – High impulsive; LI – Low Impulsive; SNI – Spared Nerve Injury; VDS – Variable delay-to-signal.

contrast for instance (Pais-Vieira et al., 2009; Leite-Almeida et al., 2012; Higgins et al., 2015). Recently, we have analyzed a large cohort of rats in the variable delay-to-signal (VDS) and found a high variability in trait impulsivity related with sex, age and even strain (Soares et al., 2018). Considering the conflicting results in chronic pain conditions and the heterogeneity observed in outbred animals, we hypothesized that baseline impulsivity can influence the impact of chronic pain on impulsive behavior. For that we first compared impulsive behavior of controls and neuropathic rats on the VDS paradigm. Next, we evaluate the impact of the neuropathic lesion on animals with high and low levels of impulsivity at the baseline. We show that the neuropathy exacerbates impulsivity in animals that present high trait impulsivity at baseline.

2. Materials and methods

2.1. Experimental design

Two independent experiments were performed in this study (Fig. 1A). In the first experiment we studied the impact of chronic pain on impulsivity. For that, a group of rats performed the VDS paradigm 4 weeks after the spared nerve injury (SNI) or the Sham surgery. In the second experiment, we studied the influence of baseline impulsivity on chronic pain-related impulsivity. For that, a group of naïve rats was divided in high (HI) and low impulsive (LI) according to their performance in the VDS (VDS I). Then, SNI was performed in 2/3 of the animals from each group and Sham surgery on the other 1/3. 6 weeks after the surgery, animals were tested again in the VDS paradigm (VDS II).

2.2. Experimental subjects

All procedures were approved by the Portuguese National Authority for Animal Health (Direção Geral de Alimentação e Veterinária – DGAV) and in accordance with the guidelines of the European Communities Council Directive 2010/63/EU. Efforts were made to ensure the well-being of the animals used. Sixty (30 + 30) male Wistar-Han rats with 7 weeks of age at the beginning of the experiments were used. Animals were kept in Specific Pathogen Free (SPF) conditions, in a room with controlled temperature (22 °C ± 1 °C) and humidity (50–60%) under a 12 h light/dark cycle (lights on at 8 am), and randomly housed in pairs in plastic cages with food (4RF21; Mucedola, SRL, Settimo Milanese, Italy) and water available *ad libitum*, except during the VDS protocol, in which food availability was restricted to 1 h/day. Weight was controlled along the entire experiment time course, particularly during the food restriction period to prevent drops below 15% of baseline values.

2.3. Variable delay-to-signal

The VDS was performed in 5-hole operant chambers (25 × 25 cm; TSE Systems, Germany) as previously described (Leite-Almeida et al., 2013). Briefly, the VDS test comprised 3 phases: habituation to the chamber and rewards (4 days), training, and VDS test. The training consisted of 10 sessions (1 per day) with a maximum of 100 trials or 30 min each. Animals which repeated the VDS, performed only 6 sessions of training in the VDS II. Trials started with the lightning of the house light for 3 s (delay period) followed by the light stimulus (3 W) in the response aperture (central hole) for 60 s (response period). Nosepokes during the response period were rewarded with the delivery of a sugar pellet (45 mg, Bioserv Inc., New Jersey, EUA). Omissions and responses in the delay period (premature responses) were punished with a timeout in complete darkness (3 s) and no reward. The order in which animals performed the training was changed along the days to exclude timing effects. The test session was composed by 120 trials similar to training. This session started with an initial block of 25 trials with 3 s-delay trials ($3s_i$) followed by 70 trials of 6- and 12-seconds delays (6 s and 12 s, respectively) presented in a random order. The VDS test session terminated with a final block with 25 trials of 3 s delays ($3s_f$). In the test, premature responses were registered but not punished.

Motor impulsivity was evaluated by the number of premature responses along training, while choice impulsivity was evaluated by the number of premature responses in the test during and after the exposure to 6 s and 12 s delays (delay intolerance). Prematurity rate (PR) was defined as the number of premature responses per time of available delay and PR_{ratio} as the PR corrected for baseline responsiveness ($3s_i$):

$$PR_{ratio} = log\frac{PR6s/12s/3s_f}{PR3s_i}$$

2.4. Spared nerve injury

Chronic neuropathic pain was induced using the SNI (Decosterd and Woolf, 2000). Rats were anesthetized via intraperitoneal administration of 1:1.5 mix (1 ml/kg) of Sededorm® (Medetomidine, 1 mg/mL – Vet-Pharma Animal Health, Spain) and Ketamidor® (Ketamine, 100 mg/mL – Richter Pharma AG, Austria), respectively (Esteves et al., 2019). A

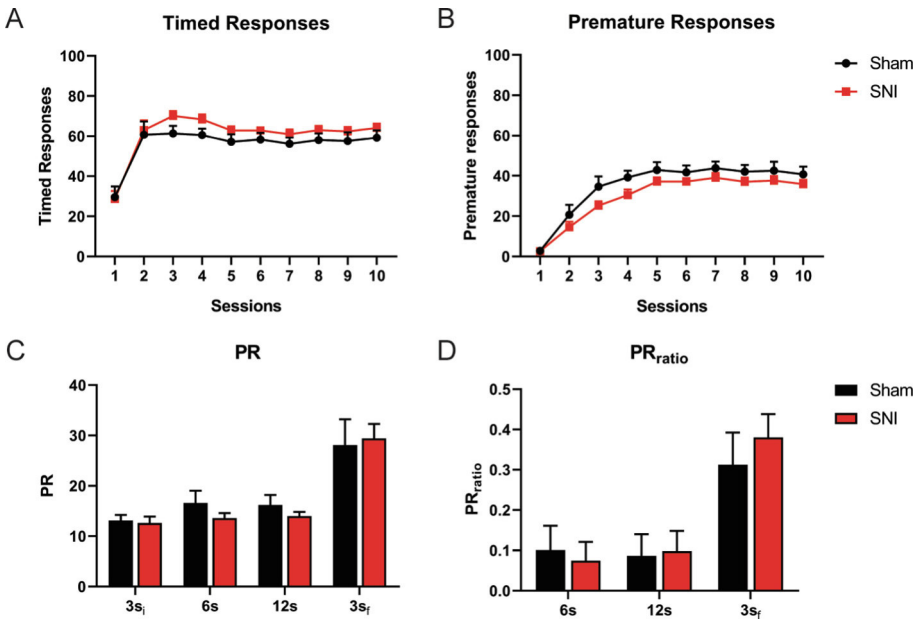

Fig. 2. Experiment 1: impact of chronic pain on impulsive behavior. Both groups learned the task equally (A) and no differences on impulsive behavior were found between the groups during VDS training (B) nor test (C, D). Data presented as mean ± SEM. $3s_i$ – initial trials with 3 s' delay; 6 s – trials with 6 s' delay; 12 s – trials with 12 s' delay; $3s_f$ – final trials with 3 s' delay; PR – Prematurity rate; SNI – Spared Nerve Injury; VDS – Variable delay-to-signal.

blunt incision was then performed to expose the three branches of sciatic nerve: common peroneal, tibial and sural nerves. A unilateral ligation and subsequent distal axotomy of the tibial and common peroneal nerves were then performed, leaving the sural nerve (spared nerve) intact. In Sham animals all the nerves were exposed but left intact. Anaesthesia was reversed with a subcutaneous injection of Antisedan® (Atipamezole Hydrochloride, ORION Corporation, Finland). In both experiments, 10 animals were used as Sham controls and 20 as SNI, 10 lesioned in the left (SNI-L) and 10 in the right hindpaw (SNI-R). In the second experiment, animals were ranked by their PR_{ratio} in VDS I and distributed according to the following sequence: Sham, SNI-L, SNI-R, SNI-R, SNI-L, Sham. After SNI, animals were left to recover in their cages and monitored for open wounds and signs of inflammation. No major problems were observed in the study.

2.5. Mechanical allodynia

Mechanical allodynia was assessed as previously described (Guimarães et al., 2018) using the up-and-down method (Chaplan et al., 1994). Briefly, Von Frey monofilaments of different forces were used: 15.0 g, 8.0 g, 6.0 g, 4.0 g, 2.0 g, 1.0 g, 0.6 g and 0.4 g (North Coast Medical Inc., USA). Each measure started with the central filament (2.0 g) and advanced upward if no response was elicited or downward if a brisk paw withdrawn was observed, until 6 measures around the turning point were obtained or the limits of the scale were reached. 50% threshold was then calculated using the formula

$$50\%g\_threshold = \frac{(10^{Xf+K.\delta})}{10000}$$

where X_f = value (in log units) of the final von Frey filament; k = tabular value corresponding to pattern of positive and negative responses; δ = mean difference (in log units) between stimuli (0.224).

2.6. Statistics

Statistical analysis was done in the JASP 0.9.2 software (JASP Team (2019), Netherlands) and graphs were obtained through GraphPad PRISM 8.0 software (GraphPad software, Inc., USA). In the second experiment, 2 animals (1 Sham HI and 1 Sham LI) were excluded from the VDS test analysis because they were considered extreme outliers according to Tukey's criterion (higher than Q3 + 3(Q3 – Q1), being Q1 and Q3, 1st and 3rd quartile, respectively) in the 6 s and in the $3s_i$ and $3s_f$ intervals, respectively. Repeated measures ANOVA was used to analyze body weight, VDS training, VDS test and re-test effects. Independent t-tests were used to compare SNI and Sham allodynia and PR or its ratios, at the different intervals, whenever a difference between the groups was revealed in the ANOVA. Mauchly's test was used to evaluate sphericity, and Levene's test to evaluate equality of variances. The Greenhouse-Geisser and the Welch corrections were used when sphericity and equality of variances were rejected, respectively. Bonferroni was used as post-hoc test. Cohens' d (d) and eta square(η^2) were used as effect size measures in t-tests and ANOVA's, respectively. Data was considered significant if $p < 0.05$. All results are represented as mean ± SEM.

3. Results

3.1. Body weight and mechanical allodynia

Body weight was controlled frequently to ensure animals' well-being and to regulate weight loss during food restriction. None of the animals lost more than 15% of its initial weight. In the first experiment, SNI rats were more affected by the surgery and weight gain remain below the levels of controls throughout the experiment ($F_{(1, 28)} = 21.61$, $p > 0.001$, $\eta^2 = 0.436$) (Fig. 1B). Importantly, after the first week, the weight evolution was identical between the 2 groups ($F_{(1, 28)} = 2.045$, $p = 0.164$, $\eta^2 = 0.068$) and, at its maximum, average weight difference between the two groups was < 4% of controls weight. In the second experiment, no differences between the groups were found (Lesion: $F_{(1, 28)} = 2.438$, $p = 0.130$, $\eta^2 = 0.080$; Lesion*Time: $F_{(1.976, 55.340)} = 1.290$, $p = 0.283$, $\eta^2 = 0.002$) (Fig. 1D).

As expected, SNI animals developed mechanical allodynia after the installation of the model in the first ($t_{(28)} = -12.678$, $p > 0.001$, $d = -4.910$) (Fig. 1C) and second experiment ($t_{(19)} = 18.52$, $p > 0.001$, $d = -5.026$) (Fig. 1E).

3.2. Experiment 1 – chronic pain does not affect impulsivity

The potential impact of chronic pain on impulsivity was accessed using the VDS test (Fig. 1A, Experiment 1).

All animals learned the task equally (Lesion: $F_{(1, 28)} < 0.001$, $p > 0.999$, $\eta^2 < 0.001$; Lesion*Time: $F_{(9, 252)} = 0.097$, $p > 0.999$, $\eta^2 = 0.001$) (Fig. 2A) as observed by the evolution of the timed responses. Also, no significant differences between groups were observed

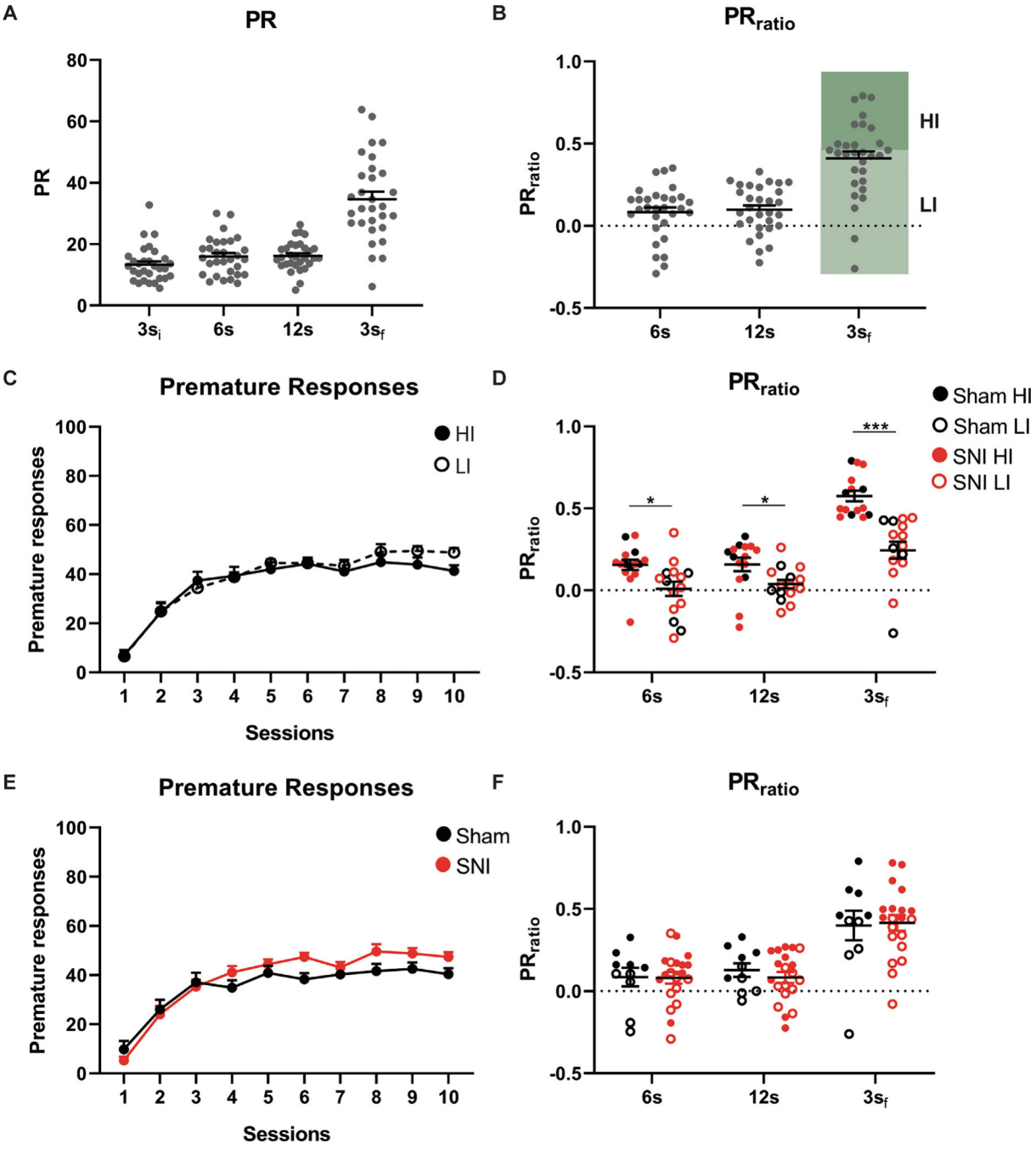

Fig. 3. Experiment 2, VDS I: determination of baseline impulsivity. Naïve rats show different levels of impulsivity in the VDS test (A). Based on the $3s_f$ PR_{ratio} (B), animals were divided in half in HI and LI. HI rats are more impulsive on the test (D) but not during training (C). Rats selected for SNI or Sham surgeries, presented similar impulsive behavior (E, F). Data presented as mean ± SEM. * $p < 0.05$ *** $p < 0.001$. $3s_i$ – initial trials with 3 s' delay; 6 s – trials with 6 s' delay; 12 s – trials with 12 s' delay; $3s_f$ – final trials with 3 s' delay; HI – High impulsive; LI – Low Impulsive; PR – Prematurity rate; SNI- Spared Nerve Injury; VDS – Variable delay-to-signal.

in the number of premature responses throughout training (Lesion: $F_{(1, 28)} = 3.654$, $p = 0.066$, $\eta^2 = 0.115$; Lesion*Time: $F_{(3.606, 100.978)} = 0.646$, $p = 0.615$, $\eta^2 = 0.007$) (Fig. 2B) or in the test sessions (PR – Lesion: $F_{(1, 28)} = 0.193$, $p = 0.064$, $\eta^2 = 0.007$; Lesion*Time: $F_{(1.400, 39.203)} = 0.643$, $p = 0.478$, $\eta^2 = 0.010$; PR_{ratio} – Lesion: $F_{(1, 27)} = 0.046$, $p = 0.831$, $\eta^2 = 0.002$; Lesion * Time: $F_{(1.374, 37.104)} = 1.103$, $p = 0.322$, $\eta^2 = 0.005$) (Fig. 2C-D).

3.3. Experiment 2

3.3.1. VDS I – determination of baseline impulsivity

To test for potential effects of baseline impulsivity, we evaluated, in a second experiment, impulsive behavior before pain onset (Fig. 1A, Experiment 2). The group presented high heterogeneity regarding PR and PR_{ratio} on the $3s_f$ of the VDS test (Fig. 3A-B). Based on the $3s_f$ PR_{ratio} animals were divided in two groups: half were considered HI and half LI (Fig. 3B). 2/3 of each group were ascribed to SNI surgery while the remaining 1/3 to Sham surgery. In a retrospective analysis accounting for the newly formed groups, no differences were observed in the

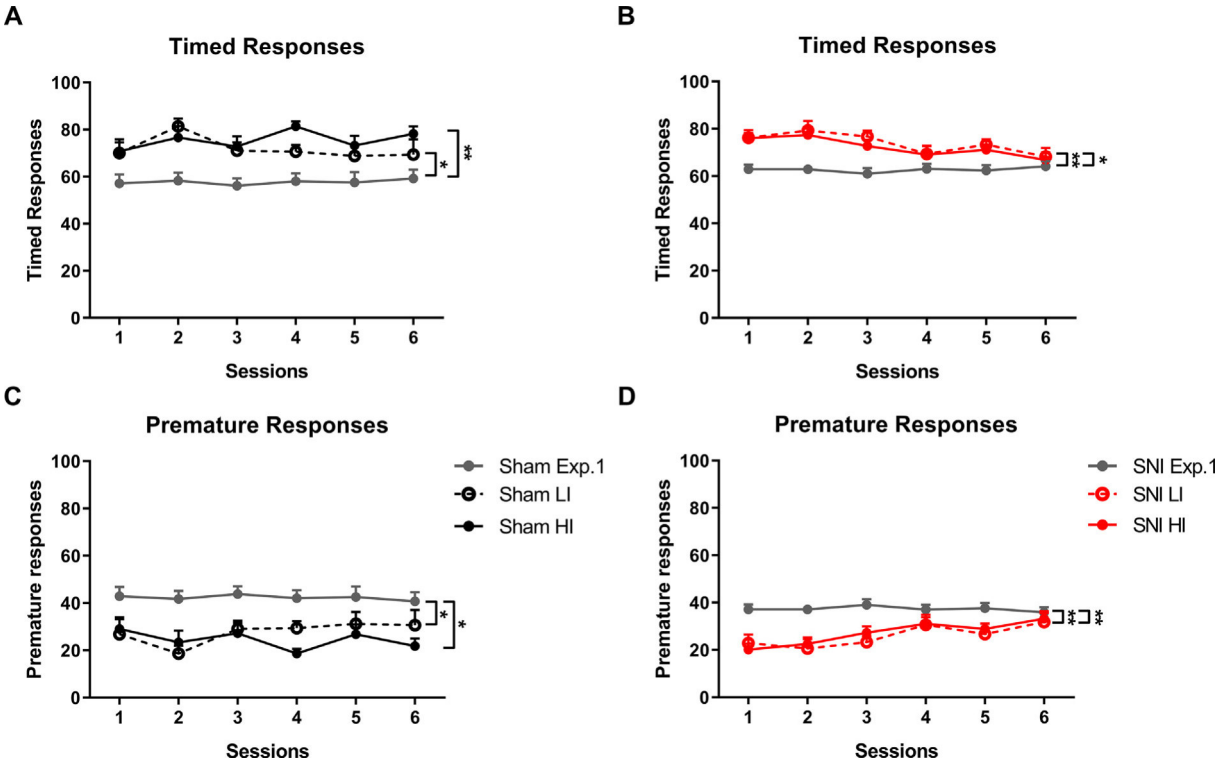

Fig. 4. Re-test effects. Both Sham and SNI increased the number of timed responses (A, B) and decrease prematurity levels (C, D) when compared with the same groups from experiment 1. No differences were found in the number of timed and premature responses between the four groups. HI – High impulsive; LI – Low Impulsive; SNI – Spared Nerve Injury.

learning curves for LI vs HI comparisons ($F_{(1,\ 28)} = 0.439$, $p = 0.513$, $\eta^2 = 0.015$) (Fig. 3C) and Sham vs SNI comparisons ($F_{(1,\ 28)} = 1.341$, $p = 0.257$, $\eta^2 = 0.046$) (Fig. 3E). Expectedly, HI presented a significantly higher PR_{ratio} than LI in all phases of the VDS test ($F_{(1,\ 28)} = 19.09$, $p < 0.001$, $\eta^2 = 0.450$; 6 s: $t_{(28)} = 2.706$, $p = 0.011$, $d = 0.988$; 12 s: $t_{(28)} = 2.454$, $p = 0.021$, $d = 0.896$; 3sf: $t_{(28)} = 5.387$, $p < 0.001$, $d = 1.967$) (Fig. 3D). No differences were however found between the animals ascribed to be Sham or SNI ($F_{(1,\ 28)} = 0.031$, $p = 0.861$, $\eta^2 = 0.001$) (Fig. 3F).

3.3.2. VDS II – CP effects on impulsivity are dependent on the baseline

After the definition of the groups, HI and LI (3.2.1), SNI surgery was performed. At 6 weeks post-surgery, animals performed 6 VDS training sessions, presenting a moderate reduction on premature responses and consequently an increase in the number of timed responses, in comparison with rats of the same groups from experiment 1 (Timed responses – Sham: $F_{(2,\ 17)} = 7.398$, $p = 0.005$, $\eta^2 = 0.465$; Exp 1 vs HI: $t = -3.453$, $p = 0.009$, $d = -0.772$; Exp 1 vs LI: $t = -2.749$, $p = 0.041$, $d = -0.615$; HI vs LI: $t = 0.610$, $p > 0.999$, $d = 0.137$ (Fig. 4A); SNI: $F_{(2,\ 37)} = 8.450$, $p < 0.001$, $\eta^2 = 0.314$; Exp 1 vs HI: $t = -3.062$, $p = 0.012$, $d = -0.484$; Exp 1 vs LI: $t = -3.607$, $p = 0.003$, $d = -0.570$; HI vs LI: $t = -0.471$, $p > 0.999$, $d = -0.075$ (Fig. 4B); Premature responses – Sham: $F_{(2,\ 17)} = 7.391$, $p = 0.005$, $\eta^2 = 0.465$; Exp 1 vs HI: $t = 3.411$, $p = 0.010$, $d = 0.763$; Exp 1 vs LI: $t = 2.810$, $p = 0.036$, $d = 0.628$; HI vs LI: $t = -0.520$, $p > 0.999$, $d = -0.116$ (Fig. 4C); SNI: $F_{(2,\ 37)} = 8.500$, $p < 0.001$, $\eta^2 = 0.315$; Exp 1 vs HI: $t = 3.171$, $p = 0.009$, $d = 0.501$; Exp 1 vs LI: $t = 3.542$, $p = 0.003$, $d = 0.560$; HI vs LI: $t = 0.321$, $p > 0.999$, $d = 0.051$ (Fig. 4D)), possibly reflecting a learning and/or re-test effect. Importantly, all groups presented no differences in timed (Group: $F_{(3,\ 26)} = 0.117$, $p = 0.949$, $\eta^2 = 0.013$; Group*Time: $F_{(10.437,\ 90.458)} = 1.557$, $p = 0.129$, $\eta^2 = 0.063$) or premature responses (Group: $F_{(3,\ 26)} = 0.168$, $p = 0.917$, $\eta^2 = 0.019$). A group*time interaction was observed in the premature responses ($F_{(11.802,\ 102.284)} = 2.711$, $p = 0.003$, $\eta^2 = 0.083$) but post-hoc tests did not reveal any differences between the groups.

In the VDS test, SNI animals showed a tendency to perform more premature responses than Sham (PR: $F_{(1,26)} = 3.887$, $p = 0.059$, $\eta^2 = 0.130$) (Supplementary data, Fig. 1). Considering trait impulsivity in these animals, we observed that while LI animals were not affected by SNI (PR: $F_{(1,12)} = 0.584$, $p = 0.459$, $\eta^2 = 0.046$; PR_{ratio}: $F_{(1,13)} = 0.344$, $p = 0.568$, $\eta^2 = 0.026$) (Fig. 5A, C), HI SNI rats were more impulsive than the corresponding Sham controls on the $3s_f$ (PR: $F_{(1,12)} = 4.928$, $p = 0.046$, $\eta^2 = 0.291$; $3s_i$: $t_{(12)} = 1.671$, $p = 0.121$, $d = 0.988$; 6_s: $t_{(12)} = 0.827$, $p = 0.424$, $d = 0.489$; 12_s: $t_{(12)} = 0.884$, $p = 0.394$, $d = 0.523$; $3s_f$: $t_{(12)} = 2.951$, $p = 0.012$, $d = 1.746$) (Fig. 5B). These results show that only animals that are more impulsive at the baseline are affected after a neuropathic lesion. Interestingly, differences between Sham and SNI in the PR on the $3s_f$ trials are mainly dependent of SNI-L ($F_{(2,11)} = 4.027$, $p = 0.049$, $\eta^2 = 0.423$; Sham vs SNI-L: $t = -2.824$, $p = 0.050$, $d = -0.755$) (Fig. 5D inset; supplementary data, Fig. 2). No effects were seen in the PR_{ratio} ($F_{(1,13)} = 0.073$, $p = 0.792$, $\eta^2 = 0.006$) (Fig. 5D). However, comparing the evolution of PR_{ratio} from VDS I to VDS II in all groups, only SNI-L LI and HI are different between each other ($F_{(5,24)} = 3.644$, $p = 0.014$, $\eta^2 = 0.432$; SNI-L HI vs SNI-L LI: $t = 3.474$, $p = 0.029$, $d = 0.634$) (Fig. 5E). It appears that while Sham and SNI-R animals corrected their impulsivity levels in the second VDS, SNI-L animals maintained them (Fig. 5E).

4. Discussion

In this work we studied SNI impact on impulsive behavior in an heterogeneous population of outbred rats (experiment 1) and in a previously characterized population regarding trait impulsivity (experiment 2). We observed no differences between lesioned and control groups in any aspect of impulsive behavior. When considering impulsivity baseline, HI SNI animals were more intolerant to delay than

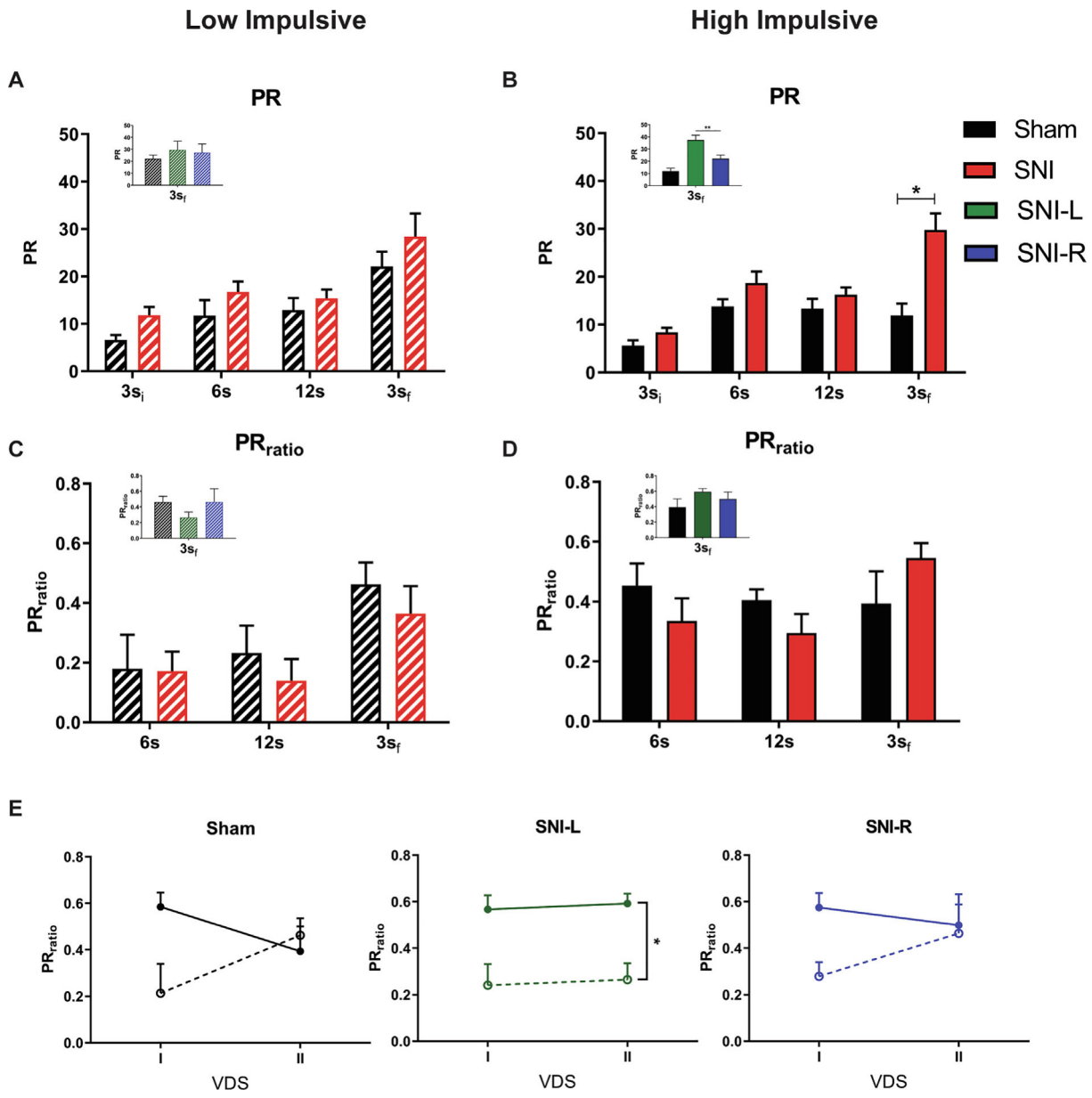

Fig. 5. Experiment 2, VDS II: Effect of chronic pain on HI and LI animals. HI rats with chronic pain, mainly SNI-L, are more impulsive than the corresponding controls in the PR (B) although not in the PR_{ratio} (D). LI animals are not affected (A, C). Sham and SNI-R animals corrected the levels of impulsivity from VDS I to VDS II, but in SNI-L, HI and LI maintained their PR_{ratio} (E). Data presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$. $3s_i$ – initial trials with 3 s' delay; 6 s – trials with 6 s' delay; 12 s – trials with 12 s' delay; $3s_f$ – final trials with 3 s' delay; HI – High impulsive; L – left; LI – Low Impulsive; PR – Prematurity rate; R – right; SNI – Spared Nerve Injury; VDS – Variable delay-to-signal.

the respective controls while LI were not affected by the neuropathy. Also, while Sham animals adjusted their behavior between the 2 VDS assessments, SNI animals failed to do so.

Results reported here are in line with a previous study of the group in which at the $3s_f$ VDS block Sham and SNI behaved similarly (Leite-Almeida et al., 2012), even though in that report SNI-R presented a higher PR_{ratio} during the longest delay trials that was not observed here. Results shown here are also in accordance with the work from Higgins and colleagues that tested SNI animals in the 5-choice serial reaction time task (5-csrtt) which bears some resemblance with the VDS training protocol (Higgins et al., 2015). On the contrary, Pais-Vieira and colleagues reported a decrease in impulsivity on the 5-csrtt (Pais-Vieira et al., 2009), in a monoarthritic inflammatory model of chronic pain, initiated in the day after chronic pain induction, suggesting some specificity regarding the type or duration of pain.

VDS II revealed, in the training, a re-test effect characterized by an increase in the number of timed responses and a decrease in motor impulsivity. Importantly, this effect was observed independently of lesion and trait impulsivity. In the test, HI SNI animals (mainly left-lesioned) were more intolerant to delay than the respective controls and when VDS I and VDS II were compared, we observed that both LI and HI Sham and SNI-R adjusted their behavior but not SNI-L. This maintenance of impulsivity levels might result from a complex interaction of re-test and/or learning effects.

Trait impulsivity differences manifest in other behavioral domains (Hayward et al., 2016). For instance, it has been observed that HI animals perform worse in working memory tasks (James et al., 2007; Renda et al., 2014) and present increased anxiety-like behavior(Stein et al., 2015) though some conflicting evidence has also been reported (Velázquez-Sánchez et al., 2014). Furthermore, HI predicts nicotine and cocaine self-administration (Anker et al., 2009; Dalley et al., 2007; Diergaarde et al., 2008; Perry et al., 2008) and increases seeking

behavior for sucrose and palatable food (Diergaarde et al., 2009; Velázquez-Sánchez et al., 2014).

Transcriptional differences between HI and LI rats have been found in the Nucleus Accumbens (NAc), ventral tegmental area, dorsomedial striatum and orbitofrontal cortex at basal conditions (Besson et al., 2013; Caprioli et al., 2014; Moloney et al., 2019). In the NAc, HI animals present a decreased availability of D1 and D2/3 receptors and of dopamine transporter (DAT) (Caprioli et al., 2015; Jupp et al., 2013) as well as a reduction in grey matter density (Caprioli et al., 2014). Interestingly, deep brain stimulation in the NAc decreases impulsivity particularly in HI animals (Schippers et al., 2017). The NAc has been involved in chronic pain – see for review (Benarroch, 2016; Mitsi and Zachariou, 2016) – and appears as a prime candidate to mediate decision-making and motivational alterations observed in chronic pain models. Moreover, it has been described in HI rats a reduction of gray matter density as well as a reduction of D2/3 receptor, glutamate decarboxylase $(GAD)_{65/67}$, microtubule-associated protein 2 (MAP2) and spinophilin in the left (but not right) NAc (Caprioli et al., 2015, 2014) which might explain some of the lateralized effects observed in animal models of chronic pain. Indeed, in this and in previous works from the group we observed lesion-side specific impairments on behavior, namely increased anxious-like behavior in SNI-L and cognitive flexibility deficits in SNI-R (Leite-Almeida et al., 2014, 2012). Interestingly, lateralized effects of chronic pain have also been observed in chronic pain patients (Gagliese et al., 1995).

In conclusion, chronic pain preclinical models manifest comorbid behaviors such as depressive- and anxiety-like behaviors, and cognitive deficits (Leite-Almeida et al., 2015; Low, 2013; Yalcin et al., 2014). These have been shown to depend on a number of factors including experimental subject age (Leite-Almeida et al., 2009), pain duration (Yalcin et al., 2011) and injury location (Leite-Almeida et al., 2014, 2012). In addition, our results indicate that trait manifestations should also be considered in the complex relation between chronic pain and comorbid behaviors.

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Authors contributions

A.M.C., M.E., J.P-M. and M.R.G. acquired data. AMC did the statistical analysis. A.M.C. and H.L-A. designed the experiment and wrote the first version of the manuscript. All authors discussed and interpreted the data and revised and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynpai.2019.100042.

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SUPPLEMENTARY DATA

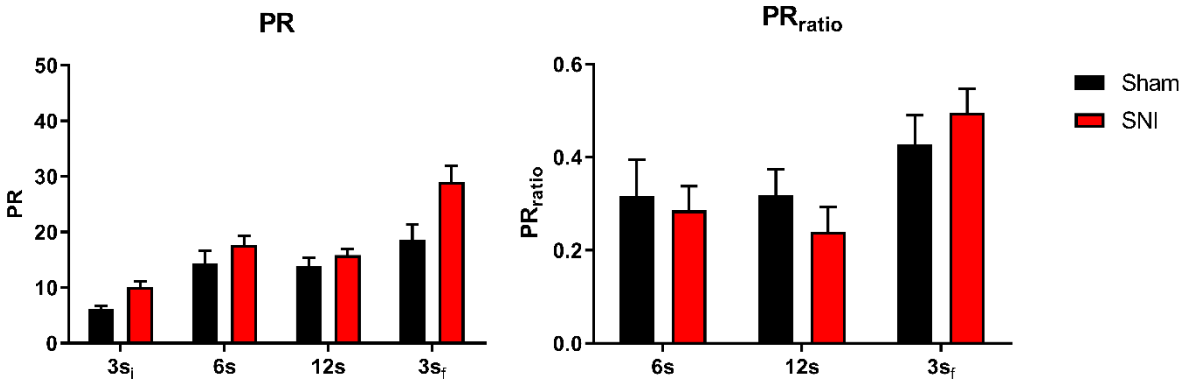

Figure 1. Experiment 2, VDS II: Effects of chronic pain on impulsivity. SNI animals show a tendency to be more intolerant to delay than controls, as they increase the PR on the $3s_f$ trials of the VDS test. $3s_i$ -initial trials with 3 seconds' delay; 6s – trials with 6 seconds' delay; 12s - trials with 12 seconds' delay; $3s_f$ - final trials with 3 seconds' delay; PR – Prematurity rate; SNI- Spared Nerve Injury; VDS- Variable delay-to-signal

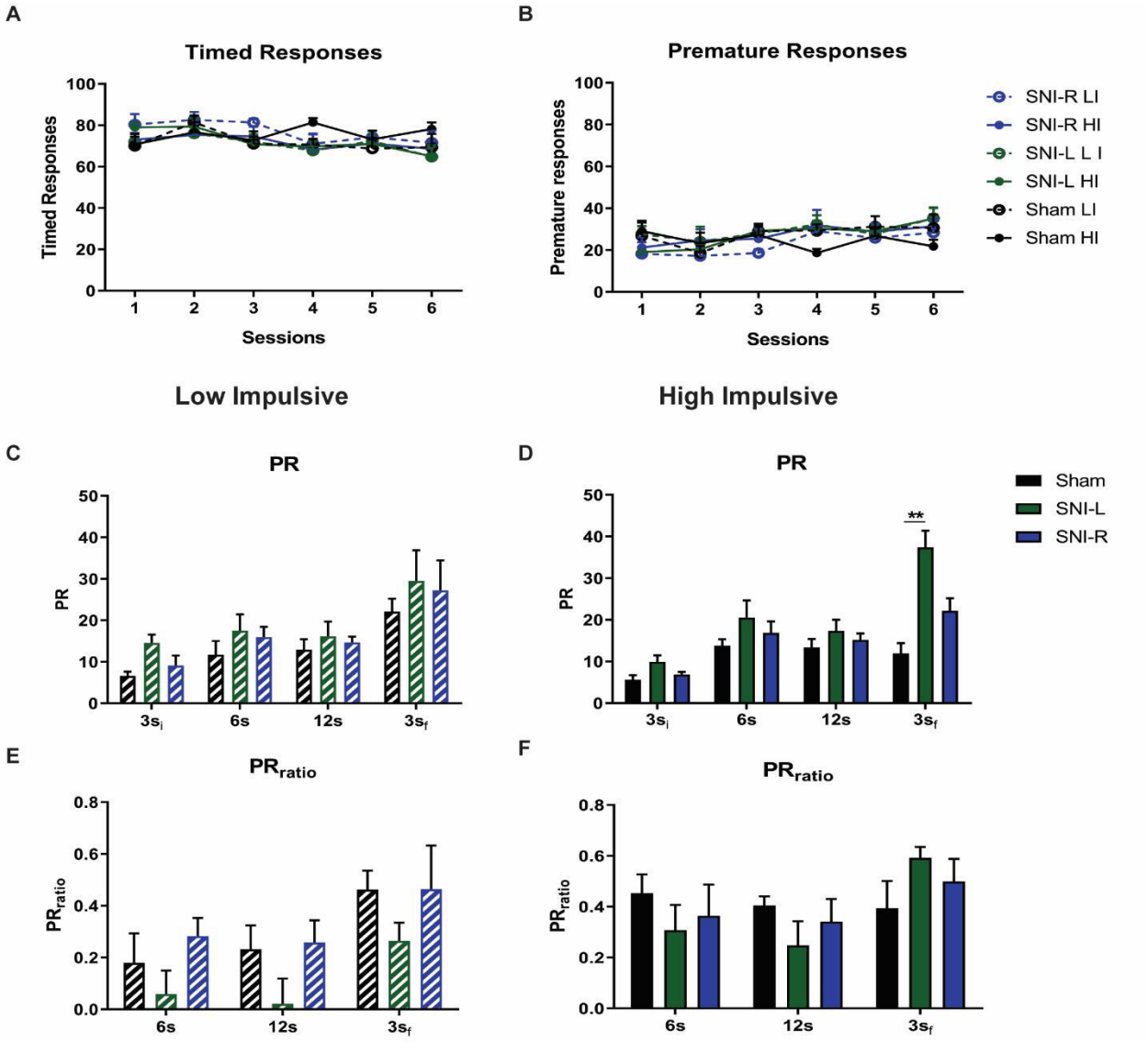

Figure 2. Experiment 2, VDS II: Effect of lesion side. No differences between the groups were found during the training in the number of timed **(A)** nor premature **(B)** responses. In the test, LI animals do not differ in the PR **(C)** but SNI-L show a tendency to be less impulsive than SNI-R and controls **(E)**. On the other hand, in HI, SNI-L are more impulsive in the $3s_f$ trials than Sham **(D)** even though this is not true for PR_{ratio} **(F)**. $3s_i$ -initial trials with 3 seconds' delay; 6s – trials with 6 seconds' delay; 12s - trials with 12 seconds' delay; $3s_f$ - final trials with 3 seconds' delay; HI- High impulsive; L- Left; LI- Low Impulsive; PR – Prematurity rate; R-Right; SNI- Spared Nerve Injury; VDS- Variable delay-to-signal

CHAPTER 2

Dopamine at the crossroad between chronic pain and decision-making impairments

CHAPTER 2.1

Dopamine at the crossroad between chronic pain and executive dysfunction- insights from rodent studies

Manuscript in preparation

Dopamine at the crossroad between chronic pain and executive dysfunction – insights from rodent research

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Abstract

Chronic pain patients frequently experience comorbid emotional and cognitive deficits. Clinical and preclinical studies have systematically shown increased prevalence of depression and anxiety, sleep impairments and executive dysfunction. Attention, working memory, impulsivity and decision-making are some of the most affected dimensions of executive function. Impairments on these behaviors can, not only affect patients' quality of life, but potentiate other conditions such as addiction. Some researchers have argued that dopamine (DA) is a molecule of interest in the interplay between chronic pain and its emotional comorbidities. Here we hypothesized that this effect is not restricted to emotional comorbidities but also comprises executive dysfunction. For that, the basics of DA and the dopaminergic system are reviewed, followed by how this system is impaired in rodent models of chronic pain, and how manipulations of dopaminergic system affect pain perception and processing. Finally, the role of the dopaminergic system in executive functions affected by chronic pain is discussed. We show that DA, and in particular the mesocorticolimbic dopaminergic system, is involved in chronic pain and executive function, and appears, in that way, as a key player in the emergence of chronic pain and its comorbidities.

1. Introduction

Pain is an unpleasant experience which comprises several components that can be organized in three complex groups: a sensory/discriminatory component which allows us to perceive the localization and the intensity of pain, an affective/motivational component involved in pain unpleasantness and affected by our emotional status, and a cognitive/evaluative component that takes into consideration our memories and context (Melzak, 1968). Acute pain, because of its unpleasant nature, plays an essential role on tissue damage avoidance and survival. On the other hand, when pain persists for long periods of time, it is no longer protective, becoming pathological.

Chronic pain is a reality for 1.5 billion people worldwide. Besides living with constant, or at least frequent, pain, chronic pain patients present poor mood, sleep less hours, present an overall decrease in the quality of life and commonly suffer from long-term disability (Bair and Robinson, 2003; Dueñas et al., 2016; Mathias et al., 2018).

Chronic pain is more than a simple extension of acute pain. In fact, acute and chronic pain present different mechanisms, effects, and treatment responses. For instance, during the transition from acute to chronic back pain (within a 1 year period), brain activity decreases in areas associated with pain (e.g. insula, thalamus and anterior cingulate cortex (ACC)) and increases in areas associated with emotion (e.g. amygdala (AMY) and medial prefrontal cortex (mPFC)), suggesting an abnormal processing of pain perception in chronic conditions (Hashmi et al., 2013). Therefore, it is important to understand the mechanisms that lead to chronic pain and the associated susceptibility factors. One hint in this direction is that increased nucleus accumbens (NAc) – mPFC functional connectivity, after a back pain episode, predicts the evolution to prolonged pain (Baliki et al., 2012; Vachon-Presseau et al., 2016), placing the corticolimbic circuitry as a central piece in chronic pain. Dopaminergic neurons, in particular from the ventral tegmental area (VTA), are an important component of this circuitry. Interestingly, chronic pain patients present an hypodopaminergic state (Jääskeläinen et al., 2001; Wood et al., 2007a) and patients with dopamine (DA)-related disorders, as Parkinson's disease (PD), present abnormal pain responses. Furthermore, chronic pain patients (and rodent models) frequently suffer from impairments in executive function, namely in attention, cognitive flexibility, decision-making and delay intolerance, functions highly associated with DA.

In this review, we provide an overview of the basic components of the dopaminergic system and how these are involved in chronic pain maintenance and in the manifestation of executive function deficits, in rodent models.

2. Dopamine, dopaminergic dysfunction and pain perception

2.1 Catecholamines

All catecholamines – DA, noradrenaline (NA) and adrenaline – derive from the amino acid tyrosine and are composed by a cathecol and an amine side-chain, to whom they owe their name. At the cytosol, L-tyrosine is converted by tyrosine hydroxylase (TH) in L-3,4-dihydroxyphenylalanine (L-DOPA) that in turn is converted in DA (3-hydroxytyramine) by L-DOPA decarboxylase (Blaschko, 1942). DA is then transported to synaptic vesicles by the vesicular monoamine transporter 2 (VMT2) (Laduron and Belpaire, 1968) where it is either stored or converted to NA (4, 5-β-trihydroxy phenethylamine) by DA β-hydroxylase (Levin et al., 1960). Further on, NA can be converted to adrenaline (β, 3, 4-trihydroxy-N-methylphenethylamine) by phenylethanolamine N-methyltransferase (Axelrod, 1962; Bulbring, 1949; Kirshner and Goodall, 1957). It is the presence or absence of the mentioned enzymes in the cells that determinates which catecholamines are produced in each cell. Most adrenalin molecules are released from adrenal glands and act as hormones while NA and DA are mostly present in the central nervous system and act as neurotransmitters (Rao et al., 2007).

2.2 Anatomical organization of DA neurons

Dopaminergic neurons are organized in restricted groups localized in different brain regions, comprising no more than 1% of the total number of neurons present in an adult brain (Chinta and Andersen, 2005; Moore and Bloom, 1978). Most of these nuclei were identified in the early 1960's in the seminal study of Dahlström and Fuxe (Dahlstroem and Fuxe, 1964). Recurring to the Falck and Hillarp fluorescence technique, they identified different areas, not anatomically defined, in the ventral midbrain (VM) and diencephalon, which they nominated A8 to A15 (Björklund et al., 1973; Dahlstroem and Fuxe, 1964). The majority of the dopaminergic neurons (75%) can be found in the VM divided in three continuously distributed and not independent nuclei - A8, A9 and A10 - corresponding respectively to retrorubral field, substantia nigra (SN) and VTA (Hegarty et al., 2013).

Later, retrograde, anterograde and lesion studies allowed the discovery of several dopaminergic pathways and their roles in brain function (Beckstead et al., 1979). The most studied are those that project from

the VM, namely the nigrostriatal pathway, which projects mostly from the SN to the striatum (STR), and the mesolimbic and mesocortical pathways – also denominated mesocorticolimbic pathway – which project mainly from the VTA to limbic and cortical areas, respectively (Björklund and Dunnett, 2007). The nigrostriatal pathway is implicated in motor control and coordination while the mesocorticolimbic pathway is involved in reward, motivation and reinforcement learning (Arias-Carrión et al., 2010; Floresco and Magyar, 2006; Lammel et al., 2014; Oswald et al., 2015; Rapanelli et al., 2015; Rogers, 2011; Saddoris et al., 2015).

2.3 Dopamine release and degradation

Dopaminergic transmission can be tonic or phasic. Tonic transmission is characterized by spontaneous spike activity at low frequencies (1-9 Hz), which maintains the baseline levels of DA. On the other hand, in response to a stimulus (reinforcing, unexpected or aversive), there is a bursting of the dopaminergic neurons at higher frequencies (>20 Hz). This phasic transmission leads to high release and fast reuptake of DA and it is believed to encode reward predicting errors (Floresco et al., 2003; Goto et al., 2007; Grace and Bunney, 1984a, 1984b; Schultz, 2016).

DA release does not have an excitatory or inhibitory effect *per se*, instead it acts by modulating the afferent cells through signaling cascades or by direct membrane interactions (see section 2.4). It can regulate neurotransmitter or neuropeptide release (including substance P and opioids, which are closely involved in pain response), gene expression and synaptic plasticity (Hervé and Girault, 2005).

After its release in the synapse, DA is reuptaken via DA transporter (DAT) and is stored in vesicles, ready to be released again. Alternatively, instead of being recycled, DA can also be degraded. This process takes place mostly in the glia cells surrounding the synapse. DA is converted in 3,4-dihydroxyphenylacetic acid (DOPAC) or 3-methoxytyramine (3-MT) and posteriorly in homovanillic acid (HVA).

2.4 DA receptors

In the late 70's two of the DA receptors were identified for the first time and named β and α, depending on the ability/inability to activate adenylyl cyclase, respectively (Kebabian, 1978). This nomenclature induced some confusion with α and β adrenergic receptors, so α and β dopaminergic receptors were respectively named D2 and D1 receptors (Kebabian and Calne, 1979). With the development of gene cloning techniques, three new receptors were found: D3, D4 and D5 (Sokoloff et al., 1990; Tiberi et al., 1991; Van Tol et al., 1991). Because these receptors share structural, pharmacological and genetic properties with D1 or D2 dopaminergic receptors, they were divided in two

families: D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors. All of them are G protein-coupled receptors (GPCRs) with seven transmembranar domains.

In terms of location, D1 and D5 are expressed postsynaptically on dopamine-receptive cells whereas D2 and D3 are also expressed presynaptically (Rondou et al., 2010; Sokoloff et al., 2006). It is believed that co-expression of D1 and D2 receptors occurs in a very small percentage of neurons. Normally D1 and D2 are segregated in different neuronal populations, namely, dynorphin- and substance P-containing neurons and enkephalin-containing neurons, respectively (Le Moine 1990,1991). D2-like receptors show in general more affinity to DA than D1-like receptors (Sokoloff et al., 1990; Sunahara et al., 1991; Van Tol et al., 1991) and because of that D2 are more sensitive to tonic DA release and D1 to phasic transmission.

2.5 Pain in DA-associated pathologies

As stated above, dopaminergic system dysfunction can impact motor performance, executive function and motivation, according to the specific pathways affected. Pain perception on conditions as PD and schizophrenia has provided important information on the relationship between DA and chronic pain.

PD is characterized by degeneration of dopaminergic neurons, mainly from the SN, and the consequent decrease of DA in the STR. It is well described that PD patients and animal models have higher prevalence of pain and decreased pain thresholds as well as alterations in pain processing (Blanchet and Brefel-Courbon, 2018; Faivre et al., 2019; Thompson et al., 2017). It is not clear if these are consequence of the lack of DA, but a higher prevalence of pain in the unmedicated OFF states implicates DA at least partially (Blanchet and Brefel-Courbon, 2018; Thompson et al., 2017). On the other hand, pain intensity does not correlate with PD severity (Thompson et al., 2017), although it is important to consider that PD diagnosis usually happens after 50-70% of the dopaminergic neurons have died (Cheng et al., 2010).

In opposition, patients with schizophrenia, a disease associated with a hyperdopaminergic status, appear to have more tolerance to pain (Potvin and Marchand, 2008). It has been argued however that these increased thresholds do not reflect a sensory phenomenon but instead differences in self-reporting (Bonnot et al., 2009; Stubbs et al., 2014). Still, healthy relatives of schizophrenia patients also report increased pain tolerance and higher pain thresholds (Hooley and Delgado, 2001).

3. Dopamine in chronic pain

3.1 The dopaminergic system in rodent models of chronic pain

Imaging studies in patients with different chronic pain conditions reveal an hypodopaminergic status of basal ganglia (Jääskeläinen et al., 2001; Wood et al., 2007a). In rodents, DA has been measured (mostly by high performance liquid chromatography (HPLC)) in different models of chronic pain but results are inconclusive. Mlost and colleagues (2018) reported no alterations in striatal DA in an osteoarthritis model (Mlost et al., 2018), while Gemikonakli and colleagues (2019) found a DA increase in the dorsolateral (but not dorsomedial) STR in a traumatic peripheral neuropathy model (Gemikonakli et al., 2019). In the NAc, data is also inconclusive: decreases (Taylor et al., 2014), increases (Sagheddu et al., 2015) and no alterations (Mlost et al., 2018; Pais-Vieira et al., 2009a) in DA levels have been reported. Importantly, the DA metabolite HVA is decreased in both STR and NAc, which could reveal that although DA levels are not altered, its metabolism is (Mlost et al., 2018). HPLC analyses also reveal a decrease of DA in hippocampus and no alterations in AMY, habenula and ACC (Mlost et al., 2018; Pais-Vieira et al., 2009b; Taylor et al., 2014). Concerning the frontal areas, no differences were found in the frontal cortex (Mlost et al., 2018), but Pais-Vieira and colleagues (2009b) report differences, specifically in the orbitofrontal cortex (OFC), 21 days after monoarthritis induction (Pais-Vieira et al., 2009b). They, however, did not find alterations 5 and 56 days after the lesion, which hints at a temporal dynamic in the impact of chronic pain in DA. It is also important to take into account that Sham surgery by itself appears to induce alterations in the levels of DA (Gemikonakli et al., 2019; Taylor et al., 2014) and that alterations in DA hemispheric distribution (Gemikonakli et al., 2019) and NA (Gemikonakli et al., 2019; Mlost et al., 2018; Taylor et al., 2014) are also reported.

Interestingly, DA is correlated with mechanical nociceptive thresholds in Sham but not in neuropathic animals (Taylor et al., 2014). Similarly, DA release after noxious stimulation is altered in chronic pain patients and does not correlate with perceived pain, as happens in healthy subjects (Baliki et al., 2010; Wood et al., 2007b). Furthermore, release of DA after rewards (pain release, cocaine, sucrose) is also impaired in neuropathic models (Kato et al., 2016; Taylor et al., 2015), which could explain the increased anhedonia observed in chronic pain patients.

Chronic pain leads also to a decrease in TH+ cells in the VTA and to electrophysiological alterations in these cells. Reports show both a decrease (Watanabe et al., 2018) and an increase (Sagheddu et al., 2015) in the number of spikes and a decrease in inhibition's duration (Sagheddu et al., 2015). Decreases and increases in spikes were observed in mice and rat, respectively, hinting at species divergencies.

Furthermore, VTA subregions can also play different roles as Huang and colleagues (2019) show a decrease in firing in the lateral but not medial VTA, after spared nerve injury (Huang et al., 2019).

Probably as a compensatory mechanism for the decreased striatal DA, positron emission tomography (PET) studies show an increase in D1 and D2 receptors in this area in chronic pain patients (Hagelberg et al., 2003b, 2003a; Martikainen et al., 2015). Surprisingly, not many have studied DA receptors expression in rodent models of prolonged pain. In neuropathic pain models, a D2 decrease in the NAc contralateral to the injured limb has been observed (Chang et al., 2014; Sagheddu et al., 2015). In the ipsilateral, and for D1, the results are less conclusive, but appear to show the same decrease even though not always statistically significant (Chang et al., 2014; Sagheddu et al., 2015). Also, Cardoso-Cruz and colleagues (2014) show that D2 is increased and D3 is decreased in dorsal and ventral hippocampus and that D1 is increased only in its ventral part, an area more associated with emotional processing (Cardoso-Cruz et al., 2014a).

3.2 Dopamine's role in pain modulation in rodent models of chronic pain

Antidepressants are one of the most used treatments for chronic pain management, particularly 5-HT and NA reuptake inhibitors but also, in recent years, DA reuptake inhibitors (Benson et al., 2015). Systemic treatment with the triple reuptake inhibitor Bicifadine and the NA and DA reuptake inhibitor Bupropion decreases allodynia in chronic pain rat models. This effect seems to be mostly dependent on D2 stimulation, as D2 antagonism before Bicifadine injection inhibits the analgesic effect (Basile et al., 2007; Hoshino et al., 2015). Accordingly, systemic injections of apomorphine, a DA agonist with preference for D2-like receptors, and systemic and intrathecal injections of Quinpirole, a D2/3 agonist, have also an analgesic effect (Cobacho et al., 2014; Sarkis et al., 2011). On the other hand, Haloperidol, a D2 antagonist, appears to have the same effect (Espinosa-Juárez et al., 2017), perhaps implying the need to balanced levels of DA.

Centrally, injection of DA in the ACC, Apomorphine in the NAc and Quinpirole in the dorsolateral STR also have analgesic effects (Ansah et al., 2007; López-Avila et al., 2004; Sarkis et al., 2011). In the same way, optogenetic activation of VTA dopaminergic neurons that project to NAc, decreases allodynia (Watanabe et al., 2018), and inhibition of these same neurons during exercise inhibits exercise-induced analgesia (Wakaizumi et al., 2016). This aligns with clinical studies showing that deep brain stimulation of VTA decreases pain frequency and intensity on chronic cluster headache (Vyas et al., 2019).

4. Dopamine role in executive dysfunction

4.1 Impulsivity

DA appears to impact impulsivity differently according to types of impulsive behavior and stimulated receptors. DA reuptake inhibitors increase impulsive action in the 5- choice serial reaction time task (5-CSRTT) but decrease impulsive choice on the delayed reward task (Baarendse and Vanderschuren, 2012; Balachandran et al., 2018a). Furthermore, antagonism of D1 receptors leads to a decrease in premature responses in the 5-CSRTT but no alterations are observed upon D2/3 antagonism (Balachandran et al., 2018b; Besson et al., 2010a). Surprisingly, agonism of both types of receptors induces the same decrease in impulsive behavior, revealing the absence of a direct correlation between DA levels and impulsivity (Winstanley et al., 2010). On the other hand, chemogenetic inhibition of VTA dopaminergic neurons leads to decreased prematurity in the 5-CSRTT although activation of the main dopaminergic niches, VTA and SN, has no effect on action impulsivity (Boekhoudt et al., 2017; Fitzpatrick et al., 2019). Most studies looking into the role of DA on impulsivity have focused on the NAc core and shell subdivisions. Increased mRNA expression of D1 at the shell and decreased mRNA expression of D2 at the core predicted increased impulsive action (Simon et al., 2013), while choice impulsivity was inversely correlated with D2/3 availability on the core (Barlow et al., 2018). Furthermore, high-impulsive animals (in the 5-CSRTT) present lower DAT and D2/3 binding in the NAc shell (cf with choice impulsivity (Barlow et al., 2018)) and lower D1 binding in the core (Jupp et al., 2013). Manipulations of NAc core and shell with specific agonists and antagonists appears to also induce different effects, but the literature is still very controversial about it (see for instance (Besson et al., 2010b) *vs* (Moreno et al., 2013) *vs* (Pattij et al., 2007)). Frontal areas as the mPFC and the OFC are also involved (Simon et al., 2013; Winstanley et al., 2010; Yates et al., 2014).

4.2 Attention

Attention, also evaluated in the 5-CSRTT, is likewise impacted by the dopaminergic system. Levels of DOPAC/DA in the right frontal cortex positively correlate with the number of correct responses in the 5-CSRTT (Puumala and Sirviö, 1998). This, along with 5-hydroxyindoleacetic acid (5-HIAA)/5-HT levels on the left frontal cortex, account for almost 50% of the variability between animals on attention performance, emphasizing the role of this region on this behavior (Puumala and Sirviö, 1998). On the other hand, systemic blockage of DA reuptake by DAT induces no effect or decreases attentional performance, hinting at opposite effects of DA on different regions (Balachandran et al., 2018b; Nikiforuk et al., 2017). On the

same line, chemogenetic activation of midbrain DA neurons induces attention impairments (Boekhoudt et al., 2017) while inhibition of VTA neurons does not impact accuracy (Fitzpatrick et al., 2019). It is not clear if systemic antagonism or agonism of DA receptors impacts attention, even though a decrease in correct responses was reported at low delays (Balachandran et al., 2018b; Besson et al., 2010a; Winstanley et al., 2010). Furthermore D2-Knock-out (KO) mice also present attentional impairments (Kim et al., 2018). Locally, however, differences have been reported, varying according with area and receptor. In the OFC, D2/3 agonist Quinpirole decreases accuracy at high doses and particularly on high impulsive rats (Winstanley et al., 2010). It is however debatable if this reflects an impairment in attention as it is accompanied by an increase in omissions, which could indicate a lack of motivation. In the NAc, D2 (but not D1) antagonism in the core slightly increases attention (Pattij et al., 2007), while in the central amygdala there is a decrease in attention upon stimulation with D1 antagonism and no effect of D2 antagonism (Smith et al., 2015). Again, an increase in the number of omissions was also observed.

4.3 Working memory

Genetic models have provided some clues about the impact of DA alterations on working memory (WM). DAT-, D2- and D3-KO animals present WM impairments (Deng et al., 2015; Glickstein et al., 2002; Leo et al., 2018; Li et al., 2010), hinting at an inverted u-shape relationship between DA and this behavior. D5-KO animals, on the other hand, do not present any WM impairments (Moraga-Amaro et al., 2016). This, added to the fact that systemic D2 agonist, but not D1, impairs WM, suggests a more relevant role of D2 than D1-like receptors in this behavior (Amico et al., 2007; Bushnell and Levin, 1993). On the other hand, the impact of systemic D1 and D2 antagonists is not clear, with some showing impairments and others no alterations (Bushnell and Levin, 1993; Deng et al., 2015; Murphy et al., 1996). Interestingly, Zhang and colleagues (2004), show that a systemic injection of D4 specific antagonist L-745.870 improves WM in bad performers and worsens WM in good performers (Zhang et al., 2004).

Centrally, treatment with L-DOPA improves WM in a dose- and time-dependent (peaking at 3 days) manner (Bezu et al., 2016) and injections of D2/3 agonist Quinpirole in the ventral hippocampus also improves animals' performance (Wilkerson and Levin, 1999).

4.4 Cognitive flexibility and decision-making

Not surprisingly, DA also appears to be important to cognitive flexibility and decision-making. Depletion of DA in the STR by 6-hidroxydopamine (6-OHDA) lesions or TH inactivation leads to impairments in reversal learning (Darvas et al., 2014; Grospe et al., 2018; Tait et al., 2017) while blockage of DAT induces

improvements in the extradimensional shift trials of the attentional set-shifting task (ASST) (Nikiforuk et al., 2017). It is not clear if D1 and D2-like neurons contribute differently to this effect but Wang and colleagues (2019) show that optogenetic activation of D1 neurons impairs reversal learning, while its inhibition improves this behavior, but manipulation of D2 neurons has no effect on cognitive flexibility (Wang et al., 2019).

Systemic agonism of D2-like receptors leads to worse decision-making in gambling tasks (Morgado et al., 2014; Pes et al., 2017), which disappears when D2, D3 and D4 receptors are selectively agonized (Di Ciano et al., 2015). Frontal areas, as well as the NAc and the AMY appear to be the regions most involved on this behavior. Animals with bilateral lesions in the mPFC perform less advantageous choices (cf with (Mai and Hauber, 2012)), which are corrected with a D1-like (but not D2-like) antagonist (Paine et al., 2013). In the same line, in naïve animals, D1-like antagonists decrease risky disadvantageous choices, while D2-like antagonists increase this type of behavior. D2/3 agonist Quinpirole leads also to disadvantageous choices, inducing a decreased preference for higher certain rewards, which is maintained even when a probability to obtain them is very low (St. Onge et al., 2011). Specific inactivation of the prelimbic and infralimbic cortices appears to lead to the same deficits observed upon mPFC lesion (Zeeb et al., 2015). OFC and ACC, on the other hand, do not seem to be involved in risky decision-making (Ishii et al., 2015; Mai and Hauber, 2015; Zeeb et al., 2015). The involvement of the NAc is less clear, while NAc lesions do not have an effect on behavioral choice (Mai and Hauber, 2015, 2012), D1 and D3 receptors on this area appear to play a role (Mai et al., 2015; Stopper et al., 2013). Furthermore, disruption of the D1 PFC-NAc pathway decreases advantageous decisions (Jenni et al., 2017). Risk decision-making is also affected by the disruption of the D2 (but not D1) neurons which project from the PFC to basolateral AMY (Jenni et al., 2017). In contrast, Larkin and colleagues (2016) show that decision-making is affected by D1 agonists and antagonists in the basolateral AMY, but not D2 (Larkin et al., 2016). Effort decision-making also appears to depend on the dopaminergic system but less is known (Bardgett et al., 2009; Morales et al., 2017; Robles and Johnson, 2017).

5. Conclusions

As shown in this review, DA is independently involved in pain perception and executive function. These relationships are not always clear and sometimes data are even contradictory, revealing the need to better understand the spatial and temporal dynamics behind them, as well as the role of the different dopaminergic receptors. It is also important to note that DA release is influenced by, and influences strongly the action of monoamines like NA and 5-HT. Anatomically, reciprocal projections between the

noradrenergic locus coeruleus, the serotonergic dorsal raphe nucleus, the VTA and/or the SN are known (Albanese et al., 1986; Beckstead et al., 1979; Deutch et al., 1986; Steinbusch, 1981), and manipulation of either one of these systems by specific agonists, lesions, or electrophysiological stimulation, leads to alterations in the others (Clement et al., 1992; Dremencov et al., 2007; Guiard et al., 2008; Linnér et al., 2001). Consequently, isolating the specific function of each neurotransmitter is difficult as its action is never independent of other neurotransmitters or molecules.

The few studies focused on chronic pain, executive dysfunction and dopamine reinforce the importance of this relation. Monoarthritic rats with impairments in risk decision-making present alterations in the levels of DA and DOPAC in the OFC (Pais-Vieira et al., 2009a). Behavioral flexibility is correlated with DA, DOPAC and HVA in the left dorsomedial STR in a positive manner, and negatively in the right dorsolateral STR of neuropathic animals (Gemikonakli et al., 2019). Furthermore, systemic administration of Quinpirole reverses the impairments on WM caused by a neuropathic lesion (Cardoso-Cruz et al., 2014b). However, more studies are needed to understand exactly which circuitries are affected by pain chronification and how those alterations impact executive dimensions as impulsivity, WM and decision-making.

Others have discussed the importance of the dopaminergic system for other chronic pain-associated comorbidities as depression, anxiety and sleep disorders (Finan and Smith, 2013; Mitsi and Zachariou, 2016; Serafini et al., 2020). In that way, DA and the dopaminergic system appear as key elements in the relation between chronic pain and its emotional and cognitive comorbidities. The mesocorticolimbic system in particular, has, in the last years, been hypothesized as an important target on the arise and maintenance of chronic pain, as well in the prevalence of depressive and addictive behaviors. For instance, Lee and colleagues (2015) show that optogenetic stimulation of PFC projections to NAc not only decreases the neuropathic-associated allodynia but also ameliorates the depressive-like behavior (Lee et al., 2015). Here, this hypothesis is reinforced as cortical and limbic areas are the ones most frequently affected by chronic pain, as well as the ones more involved on pain perception and executive dysfunction.

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CHAPTER 2.2

Unilateral accumbal dopamine depletion affects decision-making in a side-specific manner

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Research Paper

Unilateral accumbal dopamine depletion affects decision-making in a side-specific manner

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ABSTRACT

Mechanisms underlying affective and cognitive deficits in Parkinson's disease (PD) remain less studied than motor symptoms. Nucleus accumbens (NAc) is affected in PD and due to its well-known involvement in motivation is an interesting target in this context. Furthermore, PD is frequently asymmetrical, with side-specific deficits aligning with evidences of accumbal laterality. We therefore used a 6-hydroxydopamine (6-OHDA) model to study the role of left and right NAc dopamine depletion in a battery of behavioral tasks.

2 months old male rats were used in all experiments. Habitual-based and goal-directed decision-making, impulsivity, anxiety- and depressive-like behavior and motor performance were tested 3 weeks after left (6-OHDA L) or right (6-OHDA R) NAc lesion was induced. Upon contingency degradation, 6-OHDA R decrease their lever press rate less than Sham and 6-OHDA L, indicating an impairment in the shift from habit-based to goal-directed strategies. On the other hand, 6-OHDA L lesions lead to increased rates of premature responding when delays where increased in the variable delay-to-signal test. Importantly, in both paradigms task acquisition was similar between groups. In the same line we found no differences in the amount of sugared pellets eaten when freely available as well as in both general and fine motor behaviors.

In conclusion, left and right NAc play distinct roles in the contingency degradation and impulsivity. More studies are needed to understand the mechanisms behind this functional lateralization and its implications for PD patients.

1. Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease in the aged population, with a worldwide average incidence of 160 cases per 100,000 in individuals with 65 or more years (Hirtz et al., 2007). It is mostly characterized by impairments in the motor system, namely tremors, body rigidity, bradykinesia, akinesia and postural/gait problems (Sveinbjornsdottir, 2016). However, patients also present non-motor symptoms, which can either appear in later stages of the disease or precede the motor symptoms (Schapira et al., 2017). Pain (Thompson et al., 2017), mood impairments – anxiety (Broen et al., 2016), depression (Reijnders et al., 2008), apathy (den Brok et al., 2015), sleep disturbances (Raggi et al., 2013; Suzuki et al., 2015) – and cognitive deficits, particularly dysfunction of executive functions as cognitive flexibility, attention, inhibitory control, rule acquisition and general decision-making (Dirnberger and Jahanshahi, 2013; Kudlicka et al., 2011; Ryterska et al., 2013) are common non-motor symptoms of the disease. Most of these non-motor symptoms are also present in animal models of PD, specifically in rodents (Campos et al., 2013; Carvalho et al., 2013; Jimenez-Urbieta et al., 2019) and non-human primates (Decamp and Schneider, 2004) – see for review (McDowell and Chesselet, 2012). Notwithstanding, although non-motor symptoms play a significant role in the patients' quality of life (Barone et al., 2009; Martinez-Martin et al., 2011; Schrag et al., 2000; Song et al., 2014), they are still frequently undervalued, both on clinical practice and basic investigation.

Pathologically, PD is characterized by the degeneration of dopaminergic neurons, primarily at the level of the nigrostriatal circuitry (dopaminergic neurons from substantia nigra that project to dorsal striatum (STR)). The death of these neurons is the cause of most of the disease symptoms, including the motor impairments due to the lack of dopamine (DA) production and release in the STR. However, the

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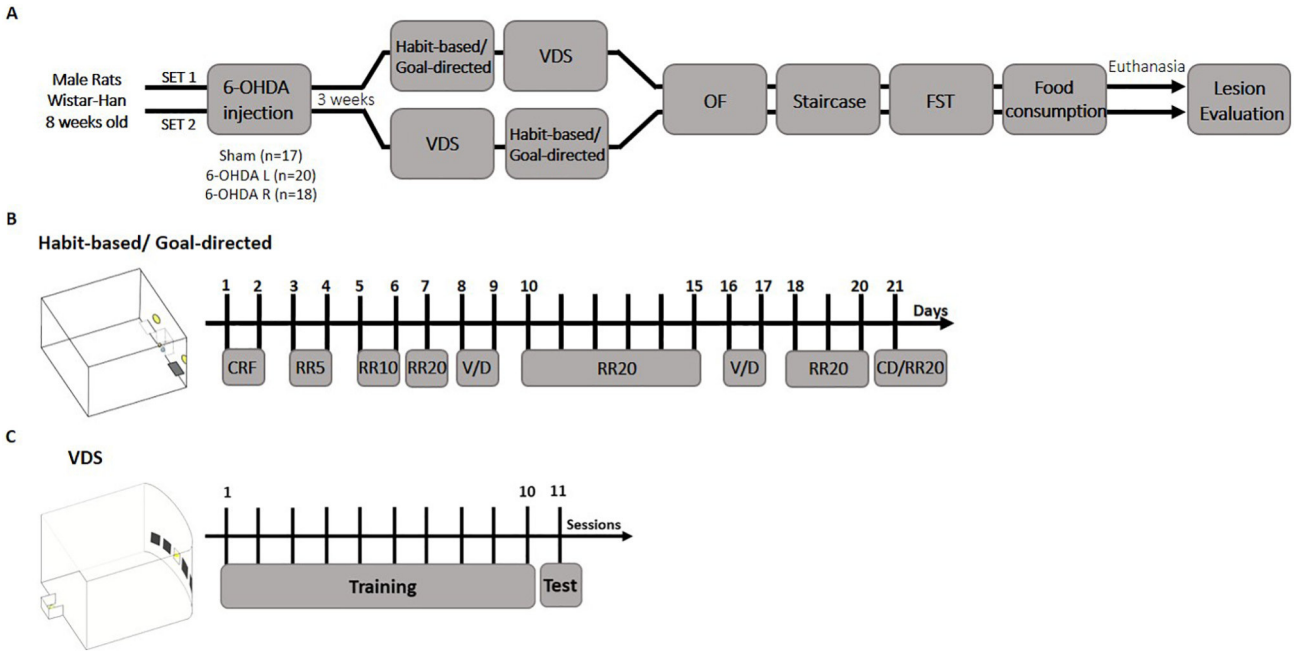

Fig. 1. Experimental design. Two sets of animals were used. In the first the animals performed the VDS test after the habit-based/goal-directed behavior and in the second they performed it the other way around (A). The following motor and emotional evaluations were all done in the same order (A). Description of habit-based/goal-directed (B) and VDS (C) tests. 6-OHDA – 6-Hydroxidopamine; CD- Contingency degradation; CRF- Continuous reinforcement; FST- Forced swimming test; L-left; OF- Open Field; R-right; RR- Random ratio; V/D- Valuation/devaluation; VDS- variable delay-to-signal.

mesolimbic circuitry, which encompasses neurons from the ventral tegmental area (VTA) that project to the ventral STR/ Nucleus accumbens (NAc), is also affected (Alberico et al., 2015; Mavridis et al., 2011). The NAc, in particular, is involved in reward, aversion, motivation and reinforcement, which can influence both the emotional and the cognitive symptoms mentioned above. Patients with major depressive disorder show lower activity of the NAc when exposed to positive stimuli/rewards (Epstein et al., 2006; Heller et al., 2009; Pizzagalli et al., 2009). On the other hand, deep brain stimulation (DBS) of this area has been correlated with the amelioration of anxious and depressive symptoms in patients and animal models, thereby indicating that NAc may be a potential target to the treatment of PD non-motor symptomatology (Bewernick et al., 2010; Bewernick et al., 2012; Falowski et al., 2011; Schlaepfer et al., 2008). Furthermore, PD patients with "dopa-resistant apathy" display an atrophy of the left NAc when compared to healthy controls or non-apathic PD patients (Carriere et al., 2014), once again indicating a role of NAc in the emotional impairments observed in PD.

Concerning cognition, NAc has been more extensively studied in its relationship with impulsive decision-making – see (Basar et al., 2010) for a detailed review. Lesions of the NAc core in rodent models lead to preference for immediate rewards instead of larger delayed ones (Bezzina et al., 2007; Cardinal and Howes, 2005; Cardinal et al., 2001; da Costa Araujo et al., 2009; Pothuizen et al., 2005). Also, DBS and pharmacological manipulations of this nucleus have been shown to affect impulsive behavior (Pezze et al., 2007; Sesia et al., 2008). Furthermore, rats with high and low trait impulsivity present marked molecular and structural differences in the NAc (Caprioli et al., 2014; Moloney et al., 2019). In humans, imaging studies report an activation of the ventral STR during the choice of more immediate rewards (McClure et al., 2004; Xu et al., 2009). In a less extent, NAc was also described as being involved in learning, cognitive flexibility, extinction and perseverative response (Aquili et al., 2014; Dalton et al., 2014; Francois et al., 2014; Pezze et al., 2007). Finally, a reduction of NAc was described in patients with comorbid PD and mild cognitive impairment in comparison to patients with no cognitive impairment and healthy controls (Hanganu et al., 2014), reinforcing the involvement of NAc in cognition (Foo et al., 2017).

Degeneration of DA neurons in PD is usually asymmetrical, with one side being more susceptible/affected than the other (Kempster et al., 1989; Tatsch et al., 1997; Wang et al., 2015). Surprisingly, few studies have been focused on the impact of left vs right degeneration. These seem to associate right-sided PD with impairments in language related tasks and left-sided PD with impairments in motor imagery and spatial tasks and no influence of laterality in executive functions, although this last one is highly dependent of the study conditions and tasks evaluated (Cooper et al., 2009; Lo Monaco et al., 2018; Verreyt et al., 2011). Also, patients with relatively greater left-sided DA deficit, minimize losses more effectively than they maximize gains; right-sided deficits presenting the opposite pattern (Maril et al., 2013; Porat et al., 2014). Interestingly, right-sided patients also presented higher levels of novelty seeking than left-sided (Harris et al., 2015). Concerning emotional dysfunction, Foster et al. (2011, 2013) show a correlation between anxiety and depression severity and the duration of the disease in right NAc PD patients but not in left, indicating a possible asymmetrical involvement of NAc. Interestingly some studies have also shown behavioral differences related with the NAc side. In humans, decreased activity/volume of right NAc is related with mood disorders (Barcia et al., 2014; Choi et al., 2017) while left NAc appears to be involved with higher sensitivity to reward than punishment (Aberg et al., 2015). Still, in naïve rats left (but not right) NAc was also found to be involved in impulsivity (Caprioli et al., 2015; Caprioli et al., 2014). Based on the information above, in the present work we have explored the role of left and right NAc DA depletion in a battery of behavioral task.

2. Methods

2.1. Study-design

Two independent experiments were carried out in which 6-hydroxydopamine (6-OHDA) lesioned animals were tested in habit-based/goal-directed paradigms – reward devaluation and contingency degradation (CD) – followed by an impulsivity task – variable delay-to-signal (VDS); in the second experiment, the sequence was inverted to

exclude potential carryover effects (Fig. 1A). In both experiments 1 and 2, decision-making paradigms were followed by open-field (OF), staircase, forced swimming test (FST) and a consumption test to evaluate animals' drive to eat (Fig. 1A).

2.2. Animals

55 male Wistar-Han rats (Charles-River Laboratories, Barcelona, Spain), 8 weeks old at the beginning of the experiment, were used. Rats were kept two per cage under a controlled environment with a 12 h light cycle (lights on at 8 a.m.), temperature of 21 ± 1 °C and humidity of 50–60%. Rats had food (4RF21; Mucedola SRL, Italy) and water ad libitum, except during the operant behavior and the staircase and food and sugared pellets consumption evaluation, in which food availability was restricted to 1 h/day. Animals handling and experiments were done after consent of the Portuguese National Authority for Animal Experimentation – Direção Geral de Veterinária (ID: DGV9457), and in accordance with the European guidelines for the care and handling of laboratory animals, by following the Directive 2010/63/EU of the European Parliament and Council. Efforts were made to minimize the number of animals used and their suffering, and body weight was controlled along the experiment to prevent weight losses superior to 15%.

2.3. 6-hydroxydopamine (6-OHDA) lesions

Rats were deeply anesthetized with a 1:1.5 mixture of Medetomidine hydrochloride (Dorbene®; SYVA Labs, Spain) and Ketamine (Imalgene®; MERIAL, France) injected intraperitoneally (ip; 1 ml/Kg) (Esteves et al., 2019). Then, they were fixed in a stereotaxic frame (Stoelting, USA) and unilaterally injected with 1 μl of 6-OHDA hydrochloride (4 μg/μl; H4381, Sigma-Aldrich, USA) with 0.2 mg/ml of ascorbic acid in 0.9% of NaCl at a rate of 0.250 μl/min in the left (6-OHDA L, $n = 20$) or right (6-OHDA R, $n = 18$) NAc – 1.2 mm (Anteroposterior, AP); +/− 2.1 mm (Medial Lateral, ML); −7.0 mm (Dorsoventral, DV) to Bregma; incisor bar set at −3.3 mm (Paxinos and Watson, 2007). Controls were injected in the left or right NAc with 1 μl of 0.2 mg/ml of ascorbic acid in 0.9% of NaCl ($n = 17$). A 10 μl syringe with a 30-gauge needle (Hamilton, Switzerland) connected to an automatic pump (World Precision Instruments, USA) was used to perform the injections. After injection, the needle was left in place for 2 min in order to avoid any backflow up the needle tract. Animals were then sutured, injected with Antisedan® (Atipamezole hydrochloride; ORION Corporation, Finland; 80 μl, subcutaneous) to reverse the anesthesia and left to recover in their home cages. Behavior assessment started 3 weeks after surgery.

2.4. Behavioral paradigms

2.4.1. Reward devaluation/contingency degradation

Goal-directed/habit-based decision-making behavior was based on a previously performed protocol (Dias-Ferreira et al., 2009). Behavior was done in an operant box (OB; 30.5 cm L × 24.1 cm W × 21.0 cm H; ENV-467, MedAssociates, USA) with 2 retractable levers and a food magazine in between (Fig. 1B). OB were placed in noise attenuating boxes. After 2 days of habituation to the OB and reinforcements, animals were trained in a continuous reinforcement schedule (CRF; 1 reward per lever press) followed by increasing reinforcement schedules: 2 days of random ratio (RR) -5, 2 days of RR-10 and 7 days of RR-20, where the probability of receiving a reward was respectively 20, 10 and 5% per lever press (Fig. 1B). Each session terminated after 30 reinforcements or 30 min. Each animal performed 2 daily sessions (at least 5 h apart) in each of which right or left levers were available. For each animal pellets (dustless precision pellets® 45 mg, PHYMEP, France) and sucrose (20%; Laborspirit, Portugal) were associated with a specific lever (pellets/right lever and sucrose/left lever or vice-versa, randomized across animals/groups) presented in alternate sessions. After the 1st (early test) and 7th (late test) RR20 sessions, animals were tested in a devaluation test (Fig. 1B). Prior to this session, animals received *ad libitium* access to one of the reinforcements (randomized across animals/experimental groups) for 1 h. Then, a 5 min' session in extinction (i.e. the levers were presented but no reward was delivered) with both levers available took place. On the following day, the same procedure was repeated changing the devalued reinforcement. The RR20 schedule was resumed for 3 additional sessions after which, the contingency was degraded in one of the levers, i.e. the reinforcement was delivered independently of amount of lever presses (Random interval (RI) 20); RR20 was maintained in the other lever (randomized across animals/groups) (Fig. 1B). MED-IV software (MedAssociates) controlled the equipment and recorded number of lever presses and session time.

2.4.2. Impulsivity

Action impulsivity and delay intolerance were tested in the VDS (Leite-Almeida et al., 2013; Soares et al., 2018). The protocol was performed in 5-hole boxes (25 × 25 cm; TSE, Germany). One of the walls was slightly curved and contained five holes (2.5 × 2.5 cm) elevated 2 cm from the grid floor. In the opposite wall, there was a food magazine connected to a pellet dispenser located outside the box. Each hole contained a 3 W lamp bulb and an infrared light sensor to detect nosepokes. During the test only the central hole and the food magazine were open (Fig. 1C). Animals were accustomed to the box in 2 daily sessions for 2 consecutive days. In the first day, rats were left exploring the box for 15 min. All the holes were closed and lights off. Sugared pellets (dustless precision pellets ® 45 mg) were left in the food magazine. In the second day, animals explored the box for 30 min. In these 2 sessions the central hole was open and sugared pellets were also presented there. Food magazine, central hole and house lights were on during the entire session.

10 training sessions, twice daily, were then performed. Each started with the delivery of a sugared pellet in the food magazine and with the house-light on. Then, after a 3 s delay, the light in the central hole was switched on (for 60 s). If the animal nosepoked in that time (timed nosepoke) it was rewarded with a pellet. But if the animal nosepoked during the 3 s delay period when the light was off (premature response) or if it did not nosepoke (omission) it was punished with 3 s in complete darkness and no reward (Figs. 1C, 4A). Each session ended after 100 complete trials or 30 min. Premature responses, timed nosepokes, omissions and perseverant responses were recorded. In the VDS test, a total of 120 trials were performed, consisting of 25 trials of 3 s delay in the beginning ($3s_i$) and in the end ($3s_f$) flanking 70 trials of 6s or 12 s delay (randomly given by the computer). Contrary to the shaping session, during the VDS animals were allowed to perform premature responses; these were registered but not punished (Figs. 1C, 4E).

2.4.3. Locomotor activity and anxious-like behavior

Locomotion and anxiety-like behavior were tested in an OF apparatus as described previously (Leite-Almeida et al., 2009). This apparatus consisted of a brightly illuminated square arena (43.2 × 43.2 cm; ENV-515, MedAssociates) with infrared beams at the floor level, which allowed to automatically monitor animals' position. The arena was enclosed in a transparent acrylic wall (30.5 cm) and placed inside a sound-attenuating box. For the test, animals were placed in a corner of the arena and left to explore it for 15 min. The arena was cleaned with 10% ethanol between animals. Using Activity Monitor software (MedAssociates), the time and distance spent in center and periphery in the first 5 min were calculated for anxious-like behavior and total distance walked was calculated for evaluate locomotor performance.

2.4.4. Independent forelimb reaching

The staircase test was used to evaluate independent paw reaching. The test was done in double staircase boxes (model n° 80,300, Campden

Instruments, UK) as described previously (Carvalho et al., 2017; Cunha et al., 2017). Briefly, the apparatus consisted of a clear Perspex chamber with a hinged lid connected to a narrower compartment with a central platform and a 19 mm wide trough on either side, in which a removable double staircase with 7 steps was placed. Each of the steps contained a small well into which five sugared pellets (dustless precision pellets ® 45 mg) were placed. The test consisted of 9 days. In the first 2 days animals were left in the box for 5 and 10 min, respectively, to get habituated to the boxes. In the next 5 days, rats were left for 15 min and pellets left in each step registered. In the last 2 days pellets were only placed on the right or left side (force choice test). Total number and percentage (success rate) of eaten pellets were calculated posteriorly.

2.4.5. Food consumption

To test the rats' drive to eat normal chow and sugared pellets (dustless precision pellets® 45 mg), they were left in individual cages with access to 20 g of normal chow and sugar pellets for 60 and 30 min, respectively. Food consumed was calculated in the end.

2.4.6. Depressive-like behavior

The FST (Porsolt et al., 1977) was performed to evaluate depressive-like behavior (learned helplessness) as previously described by the group (Guimaraes et al., 2019; Leite-Almeida et al., 2009). Rats were placed in a glass cylinder filled with water, from which they cannot escape, and left there for 5 min. Two sessions in subsequent days were performed and the second was recorded for posterior behavioral scoring by a blind researcher using Observador® (Faculty of Pharmacy, Natioanal and Kapodistrian University of Athens) software. Immobility was defined as movement only necessary for the rat to remain at surface.

2.5. Lesion evaluation

After completion of the behavioral studies rats were euthanized with pentobarbital (ip; Eutasil®; Ceva Sante Animale, France) and transcardially perfused with Phosphate Buffer Saline (PBS) followed by Paraformaldehyde (PFA; 4% in PBS; Sigma-Aldrich). Brains were then removed and after post-fixation with PFA for 48 h, were sectioned coronally (50 μm of thickness) on a vibrating microtome (VT1000S, Leica, Germany). Through free-floating immunohistochemistry, sections were immersed in PBS with 3% H_2O_2 (20 min; Scharlau, Spain), followed by blocking with 5% fetal calf serum (FCS; Thermo Fisher Scientific Life Sciences, USA) in PBS (2 h). After blocking, sections were incubated overnight with the rabbit anti-mouse Tyrosine Hydroxylase (TH) primary antibody (1:2000; Merck, USA) in 2% of FCS in PBS, followed by incubation for 30 min with a biotinylated goat anti-polyvalent antibody, and another 30 min' incubation with a Streptavidin Peroxidase complex (TP-125-HL, Thermo Fisher Scientific Life Sciences). Antigen visualization was performed using 25 mg of 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich) in 50 ml of Tris-HCl 0.05 M, pH 7.6, with 12.5 μl of H_2O_2, and stopped at the desired time. Sections were then mounted on superfrost slides (Thermo Fisher Scientific Life Sciences) and counterstained with thionine (Donovick, 1974).

The localization and extension of the lesions was calculated through images obtained in a stereo microscope (SZX7, Olympus, USA; 20×) connected to a digital camera and with the help of the Visiomorph software (V2.12.3.0, Visiopharm, Denmark) present in the linked computer. The local of injection was analyzed in ImageJ software by using a densitometry analysis protocol as described by (Febbraro et al., 2013). 3 or 6 squares (20 mm^2) were analyzed for each side of lower STR and NAc, respectively. After averaging the values of the squares, the ratio between the lesioned and the non-lesioned side was done and the percentage of lesion for each area calculated.

2.6. Statistical analysis

Statistical analyses were performed in IBM SPSS Statistics 24 (IBM software, Inc., USA) and graphs in GraphPad Prism 5.0 (GraphPad software, Inc., USA). Values above or below the Mean ± 2 x SD were considered outliers and excluded from the analysis. For repeated measures, sphericity was calculated with the Mauchly test and if violated correction was done by the Greenhouse-Geisser. Mixed-design ANOVA was used to evaluate learning curves, cumulative premature responses (VDS test) and contingency degradation (habit-based/goal directed decision-making). Devaluation in the operant behavior and staircase performance in the forced choice was tested with paired *t*-tests for each group and all the other results reported were tested by one-way ANOVA. *Post-hoc* analysis was done by using the Bonferroni test if the equality of variances was assumed and by Games-Howell if not. Equality of variances was tested with Levene's test. η_p^2 was used as measure of effect size. Data were considered significant if $p < .05$. All results are represented as mean ± SEM.

3. Results

To understand the lateralized role of dopaminergic efferents to the NAc in behavior, 6-OHDA was injected in the left or right NAc of male rats. Three weeks after the surgery, rats were evaluated in a series of cognitive, emotional and motor behaviors as described in Fig. 1A.

Upon examination of the brain slices, 6-OHDA-L animals present in average 40.1% less fibers than the control unlesioned right NAc and 6-OHDA-R animals less 43.9% than the left NAc (Fig. 2); no significant differences were observed between left- and right-sided lesions ($t_{(12)} = 0.367$; $p = .720$). Four animals (2 left side) were excluded from the analysis because the localization of the lesion could not be confirmed and 11 rats (6 6-OHDA L and 5 6-OHDA R), in which STR was affected, were also excluded (Fig. 2). Results including these animals are not different from the ones reported (supplementary data table III) but were not considered to assure the behavioral deficits observed were exclusively dependent of NAc.

Considering Sham animals, no statistically significant differences were found between rats injected with ascorbic acid in the right and in the left NAc (supplementary data table I), so they were considered a single group in all the analyses.

Body weight was controlled frequently along the experiment and used as a measure of animals' well-being and health state. None of the animals lost more than 15% of its body weight during food restriction. Furthermore, no statistically significant differences were found between the groups in the body weight alterations nor in the evolution of these alterations during the experiment (supplementary data Fig. 1).

3.1. Habit-based/goal-directed behavior

All groups learn the goal-directed task. Along the task all animals perform 2 sessions per day, one for each reward. Results shown represent the average number of lever presses per minute for both rewards along the days. Statistical analysis did not reveal differences between the groups in the number of lever presses per minute neither in their evolution along sessions (supplementary data table II; Fig. 3A).

To test for habit behavior, a devaluation test was done in 2 consecutive days, one for each reward (order was balanced across animals/groups). Presented results correspond to the average number of lever presses for the valued and devalue lever in both days. All the groups were capable of devaluate the reward in the early (Sham: $t_{(15)} = 4.214$, $p = .001$; 6-OHDA L: $t_{(9)} = 4.259$, $p = .002$; 6-OHDA R: $t_{(9)} = 3.675$, $p = .005$) (Fig. 3B) and in the late test (after habit induction) (Sham: $t_{(15)} = -6.836$, $p < .001$; 6-OHDA L: $t_{(10)} = 3.862$, $p = .003$; 6-OHDA R: $t_{(9)} = 3.803$, $p = .004$) (Fig. 3C). Importantly, there are no statistically significant differences in sucrose and pellets consumption, in early and late tests (supplementary data table II). After late

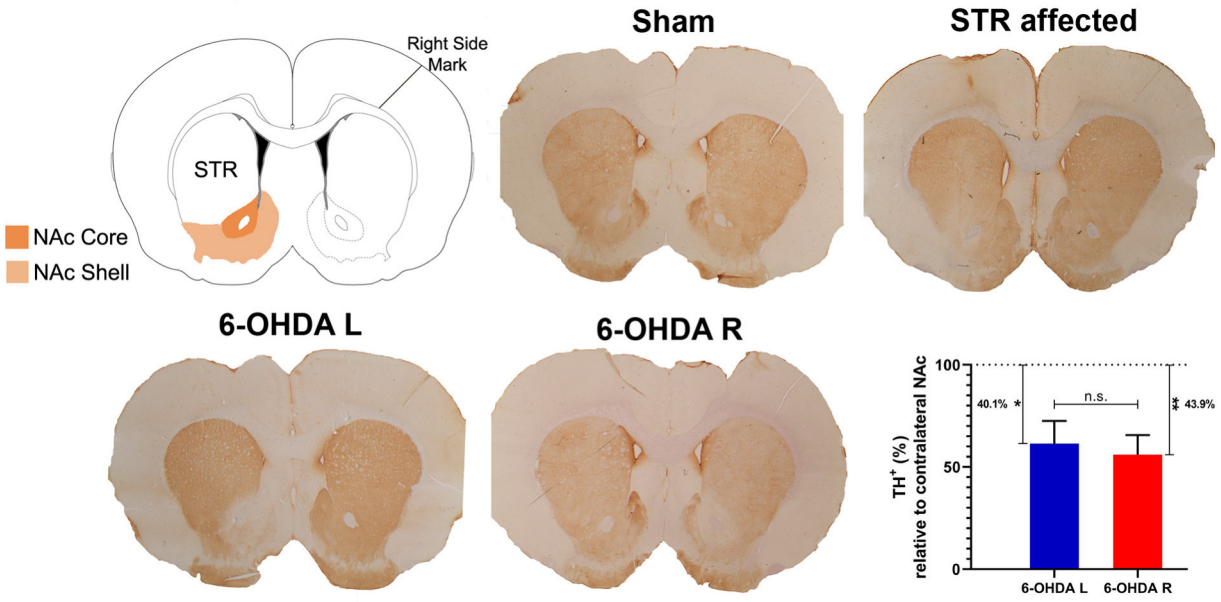

Fig. 2. Characterization of the 6-OHDA lesions. Exemplificative images of NAc lesions for Sham (n = 17 (9 L + 8 R)), 6-OHDA L (n = 12) and 6-OHDA R (n = 11) groups by immunohistochemistry against TH. 6-OHDA injected animals presented a statistically significant reduction in TH positive fibers; no differences were observed between 6-OHDA-L and 6-OHDA-R. In some cases, the STR was also affected by the 6-OHDA injection and the animals were excluded. Data presented as mean ± SEM. ** p < .01 *** p < .001. 6-OHDA – 6-Hydroxidopamine; L-Left; NAc- Nucleus Accumbens; R-Right; STR- Striatum; TH- Tyrosine hydroxylase.

devaluation, animals were submitted to a CD in one of the levers (side balanced across animals/groups). Results are presented as the percentage of degradation in relation to the RR20 in the previous day. Statistical analysis revealed an impact of degradation in the number of lever presses ($F_{(38)}$ = 30.45, p < .001, η_p^2 = 0.406). *Post-hoc* analysis shows that even though all groups decrease the number of lever presses, only Sham and 6-OHDA L present a significant decrease (Sham: p = .001, 6-OHDA L: p = .001, 6-OHDA R: p = .322) (Fig. 3D). Lever presses in the non-degraded lever did not decrease (Fig. 3D).

Considering the possibility of a carryover effect of one operant behavior to the other, we performed two independent sets of animals, one starting with the habit-based/goal directed task and the other with the VDS test. No statistically significant differences were found between the two sets in the habit-based/goal directed task (supplementary data table IV), although animals which had previously done the VDS tend to perform more lever presses per minute along the learning phase (Fig. 3E).

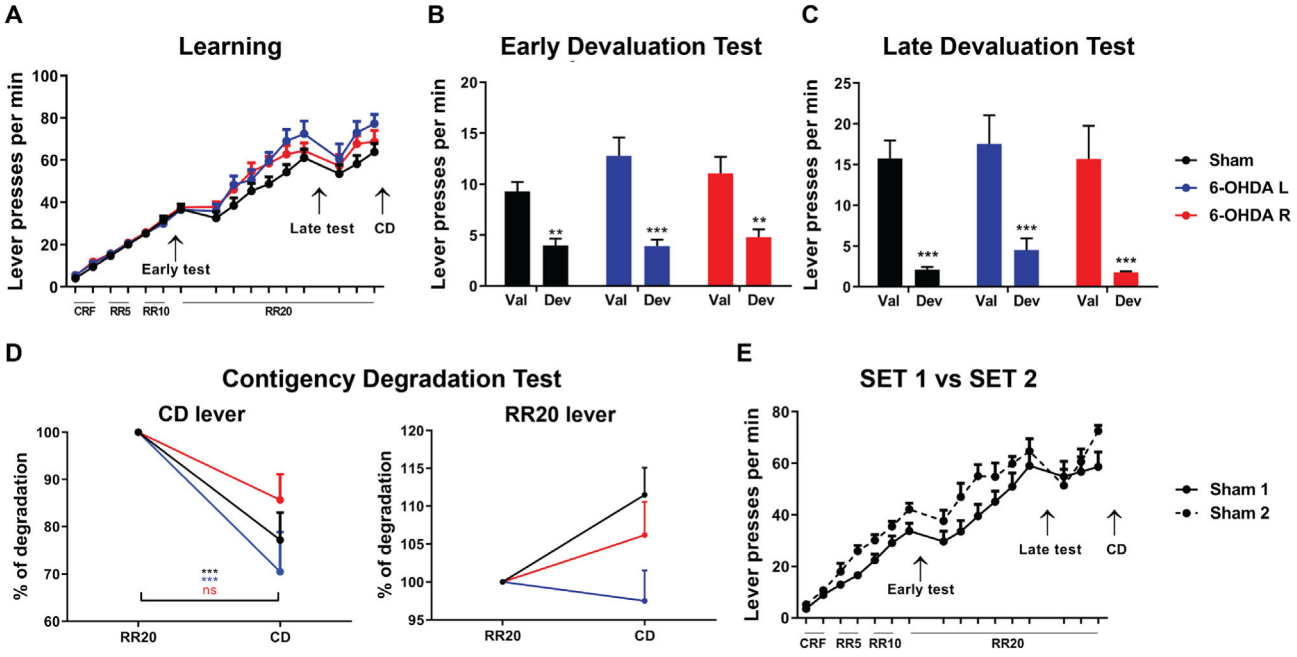

Fig. 3. Habit-based/goal-directed behavior. All the animals learned the task equally (A) and devaluated the reward before (B) and after (C) habit induction. In the CD all groups increased the number of lever presses per minute in the non-degraded lever but only Sham and 6-OHDA L significantly decrease the number of lever presses per minute in the degraded lever (D). Learning curves of set 1 and 2 (E). Data presented as mean ± SEM. ** p < .01 *** p < .001. 6-OHDA – 6-Hydroxidopamine; CD- Contingency degradation; CRF- Continuous reinforcement; Dev- Devalue; L-Left; R-Right; RR- Random ratio; Val- Value.

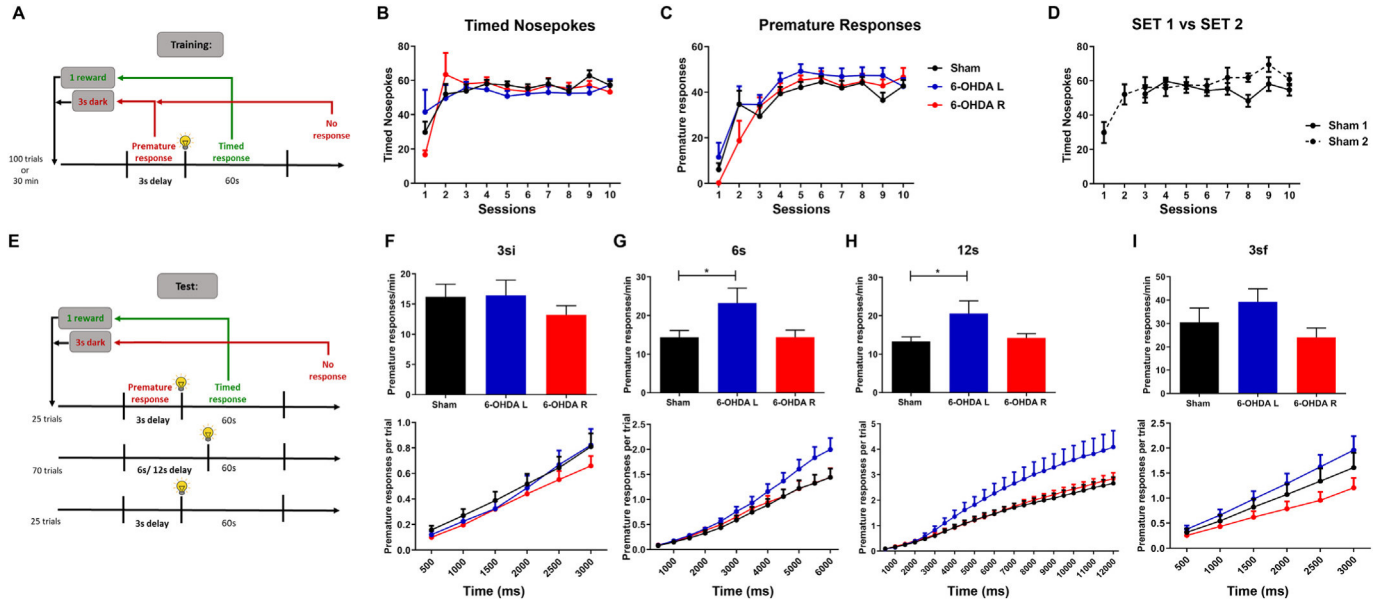

Fig. 4. Variable delay-to-signal (VDS) test. Schematics of VDS training (A) and test (E). All the animals learn the task equally (B) and perform similar number of premature responses along training (C). No statistically significant differences were found between the groups in the number of total and cumulative premature responses in the trials of 3 s delay (F, I) but 6-OHDA L perform higher number of premature responses in the longer delays than controls (G, H). Animals which had previously performed operant behavior learn quicker than the others (D). Data presented as mean ± SEM. * $p < .05$. 6-OHDA – 6-Hydroxidopamine; L-Left; R-Right; s- seconds.

3.2. Impulsive behavior

To test impulsive response, the VDS test was performed. No statistically significant differences were found between groups in the training phase regarding timed or premature responses (Supplementary data table II; Fig. 4B–C). In the test, 6-OHDA L animals present higher impulsivity particularly in the large delays, considering both the total number of premature responses (6 s: $F_{(2,34)} = 3.821$, $p = .032$, $\eta_p^2 = 0.184$; Sham vs 6-OHDA L: $p = .047$ and 12 s: $F_{(2,34)} = 3.811$, $p = .032$, $\eta_p^2 = 0.183$; Sham vs 6-OHDA L: $p = .037$; Fig. 4F, G, H, I) and the accumulation of premature responses along the delay (6 s: Group: $F_{(2,33)} = 1.336$, $p = .277$, $\eta_p^2 = 0.075$; Group*Time: $F_{(2.345,38.693)} = 2.151$, $p = .123$, $\eta_p^2 = 0.115$; 12 s: Group: $F_{(2,34)} = 3.474$, $p = .042$, $\eta_p^2 = 0.170$; Group*Time: $F_{(2.218,37.703)} = 4.740$, $p = .012$, $\eta_p^2 = 0.218$; Fig. 4F, G, H, I). Finally, no statistically significant differences were found between the groups in the VDS in the average latency to feed and response latency, revealing no motor neither motivation problems in the lesioned groups during the test (supplementary data table II).

Comparison of set 1 and 2 reveal an effect of previous operant performance on VDS training (Group*Set: $F_{(3.419,\ 51.287)} = 5.175$, $p = .002$, $\eta_p^2 = 0.257$): animals which had already performed operant behavior, learn the task quicker than the ones which did not (Fig. 4D); animals from set 1 performed only 8 sessions as they reached a steady performance immediately due to a carryover effect.

3.3. Emotional behavior

To test anxiety- and depressive-like behavior we performed the OF and the FST, respectively. No statistically significant differences were found between the groups in the immobility, swimming and climbing time (supplementary data table II; Fig. 5A). The same was true for OF, no statistically significant differences were found between the groups in the ratio center/periphery, considering both time spent and distance walked (supplementary data table II; Fig. 5B). Also, all groups eat similar amounts of normal chow and sugared pellets (supplementary data table II; Fig. 5C).

3.4. Motor performance

To control for possible motor interferences a locomotion test was done in the OF apparatus. Lesioned animals walked similar distances to control animals in the 15 min of the test (supplementary data table II; Fig. 6A). Concerning side specific paw reaching, evaluated by the staircase test, no statistically significant differences were found between the groups in the average success rate along the five days of test (Fig. 6B) neither in the forced choice trials (Fig. 6C) (supplementary data table II).

4. Discussion

In the present work, we investigated the impact of a lateralized depletion of dopaminergic efferences to the NAc in emotional behavior and decision-making, while controlling for potential motor effects. We obtained side-specific results, with right dopaminergic efferents depletion affecting the CD and left lesion affecting impulsive behavior.

Concerning the former, we observed that 6-OHDA R animals were less affected by CD than 6-OHDA L and Sham animals, presenting a smaller decrease in lever presses from the last RR20 to the first day of CD. An important aspect of this observation is that the lever press level in the non-degraded lever was maintained, indicating an impairment in the shift from habitual to goal-directed responses rather than a motivational cause. In the extinction test, 6-OHDA R distinguished devalued and non-devalued levers as 6-OHDA L and Sham controls in line with previous instrumental devaluation studies in bilateral 6-OHDA NAc injections (Lex and Hauber, 2010). In opposition to what was observed in 6-OHDA bilateral injections (Corbit et al., 2001; Lex and Hauber, 2010), no substantial differences were observed in instrumental acquisition. Indeed, 6-OHDA animals performed at slightly higher rates as the protocol proceeded through RR20 schedules, exposing the milder nature of the unilateral model and eliminating potential interpretative bias.

On the contrary, 6-OHDA L (but not 6-OHDA R) presented increased impulsivity in the VDS. The lesion specifically affected delay tolerance (increased response rate during the large delays) and had no effect in the number of premature responses during the training phase – a proxy

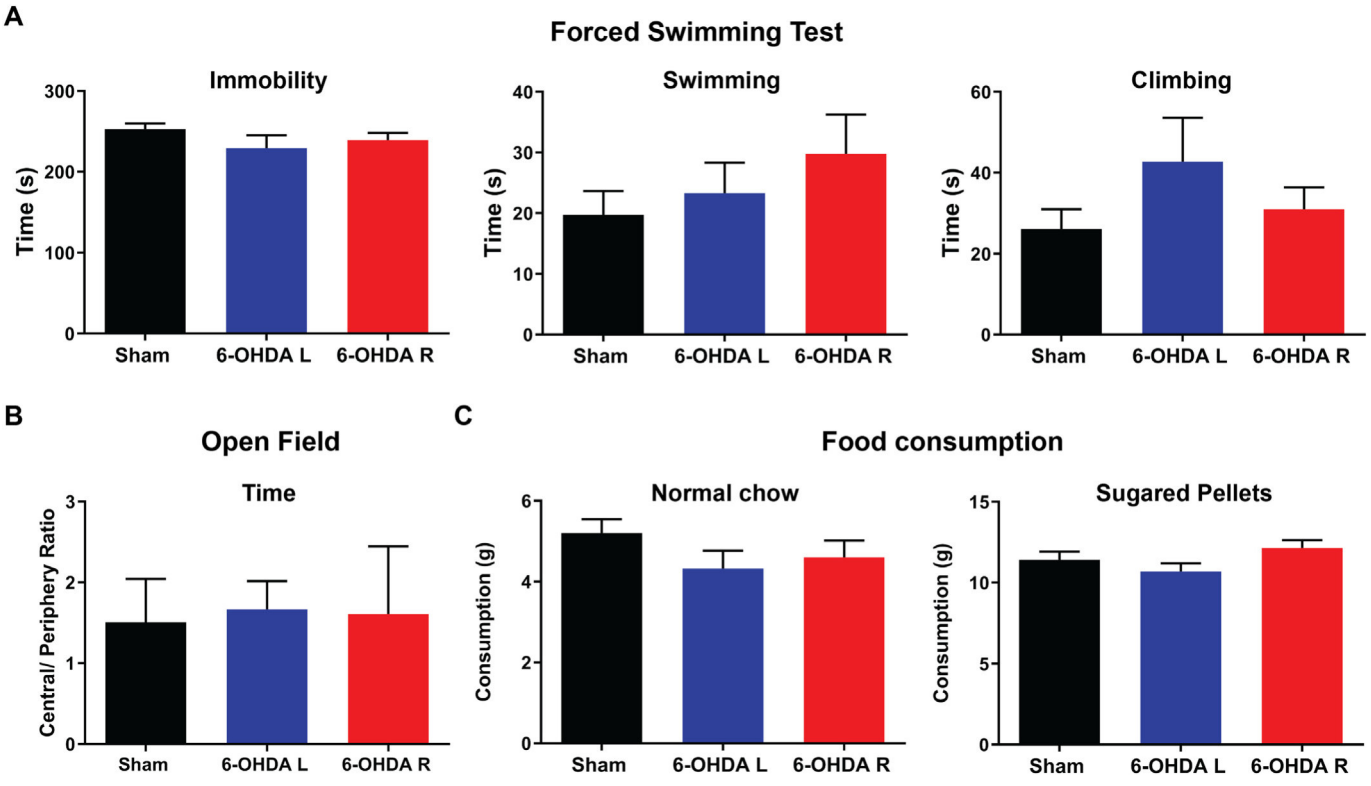

Fig. 5. Emotional behavior. All the groups performed similarly in the FST (A) and in the OF (B), revealing no impairments in depressive- and anxiety-like behaviors after NAc lesion. All the groups eat similar amounts of normal chow and sugared pellets (C). Data presented as mean ± SEM. 6-OHDA – 6-Hydroxidopamine; FST-forced swimming test; L-Left; NAc- Nucleus Accumbens; OF- Open Field; R-Right;

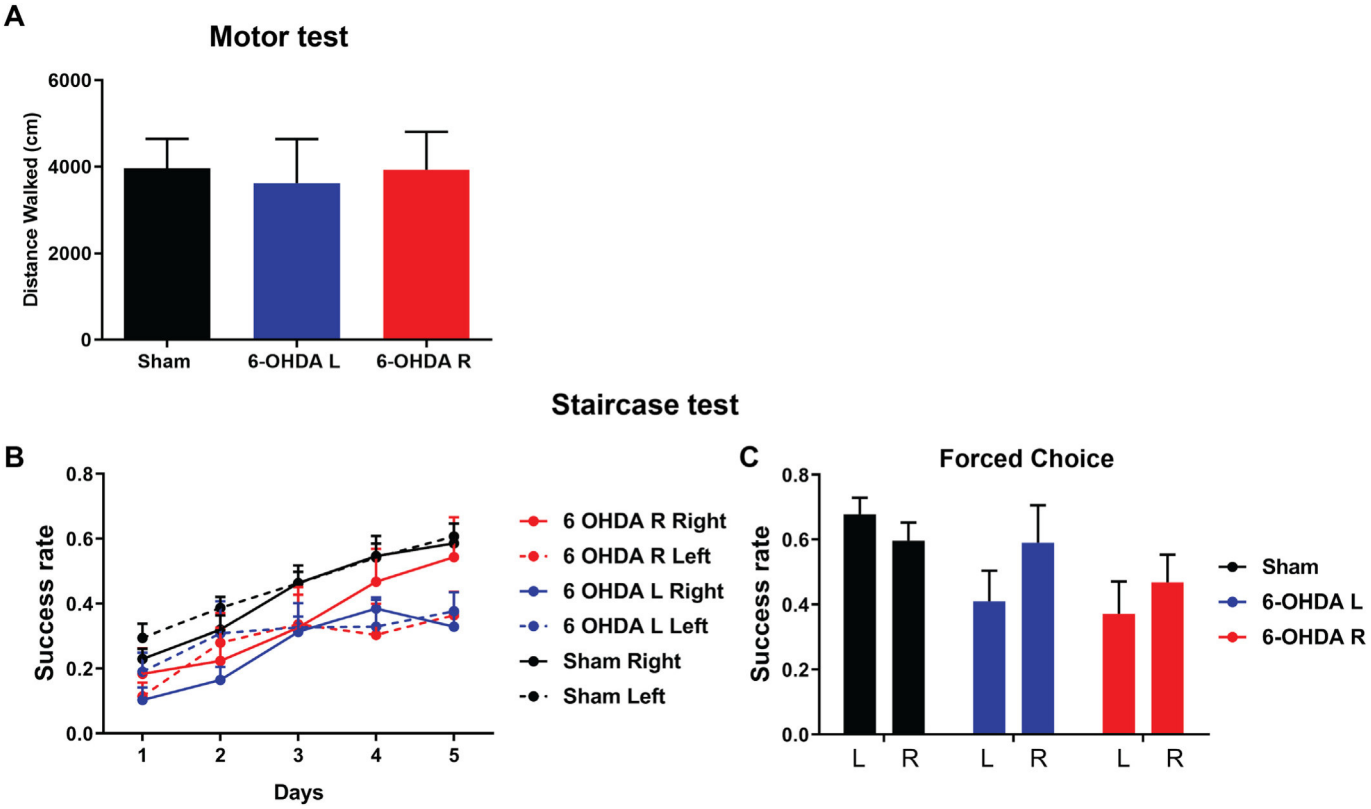

Fig. 6. Motor performance. No statistically significant differences were found between the groups in the distance walked in the OF apparatus (A). All the groups had similar performances with left and right paw in the staircase test along the 5 days (B) and in the forced choice trial (C). Data presented as mean ± SEM. 6-OHDA – 6-Hydroxidopamine; L-Left; OF- Open Field; R-Right;

of action impulsivity (Leite-Almeida et al., 2013). Previous studies assessing delay tolerance in impulsive choice (preference for smaller immediate rewards over higher but delayed ones) paradigms, e.g. delay discounting, obtained disparate results depending on the type of lesion/manipulation. Specifically, bilateral excitotoxic (quinolinic acid) lesions of NAc core increased impulsive choice (Bezzina et al., 2007; Cardinal and Howes, 2005; Cardinal et al., 2001; da Costa Araujo et al., 2009; Pothuizen et al., 2005), while its complete lesion – core + shell (Acheson et al., 2006) – or temporary inactivation with baclofen/muscimol (Moschak and Mitchell, 2014) led to the opposite effect. Specifically regarding 6-OHDA lesions, Wistanley and colleagues (2005) found no differences in delay discounting (DD). A relevant difference between this study and ours is that the DD involves a choice component (small/immediate vs large/delayed) that is absent in the VDS where the reward is constant. Another relevant aspect is that Wistanley and colleagues (2005) used bilateral 6-OHDA lesions. Indeed, Capriolli and colleagues have shown, in a series of studies, a lateralized involvement of the NAc in trait action impulsive behavior (Caprioli et al., 2013; Caprioli et al., 2015; Caprioli et al., 2014). More specifically, the left NAc of highly impulsive naïve rats presented a reduction of gray matter density, structural proteins, glutamate decarboxylase and $D_{2/3}$ receptor availability in comparison to low impulsive naïve rats.

Altogether, our results indicate that left/right dopamine balance in the NAc is critical for different decision-making domains. Potential confounders associated with the lesion were largely excluded. As shown by others (Correa et al., 2002; Liu et al., 1998; Tsutsui et al., 2011) – confront with (open-field; Winnicka, 1999) – we have not observed group differences in general locomotion and fine movements. In line with previous studies, we have not observed alterations in motivation to eat and emotional readouts in either left or right DA lesions (bilateral 6-OHDA; Tsutsui et al., 2011). Also, in a pilot-study we did not observe ipsiversive rotations in apomorphine injected animals (results not shown), a standard protocol to test for unilateral nigrostriatal lesions.

5. Conclusions

We show that 6-OHDA lesions of the right NAc lead to lower contingency degradation after habit establishment and that the same lesions in the left NAc lead to increased impulsivity. Posterior studies need to be done to test if the same is true for humans and to try to understand the mechanisms behind this functional laterality of NAc and its implication on the decision-making impairments observed on PD patients.

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Authors' contributions

AMC, FGT and HL-A design the experiment. AMC, FGT, MRG, ME and ARS perform the experiments. AMC and JP-M analyzed the behavior. AMC analyzed the extension of the lesions and performed the statistical analysis. All the authors interpreted and discussed the data. AMC, FGT and HL-A wrote the first version of the manuscript. All the authors revised and approved the final version of the article.

Declaration of Competing Interest

The authors declare that they have no competing interests to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2020.113221.

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SUPPLEMENTARY DATA

Table I- Effects of laterality on Sham animals

	Test		p
Learning of Habit-based task	Mixed-design ANOVA	Time: F (3.578) = 86.125 Time*Side: F (3.578) = 0.771 Side: F (1, 15) = 0.091	Time: <0.001 Time*Side: 0.536 Side: 0.767
Early Valuation	Independent t-test	t (15) = 1.246	0.232
Early Devaluation	Independent t-test	t (8.637) = 1.806	0.106
Late Valuation	Independent t-test	t (15) = 0.462	0.650
Late Devaluation	Independent t-test	t (15) = 1.114	0.283
Contingency Degradation	Independent t-test	t (15) = 0.328	0.747
Premature responses along VDS training	Mixed-design ANOVA	Time: F (2.648) = 14.189 Time*Side: F (2.648) = 0.514 Side: F (1, 11) = 0.065	Time: <0.001 Time*Side: 0.654 Side: 0.804
Timed nosepokes along VDS training	Mixed-design ANOVA	Time: F (2.617) = 3.837 Time*Side: F (2.617) = 0.462 Side: F (1, 15) = 0.009	Time: 0.021 Time*Side: 0.685 Side: 0.925
Premature Responses 3si	Independent t-test	t (14) = 0.372	0.715
Premature Responses 6s	Independent t-test	t (14) = -0.340	0.739
Premature Responses 12s	Independent t-test	t (14) = 0.246	0.809
Premature Responses 3sf	Independent t-test	t (14) = -0.251	0.805
OF time ratio	Independent t-test	t (15) = - 1.411	0.179
OF distance ratio	Independent t-test	t (15) = - 0.536	0.600
Immobility time in FST	Independent t-test	t (12) = - 1.734	0.109
Food consumption	Independent t-test	t (15) = - 0.165	0.871
Sugar pellets consumption	Independent t-test	t (14) = - 0.220	0.829
Motor test	Independent t-test	t (15) = 0.551	0.590
Staircase- 5 days left	Mixed-design ANOVA	Time: F (2.134) = 25.690 Time*Side: F (2.134) = 1.927 Side: F (1, 14) = 1.737	Time: <0.001 Time*Side: 0.161 Side: 0.209
Staircase- 5 days right	Mixed-design ANOVA	Time: F (2.163) = 19.442 Time*Side: F (2.163) = 0.630 Side: F (1, 14) = 0.463	Time: <0.001 Time*Side: 0.551 Side: 0.507
Staircase- Forced choice left	Independent t-test	t (14) = 0.863	0.403
Staircase- Forced choice right	Independent t-test	t (15) = -0.964	0.351

Table I. Sham animals injected with vehicle in the left or right Nucleus Accumbens do not present difference in the analyzed behaviors.

FST- Forced swimming test; OF- Open field; VDS- Variable delay-to-signal

Table II- Behavioral alterations of dopaminergic depletion in the left and right Nucleus Accumbens

	Test		p
Learning of Habit-based task	Mixed-design ANOVA	Time: F (3.514) = 205.785 Time*Group: F (7.028) = 1.682 Group: F (2, 35) = 1.849	Time: <0.001 Time*Group: 0.119 Group: 0.172
Sucrose Consumption Early	One-way ANOVA	F (2, 36) = 0.851	0.436
Pellets Consumption Early	One-way ANOVA	F (2, 36) = 0.264	0.769
Sucrose Consumption Late	One-way ANOVA	F (2, 35) = 0.773	0.469
Pellets Consumption Late	One-way ANOVA	F (2, 36) = 2.213	0.124
Premature responses along VDS training	Mixed-design ANOVA	Time: F (3.466) = 39.100 Time*Group: F (6.931) = 0.995 Group: F (2, 37) = 1.022	Time: <0.001 Time*Group: 0.438 Group: 0.370
Timed nosepokes along VDS training	Mixed-design ANOVA	Time: F (2.726) = 4.147 Time*Group: F (5.451) = 1.015 Group: F (2, 37) = 0.406	Time: 0.010 Time*Group: 0.416 Group: 0.669
Premature Responses 3si	One-way ANOVA	F (2, 34) = 0.608	0.550
Premature Responses 3sf	One-way ANOVA	F (2, 34) = 1.488	0.240
Cumulative Premature Responses 3si	Mixed-design ANOVA	Time: F (1.411) = 105.782 Time*Group: F (2.822) = 0.560 Group: F (2, 34) = 0.418	Time: <0.001 Time*Group: 0.634 Group: 0.662
Cumulative Premature Responses 3sf	Mixed-design ANOVA	Time: F (1.079) = 85.145 Time*Group: F (2.157) = 1.686 Group: F (2, 35) = 1.266	Time: <0.001 Time*Group: 0.197 Group: 0.295
Latency to Feed	One-way ANOVA	F (2, 36) = 2.539	0.093
Latency to response 3si	One-way ANOVA	F (2, 32) = 0.437	0.650
Latency to response 6s	One-way ANOVA	F (2, 32) = 1.558	0.226
Latency to response 12s	One-way ANOVA	F (2, 32) = 2.359	0.111
Latency to response 3sf	One-way ANOVA	F (2, 45) = 0.606	0.552
OF time ratio	One-way ANOVA	F (2, 35) = 0.284	0.755
OF distance ratio	One-way ANOVA	F (2, 36) = 0.363	0.698
Immobility time in FST	One-way ANOVA	F (2, 36) = 1.263	0.295
Swimming time in FST	One-way ANOVA	F (2, 35) = 1.050	0.361
Climbing time in FST	One-way ANOVA	F (2, 36) = 1.419	0.255
Food consumption	One-way ANOVA	F (2, 35) = 1.929	0.160
Sugar pellets consumption	One-way ANOVA	F (2, 35) = 1.802	0.180
Motor test	One-way ANOVA	F (2, 36) = 0.983	0.384
Staircase- 5 days Sham	Mixed-design ANOVA	Time: F (2.280) = 41.172 Time*Side: F (2.280) = 0.626 Side: F (1, 30) = 0.305	Time: <0.001 Time*Side: 0.558 Side: 0.585
Staircase- 5 days 6-OHDA L	Mixed-design ANOVA	Time: F (2.381) = 10.930 Time*Side: F (2.381) = 0.892 Side: F (1, 22) = 0.107	Time: <0.001 Time*Side: 0.432 Side: 0.747
Staircase- 5 days 6-OHDA R	Mixed-design ANOVA	Time: F (4) = 14.671 Time*Side: F (4) = 0.651 Side: F (1, 20) = 0.115	Time: <0.001 Time*Side: 0.628 Side: 0.904
Staircase- Forced choice	Paired t-test	Sham: t (15) = 0.524 6-OHDA L: t (11) = 0.533 6-OHDA R: t (10) = 1.451	Sham: 0.608 6-OHDA L: 0.605 6-OHDA R: 0.177

Table II- 6-OHDA L and 6-OHDA R animals are not different from controls in operant task acquisition (Habit-based task and VDS), neither in the emotional, drive to eat and motor tests studied.

6-OHDA L- Injection of 6-hydroxydopamine in the left NAc; 6-OHDA R- Injection of 6-hydroxydopamine in the right NAc; FST- Forced swimming test; NAc- Nucleus Accumbens; OF- Open field; VDS- Variable delay-to-signal

Table III- Behavioral alterations of dopaminergic depletion in the left and right Nucleus Accumbens (including STR damaged animals)

	Test		p
Learning of Habit-based task	Mixed-design ANOVA	Time: F (2.821) = 251.910 Time*Group: F (5.641) = 1.824 Group: F (2, 47) = 2.070	Time: <0.001 Time*Group: 0.104 Group: 0.138
Early Devaluation	Paired t-test	Sham: t (15) = 4.214 6-OHDA L: t (15) = 4.187 6-OHDA R: t (14) = 4.515	Sham: 0.001 6-OHDA L: 0.001 6-OHDA R: <0.001
Late Devaluation	Paired t-test	Sham: t (15) = 6.836 6-OHDA L: t (16) = 5.341 6-OHDA R: t (14) = 4.936	Sham: <0.001 6-OHDA L: <0.001 6-OHDA R: <0.001
Sucrose Consumption Early	One-way ANOVA	F (2, 47) = 0.680	0.512
Pellets Consumption Early	One-way ANOVA	F (2, 47) = 0.322	0.726
Sucrose Consumption Late	One-way ANOVA	F (2, 46) = 0.135	0.874
Pellets Consumption Late	One-way ANOVA	F (2, 47) = 1.680	0.197
Contingency Degradation	Mixed-design ANOVA	Time: F(46) = 29.765	<0.001 Sham: <0.001 6-OHDA L: 0.002 6-OHDA R: 0.280
Premature responses along VDS training	Mixed-design ANOVA	Time: F (3.767) = 44.970 Time*Group: F (7.535) = 1.295 Group: F (2, 48) = 1.292	Time: <0.001 Time*Group: 0.251 Group: 0.284
Timed nosepokes along VDS training	Mixed-design ANOVA	Time: F (2.782) = 3.955 Time*Group: F (5.564) = 0.915 Group: F (2, 48) = 0.278	Time: 0.012 Time*Group: 0.481 Group: 0.759
Premature Responses 3si	One-way ANOVA	F (2, 43) = 0.490	0.616
Premature Responses 6s	One-way ANOVA	F (2, 44) = 4.228	0.021 Sham vs 6-OHDA L: 0.040
Premature Responses 12s	One-way ANOVA	F (2, 45) = 3.111	0.054
Premature Responses 3sf	One-way ANOVA	F (2, 43) = 0.324	0.725
Cumulative Premature Responses 3si	Mixed-design ANOVA	Time: F (1.337) = 143.551 Time*Group: F (2.673) = 0.688 Group: F (2, 45) = 0.307	Time: <0.001 Time*Group: 0.547 Group: 0.737
Cumulative Premature Responses 6s	Mixed-design ANOVA	Time: F (1.132) = 136.4 Time*Group: F (2.264,49.819) = 4.025 Group: F (2, 44) = 3.133	Time: <0.001 Time*Group: 0.020 Group: 0.053
Cumulative Premature Responses 12s	Mixed-design ANOVA	Time: F (1.088) = 202.7 Time*Group: F (2.177, 48.978) = 4.062 Group: F (2, 45) = 2.721	Time: <0.001 Time*Group: 0.077 Group: 0.077
Cumulative Premature Responses 3sf	Mixed-design ANOVA	Time: F (1.072) = 121.760 Time*Group: F (2.144) = 0.764 Group: F (2, 46) = 0.628	Time: <0.001 Time*Group: 0.480 Group: 0.538

(continues)

Table III- Behavioral alterations of dopaminergic depletion in the left and right Nucleus Accumbens (including STR damaged animals) (continued)

	Test		p
Latency to Feed	One-way ANOVA	F (2, 48) = 0.160	0.852
Latency to response 3si	One-way ANOVA	F (2, 45) = 2.028	0.143
Latency to response 6s	One-way ANOVA	F (2, 45) = 0.894	0.416
Latency to response 12s	One-way ANOVA	F (2, 45) = 2.062	0.139
Latency to response 3sf	One-way ANOVA	F (2, 45) = 0.650	0.527
OF time ratio	One-way ANOVA	F (2, 45) = 0.752	0.477
OF distance ratio	One-way ANOVA	F (2, 47) = 0.732	0.486
Immobility time in FST	One-way ANOVA	F (2, 47) = 0.889	0.418
Swimming time in FST	One-way ANOVA	F (2, 46) = 1.004	0.374
Climbing time in FST	One-way ANOVA	F (2, 47) = 0.618	0.543
Food consumption	One-way ANOVA	F (2, 46) = 2.664	0.080
Sugar pellets consumption	One-way ANOVA	F (2, 46) = 0.039	0.962
Motor test	One-way ANOVA	F (2, 47) = 0.398	0.674
Staircase- 5 days Sham	Mixed-design ANOVA	Time: F (2.280) = 41.172 Time*Side: F (2.280) = 0.626 Side: F (1, 30) = 0.305	Time: <0.001 Time*Side: 0.558 Side: 0.585
Staircase- 5 days 6-OHDA L	Mixed-design ANOVA	Time: F (2.748) = 20.561 Time*Side: F (2.748) = 1.898 Side: F (1, 34) = 0.001	Time: <0.001 Time*Side: 0.140 Side: 0.972
Staircase- 5 days 6-OHDA R	Mixed-design ANOVA	Time: F (2.759) = 25.130 Time*Side: F (2.759) = 0.475 Side: F (1, 30) = 0.115	Time: <0.001 Time*Side: 0.685 Side: 0.736
Staircase- Forced choice	Paired t-test	Sham: t (15) = 0.524 6-OHDA L: t (17) = -0.252 6-OHDA R: t (15) = -0.210	Sham: 0.608 6-OHDA L: 0.804 6-OHDA R: 0.836

Table III-Including in the experimental groups animals in which the STR was affected by the 6-OHDA injection does not alter the results.

6-OHDA L- Injection of 6-hydroxydopamine in the left NAc; 6-OHDA R- Injection of 6-hydroxydopamine in the right NAc; FST- Forced swimming test; NAc- Nucleus Accumbens; OF- Open field; VDS- Variable delay-to-signal

Table IV- Comparison of Sham animals from SET 1 and 2

	Test		p
Learning of Habit-based task	Mixed-design ANOVA	Time: F (3.372) = 86.631 Time*SET: F (3.372) = 1.008 SET: F (1, 15) = 2.565	Time: <0.001 Time*SET: 0.403 SET: 0.130
Early Valuation	Independent t-test	t (15) = -2.066	0.057
Early Devaluation	Independent t-test	t (15) = -0.604	0.555
Late Valuation	Independent t-test	t (15) = -0.496	0.627
Late Devaluation	Independent t-test	t (15) = 0.689	0.501
Contingency Degradation	Independent t-test	t (15) = -0.657	0.521
Premature responses along VDS training	Mixed-design ANOVA	Time: F (2.461) = 18.282 Time*SET: F (2.461) = 2.399 SET: F (1, 11) = 1.184	Time: <0.001 Time*SET: 0.100 SET: 0.300
Premature Responses 3si	Independent t-test	t (14) = 0.799	0.438
Premature Responses 6s	Independent t-test	t (14) = 1.872	0.082
Premature Responses 12s	Independent t-test	t (14) = 2.123	0.052
Premature Responses 3sf	Independent t-test	t (14) = 0.978	0.345
OF time ratio	Independent t-test	t (15) = 1.864	0.082
OF distance ratio	Independent t-test	t (15) = 1.041	0.314
Immobility time in FST	Independent t-test	t (12) = 0.992	0.341
Food consumption	Independent t-test	t (15) = 0.046	0.964
Sugar pellets consumption	Independent t-test	t (14) = 0.168	0.869
Motor test	Independent t-test	t (7.116) = -0.622	0.554
Staircase- 5 days left	Mixed-design ANOVA	Time: F (2.213) = 21.862 Time*SET: F (2.213) = 0.547 SET: F (1, 14) = 2.814	Time: <0.001 Time*SET: 0.602 SET: 0.116
Staircase- 5 days right	Mixed-design ANOVA	Time: F (4) = 28.326 Time*SET: F (4) = 4.863 SET: F (1, 14) = 3.320	Time: <0.001 Time*SET: 0.002 SET: 0.090
Staircase- Forced choice left	Independent t-test	t (15) = 0.434	0.670
Staircase- Forced choice right	Independent t-test	t (15) = -0.998	0.334

Table IV- Controls of Set 1 and 2 perform very similarly in the behavioral tests studied.

FST- Forced swimming test; OF- Open field; VDS- Variable delay-to-signal

Image 1- Body weight. No differences between groups were found in the amount of weight lost along the experiment even during food restriction period (Time: F (10) = 238.3 p<0.00; Time*Group: F (20) = 0.377 p= 0.994; Group: F (2, 47) = 0.152 p= 0.859). Data shown as mean ± SEM. 6-OHDA L- Injection of 6-hydroxydopamine in the left Nucleus Accumbens; 6-OHDA R- Injection of 6-hydroxydopamine in the right Nucleus Accumbens;

CHAPTER 2.3

Mesocorticolimbic monoamines in a rodent model of chronic neuropathic pain

Submitted manuscript

Mesocorticolimbic monoamines in a rodent model of chronic neuropathic pain

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Abstract

Chronic pain manifests in multiple disorders and is highly debilitating. While its pathophysiology is not fully understood, the involvement of the mesocorticolimbic monoaminergic systems have been shown to play a critical role in chronic pain emergence and/or maintenance. In this study, we analyzed the levels of monoamines dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in mesocorticolimbic areas – medial prefrontal cortex, orbitofrontal cortex, striatum, nucleus accumbens and amygdala – 1 month after a neuropathic lesion, the Spared Nerve Injury (SNI). In SNI animals, were observed a marginal decrease of DA and 5-HT in the striatum and a rightward shift in the levels of NA in the nucleus accumbens. While mesocorticolimbic monoamines might be relevant for chronic pain pathophysiology its content appears to be relatively unaffected in our experimental conditions.

1. Introduction

Chronic pain is a multidimensional disorder that affects nearly 20% of the adult population in Western countries (Breivik et al., 2006; Kennedy et al., 2014). Furthermore, it is frequently comorbid with depression, anxiety and cognitive deficits in patients and preclinical models (Bair et al., 2003; Leite-Almeida et al., 2015; Moriarty et al., 2011). The mesocorticolimbic system (MCL) has been classically associated with motivation, reward and decision-making (Russo and Nestler, 2013) and is believed to play pivotal roles in the relation between chronic pain and comorbid manifestations.

MCL monoaminergic systems have repeatedly been implicated in chronic pain by a substantial number of clinical and preclinical studies – see for examples (Benson et al., 2015; Taylor et al., 2016). For instance, decreased Fluorodopa uptake [measure by positron emission tomography (PET)] was reported in the striatum (STR) of burning mouth syndrome patients (Jaaskelainen et al., 2001) as well as an absence of dopamine (DA) release in the basal ganglia, after a painful stimuli, in fibromyalgia patients (Wood et al., 2007). In rodents, optogenetic stimulation of neurons projecting from nucleus accumbens (NAc) to prefrontal cortex (PFC) leads to antinociception and alleviates depressive-like behaviors in a neuropathy model (Lee et al., 2015). On the same line, pain relief in a mice model of neuropathic pain triggered DA release in the NAc (Xie et al., 2014).

Finally, antidepressant pharmacotherapies based on monoamine reuptake inhibition like the selective serotonin (5-HT) reuptake inhibitors (SSRIs), the 5-HT–noradrenaline (NA) reuptake inhibitors (SNRIs) and, more recently, the NA and DA reuptake inhibitor bupropion, have been used as effective analgesics in many chronic pain conditions – see for review (Benson et al., 2015) and references within.

However, despite the cumulative evidence, information regarding MCL monoamines' availability in chronic pain is still sparse. Therefore, in the present study we measured by High Performance Liquid Chromatography with an electrochemical detector (HPLC-ED) the levels of DA, NA and 5-HT in the medial PFC (mPFC), orbitofrontal cortex (OFC), STR, NAc and amygdala (AMY) in a neuropathic model of chronic pain (spared nerve injury -SNI) and Sham controls. We hypothesize that SNI is associated with decreased monoamine content particularly in the basal ganglia.

2. Material and methods

2.1. Animals

24 Wistar-Han male rats (Charles River Laboratories, Spain) were used in this study. Animals were housed 3 per cage (one from each experimental group – Sham, SNI-L and SNI-R) with water and food (4RF21; Mucedola, SRL, Settimo Milanese, Italy) *ad libitium* and kept in a room with controlled temperature (22°C ± 1°C) and humidity (55-60%) under a 12-hour light/dark cycle (lights on at 8 am). Body weight and well-being were monitored along the entire experiment and all procedures were approved by the Portuguese National Authority for Animal Health (Direção Geral de Alimentação e Veterinária – DGAV) and made in accordance with the guidelines of the European Communities Council Directive 2010/63/EU.

2.2. Spared Nerve Injury

At 2 months of age, the SNI (Decosterd and Woolf, 2000) model was induced in the left (SNI-L; N=8) or right (SNI-R; N=8) hindpaw of the animals as previously described (Guimaraes et al., 2019). For that, rats were anesthetized with a mix of 1:1.5 (1.25 ml/kg; intraperitoneal) of Dorbene® (Medetomidine Hydrochloride®, 1 mg/mL – Laboratorios SYVA, Spain) and Imalgene® (Ketamine, 100 mg/mL – Merial, France) (Esteves et al., 2019), respectively. Then, an incision to expose the three branches of the sciatic nerve was performed. In SNI animals, a ligation and axotomy of the peroneal and tibial nerves was done, while in Sham controls all nerves were left intact (N=8; 4 in the left hindpaw). Muscle and skin were then closed in two layers and the animals were left to recover in their cages. One animal (Sham left) died during the surgery.

2.3. Mechanical allodynia

Evoked pain was tested with Von Frey (VF) monofilaments by the up-and-down method (Chaplan et al., 1994). As previously described (Guimaraes et al., 2019), one at a time, rats were placed in the VF apparatus and left undisturbed for approximately 20 seconds (s). Then, a monofilament (2.0 g) was applied to the lesioned paw for no more than 4s. If the animal withdrew the paw (positive response) a monofilament of lower force was applied; if not (negative response) a monofilament of higher force was applied. The same strategy was used until the extremes have been achieved or 6 measures around the turning point were obtained. The range of VF monofilaments used was the follow: 0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0 and 15.0 g.

50% threshold was calculated using the following formula:

$$50\%g\_threshold = \frac{(10^{Xf+K.\delta})}{10000}$$

where Xf= value (in log units) of the final VF monofilament; k= tabular value corresponding to pattern of positive and negative responses; δ = mean difference (in log units) between stimuli (0.224).

2.4. Euthanasia

Animals were euthanized 1 month after SNI (Figure 1A). Each animal was brought to the sacrificed room individually and decapitated with a guillotine in less than 5s. The brain was then careful removed and placed in a cooled brain slicer (Zivic Instruments). The mPFC, OFC, STR, NAc and AMY from each brain hemisphere were macrodissected from 1 mm thick coronal slices based on major anatomical references (Paxinos and Watson, 2007). The obtained tissue was weighted and stored at -80°C. Great care was taken to assure a quick as possible macrodissection and to keep the tissue cold and clean. All material was disinfected with 10% ethanol between animals.

2.5. High Performance Liquid Chromatography with electrochemical detector (HPLC-ED)

Monoamines quantification was done as previously described (Gemmel et al., 2016; Morgado et al., 2015). Perchloric acid ($HCLO_4$) was added to each sample (50µl/ng of tissue; never <100µl) and then the samples were homogenized using ultrasound sonification. After centrifugation (19,500g, 45 minutes (min), 4°C), the supernatants were collected to a separated tube and sent to the National and Kapodistrian University of Athens on dry ice for the HPLC-ED analysis. The tissue pellets were used for protein concentration determination.

HPLC-ED analysis was carried out using a GBC LC1150 pump (GBC Inc, Australia) and an Aquasil C18 column (150mm X 2.1 mm; 5µm particle size; Thermo Fisher Scientific, USA) coupled to an electrochemical detector set at +800 mV (BAS LC4C, Bioanalytical Systems, USA). Reverse-phase ion pair chromatography (mobile phase: 50 mM phosphate buffer at pH 3.0, containing 300 mg/L sodium octylsulfate, 20 mg/L Na_2EDTA, and acetonitrile added at a concentration of 6-9%) was used to assay NA, DA and its metabolites 3,4 dihydroxyphenylacetate (DOPAC) and homovanillic acid (HVA), as well as 5-HT and its metabolite 5-hydroxyindoleatic acid (5-HIAA). Quantification was done by comparing the areas under the curve of external reference standards using the Clarity software (Data-Apex, Czech Republic). Additionally, an index of serotonergic and dopaminergic activity was obtained after calculating the turnover rates of 5-HT (5-HIAA/5-HT ratio) and DA [HVA/DA , DOPAC/DA and (HVA + DOPAC)/DA ratios]; these

turnover rates reflect transmitter release and/or metabolic activity (Kyratsas et al., 2013; Mikail et al., 2012). Each sample was normalized for the total protein concentration.

Total protein concentration was calculated by the Bradford method. For that, 200µl of phosphate buffer (0.2 M) were added to each pellet and samples were homogenized with ultrasound sonification. A centrifugation (4,000g, 5min, 4°C) was then performed and the supernatant was used for protein quantification. Bradford reagent was added to each sample and, after 10 min at room temperature, absorbance at 595nm was read (Model 680, Bio-rad). Protein concentration was calculated based on a Bovine Serum Albumin (BSA) standard curve.

2.6. Statistical analysis

Statistical analysis was done in the JASP 0.9.2 software (JASP Team (2019), Netherlands) and graphs were obtained through GraphPad PRISM 8.0 software (GraphPad software, Inc., USA). 12 samples were lost during the extraction or the processing: 4 from Sham (1 L NAc, 1 R NAc, 2 R AMY), 5 from SNI-L (1 L OFC, 2 L STR, 1 R STR, 1 R AMY) and 3 from SNI-R (1 R OFC, 1 L STR, 1 R NAc). Other were considered outliers (>Mean+3*SD or <Mean-3*SD) and also excluded from the analysis. For the radar charts, z-scores were calculated for each area and each monoamine. Independent t-tests were used to study the differences between Sham and SNI and between the ipsilateral and the contralateral hemispheres. One sample t-tests were used to study if the Laterality index (LI) were lateralized and ANOVA's to compare the LI and the 50% threshold between the groups.

Equality of variances was test by Levene's test and Welch correction was used if equality of variances was rejected. Tukey was used as post-hoc test unless the variances were not equal, in which case Games Howell test was utilized. Cohens' d (*d*) and eta squared (η^2) were used as effect size measures in t-tests and ANOVA's, respectively. Data were considered significant if $p<0.05$. All results are represented as mean ± SEM.

3. Results

To study the impact of SNI on MCL monoamines, 1 month after lesion, rats were euthanized and the levels of monoamines in several MCL areas quantified (Figure 1A). During the follow up period the mean body weight increased similarly in the 3 groups (Time: $F_{(2, 40)}$ =302.694, p<0.001, η^2=0.930; Time*Group: $F_{(4, 40)}$ =1.342, p=0.271, η^2=0.008; Group: $F_{(2, 20)}$ =0.138, p=0.872, η^2=0.014) (Figure 1B). However, SNI induced a marked allodynia, i.e. a decrease in the threshold to VF monofilament stimulation, in both SNI groups ($F_{(2, 11.192)}$ =19.23, p<0.001, η^2=0.818; SNI-L vs Sham: t=-6.351, p=0.002; SNI-R vs Sham: t=-6.344, p=0.001) (Figure 1C). Importantly, no differences in allodynia were found between SNI-L and SNI-R animals (SNI-L vs SNI-R: t=0.084, p=0.996).

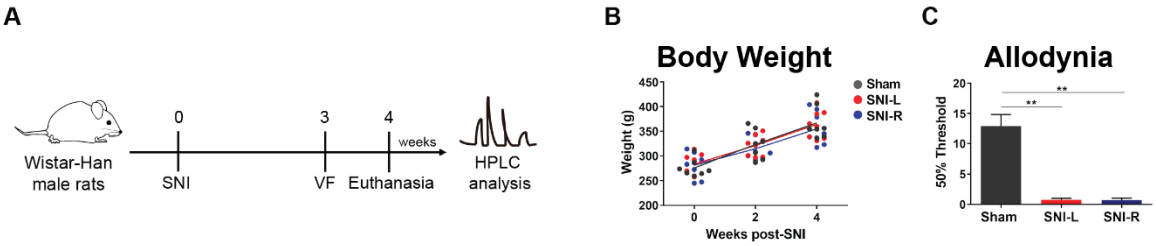

Figure 1. Experimental design, body weight and allodynia –One month after spared nerve injury (SNI), animals were sacrificed and tissues collected for HPLC **(A)**. During the experimental time course, weight was measured **(B)** and Von Frey (VF) monofilaments were used to assess the manifestation of mechanical allodynia, a common effect of neuropathic pain models in rodents **(C)**. Data is plotted as mean ± SEM.

HPLC-ED overall results revealed that while Sham, SNI-L and SNI-R present different patterns of monoamine distribution (Figure 2 A-C), essentially characterized by an increase in monoamine levels in SNI, these are below significance level (Table I). In the STR however, SNI present a strong tendency for a reduction in DA and 5-HT (Figures 2 D, F; Table I). These alterations are not related with monoamine metabolism as no effects were observed in DA, NA and 5-HT turnover readouts as evaluated by the DOPAC/DA, HVA/DA, (DOPAC +HVA)/DA and 5-HIAA/5-HT ratios (Table I). Similarly, even though the effect of SNI-L and SNI-R appears quite different (Figure 2 A-C), no differences between the groups were found in the analyzed areas.

In an attempt to study the distinct impact of SNI on ipsilateral and contralateral hemispheres to the lesion, levels of neurotransmitters in the left hemisphere of SNI-L and right hemisphere of SNI-R were compared with the levels of the opposite hemispheres. Results reveal no differences on the impact of SNI (Figure 2 G-I; Table II).

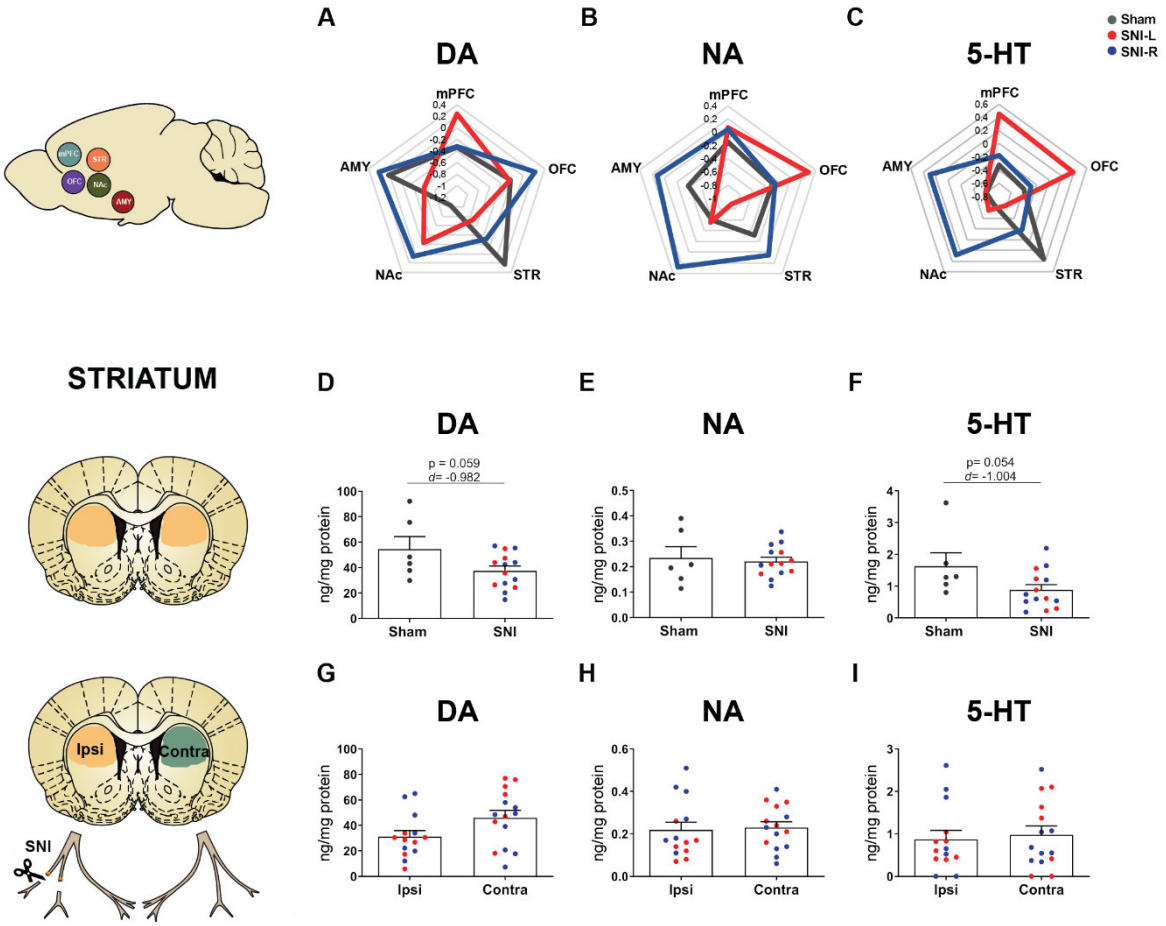

Figure 2. Mesocorticolimbic monoamine quantification in chronic pain - The monoamine content – dopamine (DA), noradrenaline (NA) and serotonin (5-HT) – of medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), striatum (STR), nucleus accumbens (NAc) and amygdala (AMY) in Sham and spared nerve injury in the left (SNI-L) and right (SNI-R) hind paw were analyzed **(A-C)**. Variations in the three monoamines were observed not only between controls and SNI but also between SNI-L and SNI-R (data presented after z-score conversion) **(A-C)**. Striatal monoamine content was analyzed in both hemispheres **(D-F)**; a marginal reduction in DA and particularly 5-HT was observed in SNI animals. SNI effects on ipsi- and contralateral STR were also analyzed **(G-I)**. Data is plotted as mean ± SEM.

Accounting for potential asymmetries and lateralized effects on monoamines levels – see for instances (Gemikonakli et al., 2019; Sullivan et al., 2014) – the laterality index (LI = ($[NTransm]_{right}$ - $[NTransm]_{left}$)/($[NTransm]_{right}$ + $[NTransm]_{left}$)) was compared between groups as well as group deviations from symmetry (i.e. LI=0) (Figure 3; Tables III and IV, respectively). The LI of NA in the NAc of SNI-L and SNI-R was different from Sham, with Sham presenting a lateralization towards the left and SNI towards

the right (Figure 3D; Table III). Furthermore, individually Sham-operated animals presented higher levels of DA in the right than in the left mPFC and this lateralization was absent in SNI-L and SNI-R animals (Figure 3A; Table IV). On the other hand, OFC and AMY DA levels were symmetrical on controls but lateralized on SNI animals; SNI-L presented a rightward lateralization while SNI-R have leftward bias on the AMY (Figure 3B, C; Table IV).

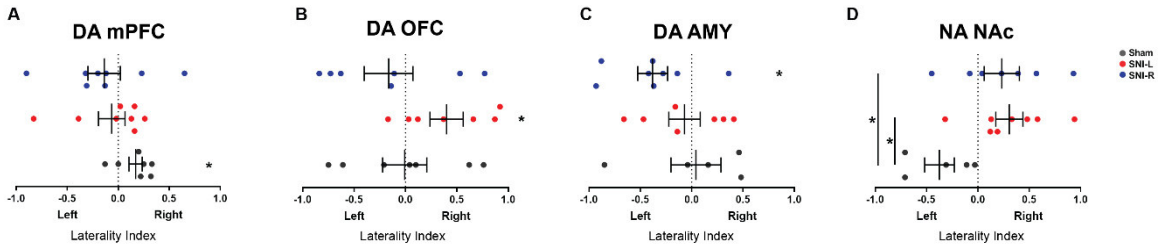

Figure 3. Laterality Index -The laterality index – LI = ($[NTransm]_{right}$ - $[NTransm]_{left}$)/($[NTransm]_{right}$ + $[NTransm]_{left}$) – was used to analyze neurotransmitter asymmetry. Dopamine (DA) in the medial prefrontal cortex (mPFC) is lateralized in Sham animals but this lateralization is lost after the spared nerve injury in the left (SNI-L) or right paw (SNI-R) **(A)**. On the other hand, DA in orbitofrontal cortex (OFC) and amygdala (AMY) becomes lateralized after SNI-L and SNI-R, respectively **(B, C)**. In the nucleus accumbens (NAc), the LI of noradrenaline (NA) from Sham is different from SNI-L and SNI-R **(D)**. Data is plotted as mean ± SEM. *p<0.05

4. Discussion

Altogether, no major alterations in monoamine levels were observed after SNI. In general, our findings are aligned with previous studies though some conflicting evidences were found. A reduction in DA and DOPAC in the OFC has been reported in a monoarthritic inflammatory chronic pain model 21 days after complete Freud's adjuvant administration into the ankle joint (Pais-Vieira et al., 2009); no differences were detected on other time points (day 5 and day 56 after administration) or areas (AMY and NAc) analyzed. In a different study using a similar model, no differences were found regarding DA availability in the PFC, NAc and STR but a diminished metabolism of this monoamine was detected in the NAc and STR 28 days after administration of Mono-iodoacetate into the ankle joint (Mlost et al., 2018); no differences were found regarding NA. On the other hand, a significant increase of DA was reported in the NAc 14 days after SNI (Sagheddu et al., 2015). Contrary to this study in which essentially extracellular DA levels were measured (microdialysis; (Sagheddu et al., 2015)), we measured all tissue levels including presynaptic and synaptic DA, which could explain some discrepancies. It is also important to notice that the cited above studies were performed at different time points after model induction therefore capturing different stages of the pathophysiology and some used inflammatory pain models. Finally, except for

occasional handling and VF test at the 3rd week, in our experimental conditions, animals were essentially undisturbed.

In our experiment, the STR was the region more susceptible to the effects of the lesion, presenting a marginal reduction of DA and 5-HT, though both with large effect sizes (Figure 2 D, F). A reduction of DA content in the ventral STR has also been shown in mice 2 weeks after the installment of a cuff in the sciatic nerve (Taylor et al., 2014). These observations are in line with a previous study in which DA receptor 2 agonism in the ipsilateral STR ameliorates pain scores in SNI (Ansah et al., 2007) and clinical data, which showed decreased Fluorodopa uptake (PET) in the STR of burning mouth syndrome patients (Jaaskelainen et al., 2001).

Our results also raise questions regarding monoamines lateralization. In the one hand, when ipsilateral and contralateral areas to the injury were compared regardless of their hemisphere, no differences were observed. On the other hand, particularly in the case of DA, the SNI lesion alters the balance observed in controls. For instance, the asymmetric DA content present in the mPFC of Sham controls is lost in both SNI groups. Also, SNI-L present a rightward bias on DA content that is absent in controls and SNI-R. Such might provide a neurobiological substrate for the behavioral differences observed between SNI-R and SNI-L in emotional and cognitive tasks (Cunha et al., 2020a; Leite-Almeida et al., 2012; Leite-Almeida et al., 2014) – see (Gagliese et al., 1995; Schiff and Gagliese, 1994) for side-specific impact of lateralized pain in humans. Indeed, previous studies demonstrated that the disruption of interhemispheric monoamine balance in MCL areas can impact behavior, particularly anxiety (Sullivan et al., 2014) and decision-making (Cunha et al., 2020b).

In conclusion, despite cumulative evidence from clinical studies implicating MCL circuitry in chronic pain development/protection (Baliki et al., 2012), preclinical evidence indicates no major alterations in monoamine content in these areas 28 days after SNI

Table I. Neuropathic pain effect on monoamine content

	N (Sham/SNI)	Mean ± SD (Sham/SNI) (ng/mg protein)	Levene's Equality of variances test	T	df	p	Cohens' *d*
DA							
PFC	7/15	0.240 ±0.04/0.283 ±0.10	F=6.677, p=0.018*	1.394	19.913	0.179	0.550
OFC	7/14	0.798±0.98/1.215±1.32	F=0.926, p=0.348	0.737	19.000	0.470	0.341
STR	6/14	54.47±24.2/37.48±13.8	F=4.043, p= 0.060	-2.012	18.000	0.059	-0.982
NAc	5/14	13.53±8.2/20.50±8.5	F=0.269, p=0.611	1.584	17.000	0.132	0.825
AMY	5/15	1.120±1.19/0.654 ±0.62	F=1.492, p= 0.238	-1.156	18.000	0.263	-0.597
NA							
PFC	7/16	0.808 ±0.10/0.875±0.38	F=5.251, p= 0.032*	0.633	19.138	0.534	0.233
OFC	7/14	0.505±0.16/0.589±0.13	F=0.618, p=0.442	1.260	18.000	0.224	0.591
STR	6/14	0.234±0.11/0.221±0.06	F=4.461, p=0.049*	-0.282	6.365	0.787	-0.151
NAc	5/15	1.63 ±0.98/1.95±1.3	F=0.156, p=0.697	0.508	17.000	0.618	0.265
AMY	5/15	0.951±0.40/0.732 ±0.23	F=3.897, p=0.064	-1.534	18.000	0.142	-0.792
5-HT							
PFC	7/16	1.42±0.34/1.80±0.96	F=2.356, p=0.140	1.002	21.000	0.328	0.454
OFC	7/14	1.35±0.6/2.72±2.7	F=1.559, p=0.227	1.319	19.000	0.203	0.611
STR	6/14	1.63 ±1.02/0.886±0.60	F=0.892, p= 0.357	-2.058	18.000	0.054	-1.004
NAc	5/14	4.10 ±3.04/5.10±2.9	F=0.080, p=0.781	0.661	17.000	0.517	0.345
AMY	5/15	3.00±1.1/2.94±1.5	F=0.276, p= 0.606	-0.082	18.000	0.936	-0.042
DOPAC/DA							
PFC	7/16	0.706 ±0.22/0.586 ±0.17	F=0.801 p=0.381	-1.394	21.000	0.178	-0.632
OFC	7/14	1.018±0.62/0.836±0.41	F=4.234, p=0.054	-0.812	19.000	0.426	-0.376
STR	6/14	0.431±0.39/0.392±0.23	F=0.846, p=0.370	-0.283	18.000	0.780	-0.138
NAc	5/15	0.670±0.23/0.645±0.27	F=0.513, p=0.483	-0.185	18.000	0.855	-0.096
AMY	5/715	0.556±0.21/0.651±0.49	F=1.133, p=0.301	0.418	18.000	0.681	0.216
HVA/DA							
PFC	7/16	0.841±0.24/0.631±0.30	F=0.062, p=0.805	-1.640	21.000	0.116	-0.743
OFC	7/14	1.449 ±1.25/0.995±0.68	F=0.062, p=0.805	-1.090	19.000	0.289	-0.505
STR	6/14	0.097±0.27/0.088±0.044	F=1.093, p=0.310	-0.314	18.000	0.757	-0.153
NAc	5/15	0.154±0.029/0.141 ±0.048	F=1.934, p=0.181	-0.565	18.000	0.579	-0.292
AMY	5/15	0.273±0.15/ 0.468±0.45	F=1.565, p=0.227	0.938	18.000	0.361	0.484
DOPAC+HVA/DA							
PFC	7/16	1.548±0.45/1.218±0.44	F=0.034, p=0.855	-1.656	21.000	0.113	-0.750
OFC	7/14	2.467±1.86/1.831±0.91	F=10.111, p=0.005**	-0.853	7.462	0.420	-0.434
STR	6/14	0.528±0.46/0.480±0.27	F=0.864, p=0.365	-0.290	18.000	0.775	-0.142
NAc	5/15	0.824±0.26/0.786±0.32	F=0.690, p=0.417	-0.243	18.000	0.811	-0.125
AMY	5/15	0.828±0.32/0.428±0.23	F=1.632, p=0.218	0.724	18.000	0.478	0.374
5-HIAA/5-HT							
PFC	7/16	0.665±0.28/0.556 ±0.27	F=0.013, p=0.909	-0.875	21.000	0.391	-0.397
OFC	7/14	0.648±0.23/0.574±0.28	F=0.150, p=0.702	-0.605	19.000	0.300	-0.493
STR	6/14	1.108±0.33/1.238±0.63	F=0.821, p=0.380	0.460	14.000	0.625	0.238
NAc	5/15	0.514±0.26/0.478±0.14	F=5.270, p=0.034*	-0.291	4.867	0.783	-0.169
AMY	5/15	0.425±0.17/0.428±0.23	F=0.191, p=0.667	0.028	18.000	0.978	0.015

Table I. Dopamine (DA), noradrenaline (NA) and serotonin (5-HT) as well as some of its metabolites homovanillic acid (HVA), 3, 4-Dihydroxyphenylacetic acid (DOPAC) and hydroxyindoleacetic acid (5-HIAA) were compared by independent t-tests across control (Sham) and neuropathic (SNI) rats.

AMY- amygdala; df- degrees of freedom; NAc- nucleus accumbens; OFC- orbitofrontal cortex; PFC- prefrontal cortex; SD- standard deviation; SNI- spared nerve injury; STR- striatum.

Table II. Ipsi- and contralateral monoamine content in chronic pain

		N (Ipsi/Contra)	Mean ± SD (Ipsi/Contra) (ng/mg protein)	T	df	p	Cohens' *d*
DA							
	PFC	16/15	0.300±0.22/0.259 ±0.12	0.658	14	0.521	0.170
	OFC	14/16	1.08±1.8 /1.19±1.2	-0.588	13	0.567	-0.157
	STR	14/15	31.2±17.2/46.0±22.1	-1.750	13	0.104	-0.468
	NAc	15/15	20.1±11.9/19.7 ±12.3	0.290	13	0.776	0.078
	AMY	16/15	0.491±0.53/0.790 ±0.82	-1.659	14	0.119	-0.428
NA							
	PFC	15/16	0.818±0.432/0.789±0.42	0.107	14	0.916	0.028
	OFC	14/15	0.595±0.19/0.578±0.168	-0.365	12	0.721	-0.101
	STR	14/15	0.219±0.14/0.230±0.10	-0.076	13	0.940	-0.020
	NAc	15/15	2.207±2.23/1.60 ±0.76	1.125	13	0.281	0.301
	AMY	16/15	0.698±0.27/0.780±0.278	-1.195	14	0.252	-0.309
5-HT							
	PFC	15/16	1.553±0.75/1.584±0.92	-0.465	14	0.649	-0.120
	OFC	14/15	1.773±1.05/2.096±1.22	-0.993	12	0.340	-0.275
	STR	14/15	0.874±0.78/0.979±0.80	-0.100	13	0.922	-0.027
	NAc	14/15	4.353±2.29/4.163±1.94	0.283	12	0.782	0.079
	AMY	16/15	2.688±1.55Contra3.195 ±1.89	-1.009	14	0.330	-0.261

Table II. Ipsi- and contralateral hemispheres to the SNI injury were compared using paired sample t-tests regarding their Dopamine (DA), noradrenaline (NA) and serotonin (5-HT) content.

AMY- amygdala; df- degrees of freedom; NAc- nucleus accumbens; OFC- orbitofrontal cortex; PFC- prefrontal cortex; SD- standard deviation; SNI- spared nerve injury; STR- striatum.

Table III. Monoamine laterality index

		N (Sham/SNI-L/SNI-R)	Mean ± SD (Sham/SNI-L/SNI-R) (ng/mg protein)	Levene's Equality of variances test	F	df	p	η^2
DA								
	PFC	7/8/8	0.170±0.17/-0.064 ±0.37/-0.138	F= 1.005, p=0.384	1.498	2, 20	0.248	0.130
	OFC	7/7/7	-0.0071±0.57/0.400±0.43/-	F=0.376, p=0.692	1.978	2, 18	0.167	0.180
	STR	6/6/8	0.238±0.59/0.345±0.36/-0.010±0.36	F=0.868, p=0.437	1.229	2, 17	0.317	0.126
	NAc	5/8/7	0.250±0.406/0.209±0.40/0.171±0.37	F=0.157, p=0.856	0.058	2, 17	0.944	0.007
	AMY	5/7/8	0.042±0.54/-0.07±0.40/-0.380±0.41	F=0.297, p=0.747	1.651	2, 17	0.221	0.163
NA								
	PFC	7/8/8	0.117±0.22/-0.066±0.36/0.025±0.29	F=0.166, p=0.848	0.701	2, 20	0.508	0.065
	OFC	7/7/7	-0.114±0.30/0.048±0.36/-0.088±0.18	F=0.810, p=0.460	0.649	2, 18	0.534	0.067
	STR	6/6/8	0.203 ±0.51/0.255 ±0.28/0.100	F=1.114, p=0.351	0.235	2, 17	0.793	0.027
	NAc	5/8/7	-0.374 ±0.32/0.306 ±0.37/0.233	F=0.209, p=0.814	5.138	2, 17	**0.018***	0.377
					SNI-L vs SNI-R (t=0.361)		0.931	
					SNI-L vs Sham (t=3.038)		0.019*	
					SNI-R vs Sham (t=2.369)		0.043*	
	AMY	5/7/8	-0.066±0.35/0.101±0.13/-0.044±0.28	F=2.570, p=0.106	0.809	2, 17	0.462	0.087
5-HT								
	PFC	7/8/8	0.0657±0.26/-0.186±0.38/-	F=0.621, p=0.547	1.236	2, 20	0.312	0.110
	OFC	7/7/7	-0.096±0.27/0.146±0.48/-0.180±0.35	F=2.446, p=0.115	1.399	2, 18	0.273	0.135
	STR	6/4/6	0.358±0.54/0.220±0.39/0.127±0.44	F=0.208, p=0.815	0.365	2, 13	0.701	0.053
	NAc	5/8/7	0.038±0.18/0.201±0.35/0.254±0.41	F=1.260, p=0.309	0.602	2, 17	0.559	0.069
	AMY	5/7/8	-0.030±0.37/0.0514±0.41/-	F=0.118, p=0.889	0.109	2, 17	0.897	0.013

Table III. Dopamine (DA), noradrenaline (NA) and serotonin (5-HT) laterality indexes (LI). LIs were obtained according to the formula LI = $[NTransm]_{right}$ - $[NTransm]_{left}$/$[NTransm]_{right}$ + $[NTransm]_{left}$ and compared with one-way ANOVAs; when necessary post-hoc Tukey test was performed.

AMY- amygdala; df- degrees of freedom; NAc- nucleus accumbens; OFC- orbitofrontal cortex; PFC- prefrontal cortex; SD- standard deviation; SNI- spared nerve injury; STR- striatum.

Table IV. Monoamines' laterality in control and neuropathic animals

		Sham				SNI-L				SNI-R			
		t	df	p	Cohens' *d*	t	df	p	Cohens' *d*	t	df	p	Cohens' *d*
DA													
	PFC	2.620	6	**0.040***	0.990	-0.491	7	0.639	-0.174	-0.868	7	0.414	-0.307
	OFC	-0.033	6	0.975	-0.013	2.472	6	**0.048****	0.934	-0.694	6	0.514	-0.262
	STR	0.995	5	0.365	0.406	2.348	5	0.066	0.958	-0.079	7	0.939	-0.028
	NAc	1.378	4	0.240	0.616	1.459	7	0.188	0.516	1.217	6	0.269	0.460
	AMY	0.173	4	0.871	0.077	-0.458	6	0.663	-0.173	-2.630	7	**0.034****	-0.930
NA													
	PFC	1.414	6	0.207	0.534	-0.521	7	0.619	-0.184	0.242	7	0.816	0.086
	OFC	-1.022	6	0.346	-0.386	0.377	7	0.717	0.133	-1.218	5	0.278	-0.497
	STR	0.979	5	0.373	0.400	2.214	5	0.078	0.904	0.612	7	0.560	0.216
	NAc	-2.587	4	0.061	-1.157	2.316	7	0.054	0.819	1.363	6	0.222	0.515
	AMY	-0.420	4	0.696	-0.188	2.009	6	0.091	0.759	-0.444	7	0.671	-0.157
5-HT													
	PFC	0.679	6	0.523	0.256	-1.378	7	0.211	-0.487	-1.224	7	0.261	-0.433
	OFC	-0.933	6	0.387	-0.353	0.860	7	0.418	0.304	-1.273	5	0.259	-0.520
	STR	1.622	5	0.166	0.662	1.132	3	0.340	0.566	0.701	5	0.515	0.286
	NAc	0.463	4	0.667	0.207	1.604	7	0.153	0.567	1.646	6	0.151	0.622
	AMY	-0.181	4	0.865	-0.081	0.335	6	0.749	0.126	-0.268	7	0.796	-0.095

Table IV. Dopamine (DA), noradrenaline (NA) and serotonin (5-HT) laterality i.e. difference to LI=0 by t-tests.

AMY- amygdala; df- degrees of freedom; NAc- nucleus accumbens; OFC- orbitofrontal cortex; PFC- prefrontal cortex; SD- standard deviation; SNI- spared nerve injury; STR- striatum.

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Authors Contributions

AMC and HLA designed the experiment and wrote the first version of the manuscript. AMC, MRG and HLA performed the experiences with the rats and AMC, IS, NK and CD performed the molecular analysis. AMC did the statistical analysis. All authors discussed and interpreted the data and revised and approved the final version of the manuscript.

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CHAPTER 3

General discussion

General discussion

Chronic pain (CP) impact on decision-making remains poorly studied in clinical and preclinical research. The work developed in this thesis focused on CP effects in decision-making, particularly on goal-directed/habit-based and impulsive decisions, and on their relation with the mesocorticolimbic dopaminergic system.

Although relatively scarce, several studies demonstrated that CP impairs decision-making, particularly emotional decision-making as evaluated by the Iowa gambling task (IGT). CP patients performed poorly on this task, making more disadvantageous choices than healthy controls (Apkarian et al., 2004; Berger et al., 2014; Verdejo-Garcia et al., 2009; Walteros et al., 2011). These impairments reflected a hypersensitivity to monetary gains in CP patients, which was related with decreased connectivity between the Nucleus accumbens (NAc) and frontal areas (Apkarian et al., 2004; Berger et al., 2014; Verdejo-Garcia et al., 2009). In animal models of monoarthritis, similar impairments were observed, with lesioned rats preferring the high risk but less profitable lever (Ji et al., 2010; Pais-Vieira et al., 2012, 2009). Looking specifically to the decision-making dimensions studied on this thesis, clinical studies showed that impulsivity was not affected by CP (Glass et al., 2011; Margari et al., 2014; Pidal-Miranda et al., 2019) and, to the best of our knowledge, no studies have focused on habitual behavior. In animal models, however, we showed that both goal-directed/habit-based and impulsive decisions are impaired in CP conditions. According to our data, CP lead to increased intolerance to delay and higher reliance on habit-based decisions. These behaviors have been associated with addiction and substance abuse (Dawe and Loxton, 2004; Meyer et al., 2016) and, in that way, are highly relevant in the context of CP, as it is estimated that around 25% of CP patients misuse opioids and around 10% develop addiction to them (Vowles et al., 2015). Other rodent studies showed that motor impulsivity, evaluated by the 5-CSRTT and the stop signal task, and reward devaluation were not affected by CP (Higgins et al., 2015; Kniffin et al., 2015; Mor et al., 2017). These results are aligned with our observation as, in fact, we do not observe differences between the groups in the number of premature responses during the VDS training, which is akin to the 5- choice serial reaction time task (5-CSRTT). Similarly to Mor and colleagues (2017), we did not observe devaluation deficits on rats with a neuropathic lesion on the right hind paw (Mor et al., 2017). Instead, our results showed that only SNI-L rats presented impairments in reward devaluation and/or contingency degradation after habit induction. Furthermore, alterations in delay intolerance after CP were only present in high-impulsive animals.

These results hint at the importance of trait and lesion characteristic to the emergence of behavioral manifestations. The importance of individual characteristics is not new nor exclusive to CP, particularly in

outbred populations as the one used in our studies. Other authors reported that only a percentage of animals develop comorbidities as anhedonia or social impairments after CP (Monassi et al., 2003; Xie et al., 2017). Similarly, when exposed to stress some animals do not develop depressive-like behaviors, presenting increased resilience (Magalhães et al., 2019). Also, some rats remain non-allodynic after a neuropathic lesion and the percentage of affected animals varies according to strain, clearly indicating the presence of a genetic component (De Felice et al., 2011; Guimarães et al., 2018). On the same line, not all the individuals who suffer a lesion or are subjected to surgery develop CP and not all CP patients present comorbidities.

Psychological factors directly related with pain as pain catastrophizing, pain expectancy/fear of pain and coping strategies have an impact on the transition from acute do CP, pain intensity and emergence of emotional comorbidities (Hruschak and Cochran, 2018; Meints and Edwards, 2018). Furthermore, emotional factors not directly associated with pain as depression, anxiety and emotional distress present a risk to CP development in patients (Hruschak and Cochran, 2018; Meints and Edwards, 2018) and increased allodynia in rodent models of CP (Burston et al., 2019; Roeska et al., 2009). Even though we showed that CP impact on impulsivity is dependent of trait impulsivity, it is not clear if emotional factors can explain some of the variability observed in our results, as these were not tested. However, in previous works from the group, it was shown that spared nerve injury in the left (SNI-L) but not right hind paw (SNI-R) present an anxious phenotype (Guimarães et al., 2018; Leite-Almeida et al., 2012). In that way, it is possible that the results observed, particularly regarding habitual decision-making, were influenced by, or even dependent on, anxiety-like behaviors. On the other hand, it is important to note that left and right lesioned and high and low impulsive animals do not present different thresholds of allodynia and that Sham and SNI (left and right) present similar levels of corticosterone, suggesting that population-level differences regarding pain and stress resilience are absent or at least cannot be discriminated.

Besides the emotional characteristics mentioned in the previous paragraph, other baseline differences have been pointed out as being important for the emergence of CP. For instance, some polymorphisms in genes associated with the dopaminergic system such as COMT have been suggested as risk factors to the prevalence of CP after surgery (van Reij et al., 2019; Xu et al., 2019). Moreover, Apkarian lab showed that the functional connectivity between the NAc and the prefrontal (PFC) can predict pain chronification in patients with low back pain (Baliki et al., 2012; Vachon-Presseau et al., 2016). These, place the dopaminergic system, and the mesocorticolimbic system in particular, as a fundamental piece on the susceptibility to CP. Interestingly, we observed, in animals injected unilaterally with 6- hydroxydopamine in the left (6-OHDA L) or right NAc (6-OHDA R), deficits in goal-directed/habit based and impulsive

decision-making very similar to those observed in SNI rats. These impairments were lateralized, with 6-OHDA R lesions impairing the shift from goal-directed to habit-based decisions, but not impulsive behavior, and 6-OHDA L lesions increasing delay intolerance, but not affecting contingency degradation. Accordingly, it had been previously shown that high impulsive rats presented decreased availability of Dopamine (DA) receptors as well as a reduction in gray matter density, specifically in the left NAc, reinforcing the differential contributions of left and right NAc to this behavior (Caprioli et al., 2015, 2014). On the other hand, preliminary results with D1 and D2 antagonists (Appendix C) revealed a non-lateralized effect of NAc DA depletion on impulsivity. This pharmacological inactivation of D1 and D2 neurons is obviously very different from DA depletion by 6-OHDA injections, namely in its transient effect. Similar to NAc, lateralized lesions of the PFC with 6-OHDA also induce different behavior impairments, in this case on anxiety-like behaviors. Right but not left 6-OHDA lesions of the PFC lead to increased anxiety-like behaviors on males (Sullivan et al., 2014). As mentioned earlier, these same impairments are present on SNI-L but not SNI-R rats. In this way, we raise the hypothesis that the deficits (emotional and cognitive) observed on CP conditions result from lateralized alterations in the mesocorticolimbic dopaminergic pathway after neuropathic lesion. However, HPLC analysis revealed no alterations in basal DA of Sham and SNI-lesioned animals, nor different levels of this neurotransmitter in ipsi- and contralateral hemispheres, on mesocorticolimbic areas. It is however important to note that in this study we are measuring basal off-task level of monoamines, which dismisses the impact of the behavioral task, food restriction and reward. In order to achieve more conclusive evidence, pre- and post-SNI and on-task voltammetry should be performed to analyze the impact of CP on NAc's DA release at basal levels and during goal-directed and impulsive decision-making.

Conclusions and future perspectives

Executive dysfunction remains poorly studied in CP research, particularly preclinical research, even though it comprises important dimensions of patients' lives as working memory and decision-making. Here, we showed that SNI leads to an increased reliance on habitual behaviors and heightened impulsivity. The observed impairments constitute two important dimensions of addiction and, as such, it would be relevant to understand if this increase in habitual and impulsive behaviors also reflects a higher risk of addiction/substance abuse. Furthermore, we observed that these impairments manifested in specific conditions, reinforcing the importance of trait behavior characterization and population diversity in CP studies. It is known that basal conditions impact on the transition from acute to chronic pain as well as the appearance of comorbidities. Higher focus of the preclinical research on understanding the

molecular, structural and behavioral characteristic relevant to CP emergence, will be essential to identify individuals at risk to develop CP, which would represent a step forward in the prevention and early intervention. The NAc, and more generally the reward system, appears to play a central role on this context and future studies targeting NAc's function prior to pain onset will be critical to our understanding of susceptibility/resistance factors to CP development and maintenance.

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APPENDIX A

Pawedness Trait Test (PaTRaT)-A New Paradigm to Evaluate Paw Preference and Dexterity in Rats

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METHODS
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Pawedness Trait Test (PaTRaT)—A New Paradigm to Evaluate Paw Preference and Dexterity in Rats

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In rodents, dexterity is commonly analyzed in preference paradigms in which animals are given the chance to use either the left or the right front paws to manipulate food. However, paw preference and dexterity at population and individual levels are controversial as results are incongruent across paradigms. We have therefore developed a semi-quantitative method—the **pa**wdeness **tra**it **t**est (PaTRaT)—to evaluate paw preference degree in rats. The PaTRaT consists in a classification system, ranging from +4 to −4 where increasingly positive and negative values reflect the bias for left or right paw use, respectively. Sprague-Dawley male rats were confined into a metal rectangular mesh cylinder, from which they can see, smell and reach sugared rewards with their paws. Due to its size, the reward could only cross the mesh if aligned with its diagonal, imposing additional coordination. Animals were allowed to retrieve 10 rewards per session in a total of four sessions while their behavior was recorded. PaTRaT was repeated 4 and 8 weeks after the first evaluation. To exclude potential bias, rats were also tested for paw fine movement and general locomotion in other behavioral paradigms as well as impulsivity (variable delay-to-signal, VDS), memory and cognitive flexibility (water maze). At the population level 54% of the animals presented a rightward bias. Individually, all animals presented marked side-preferences, >2 and <−2 for left- and right-sided bias, respectively, and this preference was stable across the three evaluations. Inter-rater consistency was very high between two experienced raters and substantial when two additional inexperienced raters were included. Left- and right-biased animals presented no differences in the ability to perform fine movements with any of the forelimbs (staircase) and general locomotor performance. Additionally, these groups performed similarly in executive function and memory tasks. In conclusion, PaTRaT is able to reliably classify rats' pawedness direction and degree.

Keywords: motor preference, laterality, handedness, cognition, impulsivity, memory, behavior

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INTRODUCTION

Pawedness reflects the preferential use and/or an increased capacity to perform tasks more efficiently with a specific paw. It corresponds, in general terms, to animals' handedness. Pawedness/handedness is thought to be associated with brain asymmetries, present both at morphological, cellular and molecular levels (see for reviews

Galaburda et al., 1978; Toga and Thompson, 2003; Sun and Walsh, 2006; Rogers, 2009, 2014; Hugdahl, 2011). Regarding morphology most studies have so far excluded any association (Good et al., 2001; Narr et al., 2007; Guadalupe et al., 2014, 2016; Ocklenburg et al., 2016); however at cellular and molecular levels, contralateral parietal spine density has been linked to skilled reaching (Ambeskovic et al., 2017) and dopaminergic system lateralization has been shown to be associated with hand/paw preference in humans (de la Fuente-Fernández et al., 2000) as well as in rodents (Uguru-Okorie and Arbuthnott, 1981; Schwarting et al., 1987; Barnéoud et al., 1990; Cabib et al., 1995; Nielsen et al., 1997; Budilin et al., 2008). Additionally, peripheral human (Lengen et al., 2009) and central rodent (Neveu, 1990; Shen et al., 2005) data have shown differences in the immunological system, while additional monoamines (norepinephrine; Barnéoud et al., 1990) and enzymes (angiotensinases; Wu et al., 2010) were also associated with rodent pawedness. It has therefore been hypothesized that differences in this trait might be associated with other behavioral outcomes particularly cognition. Indeed, a small advantage of right-handed people in spatial ability has been reported (Somers et al., 2015) and pawedness/memory associations have been found in monkeys (Hopkins and Washburn, 1994) and mice (Wu et al., 2010). Furthermore, Prichard et al. (2013) reported that cognitive data is associated with the degree of handedness (and not its direction) as inconsistent handedness seems to be related with better episodic memory and improved belief updating/cognitive flexibility.

While handedness assessment in humans is simple, determination of associated behavioral and molecular differences poses several challenges: (i) the distribution of left- and right-handers in the population is uneven (Teng et al., 1976; Guadalupe et al., 2016) imposing the creation of specific left-enriched cohorts; (ii) social context may alter behavior (Teng et al., 1976), therefore increasing the percentage of strong right-handers and weak left-handers; and (iii) assessment of central molecular correlates is limited. The utilization of animal models became therefore very useful in this regard.

The Collins' (Collins, 1968) and the lateral paw preference tests (LPP; Waters and Denenberg, 1991) are amongst the most used tests to assess pawedness in rodents. The number of times a paw is used to retrieve food from an elevated tube and the amount of food retrieved from two lowered hoppers placed side-by-side are employed, respectively, as behavioral readouts. Despite the construct similarities, different results have been obtained between these two tests (Waters and Denenberg, 1991, 1994; Rogers and Bulman-Fleming, 1998) not only at the population level but, more importantly, at the individual level. Furthermore, both tests rely in the exclusive right/left paw use disregarding paw movement precision and possible intermediary strategies implicating the simultaneous use of both paws. Additionally, as measured by these methods, preference in rodents appears to a certain extant to be training/learning dependent (Collins, 1988; Stashkevich and Kulikov, 2001; Tang and Verstynen, 2002; Ribeiro et al., 2011).

To surpass these limitations, we have designed and validated an alternative test requiring minimal equipment for a fast determination of pawedness degree—the **pa**wdeness **tra**it **t**est (PaTRaT). In this test, a circular grid separates the animal from a receptacle containing sugared items. Contrary to previous tests, the PaTRaT allows the simultaneous use of the two paws for reward handling reducing potential selection biases and making it more ethologically relevant. Also, the large size of the reward compared to the grid slits, imposes higher movement complexity for successful retrieval. Finally, the PaTRaT uses a classification system for dexterity degree that goes beyond the simple quantification of left-/right-paw retrievals. Additionally, we assessed potential associations between pawedness and behavioral outcomes, namely with impulsivity, memory and fine motor skills.

MATERIALS AND METHODS

Animals

Thirty male Sprague-Dawley rats (Charles-River Laboratories) with 6 months of age were kept under standard laboratory conditions: 12 h light/dark cycle (lights on at 8 a.m.), relative humidity of 55%, 22°C and *ad libitum* access to water. Food (4RF21, Mucedola SRL) was restricted to 1 h per day (last hour of the cycle light phase) during experimental protocols otherwise access was *ad libitum*. Body weight was controlled on a weekly basis to prevent weight losses superior to 15%. Animals that failed to learn the PaTRaT in the training sessions (see below) were excluded from further analysis. Procedures involving animals were approved by local authorities and followed the EU Directive 2010/63/EU.

Pawedness Trait Test (PaTRaT)

Apparatus

The PaTRaT apparatus consisted of a custom-made plexiglass box open at the top. In the center, a metal wire mesh cylinder was used to confine the animal. Externally, accompanying cylinder's curvature, a plexiglass transparent piece was fixed to the bottom forming a receptacle for the rewards (**Figure 1A**).

Experimental Protocol

Training

Two daily sessions were performed, separated by a minimum of 4 h. Animals were habituated to the apparatus (1 session, 10 min) and to the reward (Cheerios®, Nestlé; 1 session, 10 min). On the next sessions, animals were motivated to reach for the reward with their paws by placing single rewards close to the grid (1–2 sessions, 15 min). The diameter of the reward was larger than the horizontal width of the metal mesh, increasing the demanding for successful retrievals.

PaTRaT

Four experimental sessions were performed, in which the animal had to retrieve 10 rewards in a maximum time of 10 min while the session was being recorded. Sessions were performed during

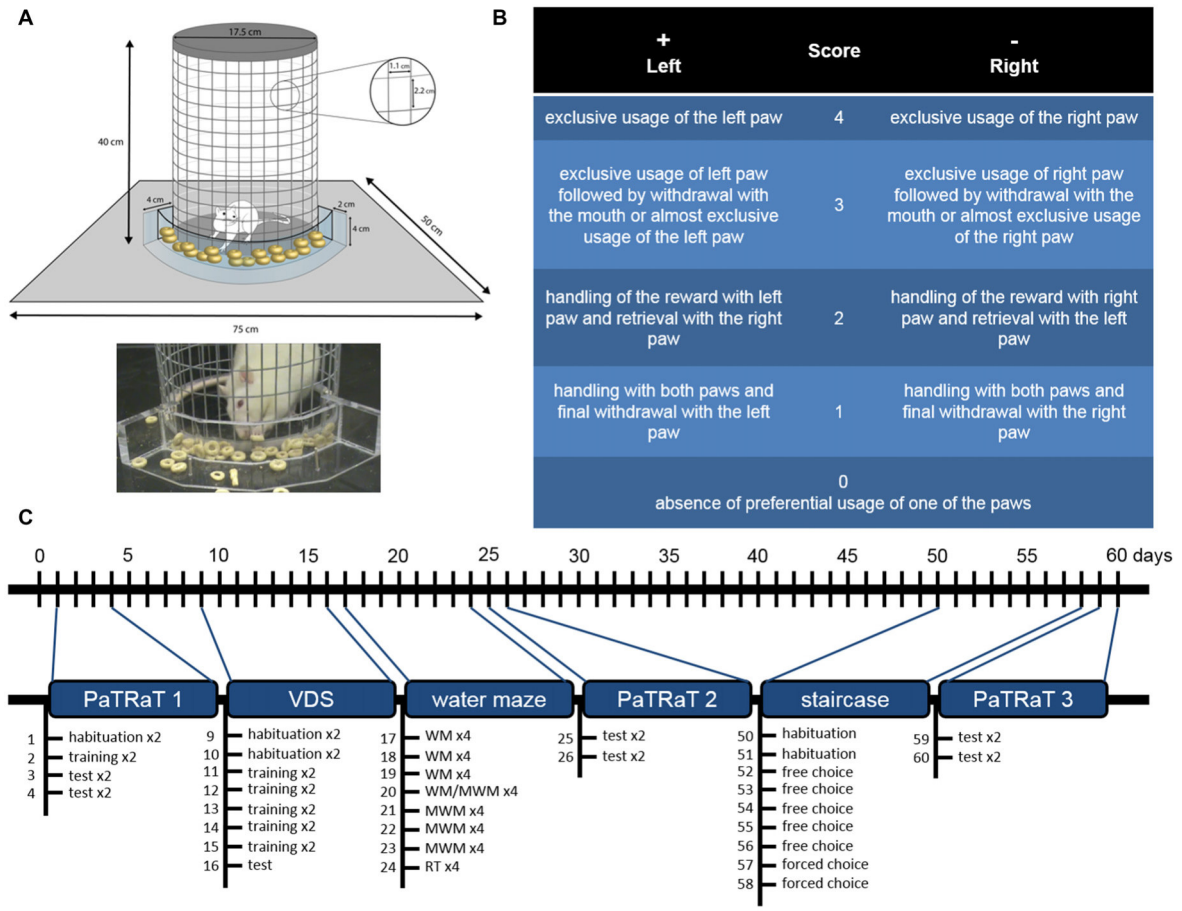

FIGURE 1 | Methods. **(A)** Scheme (top) and picture (bottom) depicting the pawedness apparatus. The animal is enclosed in a gridded cylinder. An exterior plexiglass structure contains the rewards. **(B)** Schematic table of classification for each retrieved reward. Positive and negative values correspond respectively to preferential usage of left and right paw and higher values are associated to more exclusive usage of said paw. **(C)** Timeline of behavioral analysis. Pawedness was assessed three times approximately 4 weeks apart. VDS and water mazes were performed between the two first evaluations and staircase test was performed before the third pawedness evaluation. Individual sessions are specified. VDS, Variable Delay-to-Signal; WM, working memory; MWM, Morris Water Maze; RT, reversal test.

the light period (8:00–12:00 and 14:00–18:00 for morning and afternoon sessions, respectively). Between animals the apparatus was cleaned with 10% ethanol.

To evaluate phenotype stability, two additional evaluations were performed 4 and 8 weeks after the first evaluation. Sessions were recorded and later evaluated independently by two observers. Additionally, the first evaluation was also rated by two inexperienced observers to assess inter-rater reliability.

Behavioral rating

Paw dexterity was determined by averaging the 40 trials (i.e., reward retrievals) of each evaluation. Each successful reward withdrawal was classified in a scale of +4 to −4. Positive and negative values reflected preferential use of the left or right paw respectively and increasing values were associated with increasing preference. Classification was only attributed when a reward was retrieved; unsuccessful attempts were not rated. Rewards withdrawn without usage of any of the paws (i.e., with the mouth) were also not classified.

Score 4 corresponded to exclusive usage of the left paw; score 3 to exclusive usage of left paw followed by withdrawal with the mouth or to almost exclusive usage of the left paw (e.g., right paw used as support); score 2 was attributed when the animal handled the reward with its left paw but retrieved it with the right paw; and score 1 corresponded to handling with both paws and final withdrawal with the left paw. Score 0 was associated with absence of preferential usage of one of the paws, namely equal usage of both paws for reaching the reward and final withdrawal with the mouth. Symmetrical values corresponded to similar classification for the opposite paw (**Figure 1B**).

Additional Behavioral Tests

In order to assess potential lateral preference-behavior associations, other behaviors were assessed (**Figure 1C**).

Variable Delay-to-Signal Test (VDS)

The variable delay-to-signal (VDS) task was used to assess impulsive behavior. It was previously validated by drug-induced

changes in impulsivity and by comparison with reference paradigms (Leite-Almeida et al., 2013). In short, the test was performed in a 5-hole operant chamber in which only the middle nosepoke aperture was available. Animals performed two daily sessions separated by a minimum of 5 h for a total of 8 days. There were four habituation sessions: the first two had the duration of 15 min, lights were off, all nosepoke apertures were blocked and sugared pellets (45 mg, Bioserv Inc., Flemington, NJ, USA) were available at the food magazine. The latter two had the duration of 30 min, all lights were on and pellets were available at both the food magazine and the nosepoke aperture.

Training consists of 10 sessions of 100 trials (or 30 min) in which there was a 3 s' interval between the beginning of the trial and the lightning of the nosepoke aperture (delay period). If a nosepoke was performed in this period, it was considered a premature response and it was punished with a 5 s' timeout in complete darkness. If the nosepoke was performed in the 60 s' period in which the light in the aperture was on, it was considered as a correct response and a reward was retrieved in the food magazine, initiating a new trial. If no nosepoke was performed, it was considered an omission and the animal was punished with a timeout.

Testing consisted of a single session at the end of the training. It was constituted by a total of 120 trials (or 90 min), in which premature responses were not punished and the intervals were variable. It started with 25 3 s' trials (3si), followed by 70 6 s (6 s) and 12 seconds' (12 s) (randomized) trials and again by 25 3 s' trials (3sf). During the testing session multiple premature responses were allowed during the delay periods and the rate of premature responses per time of available delay was calculated (Leite-Almeida et al., 2013).

Water Maze Test

In order to test memory, animals were subjected to a modification of the Morris Water Maze (MWM) test (Morris, 1984), for which the procedures have been previously described (Cerqueira et al., 2007). Briefly, animals were placed in a black tank 170 cm in diameter filled with 31 cm of water at 22°C. The tank was divided in four virtual quadrants, each associated with an external visual clue. A non-visible platform (black, 12 cm diameter, 30 cm high) was placed inside the tank and all movements were recorded through a video camera on the ceiling and tracked using a video-tracking system (Viewpoint, Champagne au mont d'or, France). In all trials the animal had 120 s to find the platform, at the end of which it was gently pushed towards if unable to complete the task. After reaching the platform, the animal was allowed 20 s on it before starting a new session.

Evaluation of working memory (WM) consisted of 4 days of evaluation, four sessions each. The position of the platform was maintained within each day, but changed on consecutive days, while the animal initiated each session on a different quadrant. The last day of WM evaluation was also the first of 4 days of MWM testing, in which the platform remained on the same place throughout all days of testing, while all remaining parameters were similar to the WM test. On the final day of testing, a reversal test (RT) was performed. Here, the platform (which had been in the same quadrant for 4 days) was moved to the opposite quadrant and four sessions equal to the ones above described were performed.

In all modalities, time to reach platform was evaluated. For WM and MWM, the average evolution curves throughout first to fourth trial or day were assessed respectively. For RT, the comparison was between time spent in the new and old quadrants.

Staircase Test

Aiming to assess potential differences in motor skills between left and right pawed animals, a modified version of the original staircase test (Montoya et al., 1991) was performed, for which most procedures have been previously described (Teixeira et al., 2017). This test required the usage of double staircase boxes (Campden Instruments, Lafayette, IN, USA), which consists of a narrow platform connected to a larger chamber with a moveable lid. A double removable seven-step staircase was inserted along both sides of the platform. In each session, five sugared pellets (45 mg, Bioserv Inc., Flemington, NJ, USA, EUA) were placed in each step and one daily session was performed. The first two sessions aim to habituate the animals and last respectively 5 and 10 min each, after which five test sessions were performed, each lasting 5 min. The last two evaluations were forced choice sessions, in which only one of the staircases (left or right) had pellets in it. In all cases, at the end of the session, the remaining pellets were counted.

Measures of interest were reached level at each side (lowest level from which pellets were withdrawn) and success rate (number of pellets eaten/total number of pellets) in both normal and forced sessions.

Statistical Analysis

All statistical analyses were performed on Matlab R2009b software. $P < 0.05$ was always considered the significance threshold. For assessment of inter-rater reliability Kappa statistics was used, namely Fleiss' Kappa when comparing between four observers and Cohen's Kappa when comparing the two experienced raters. Potential time-dependent differences in pawedness were assessed by comparing three separate time points using a repeated measures ANOVA.

For analysis of behavior mixed design ANOVAs were conducted. For VDS, the between subjects factor was pawedness group and the within subjects factor was session (training) or interval (test). For water maze, the between subjects factor was also pawedness group and the within subjects factor was either trial (WM), day (MWM) or quadrant (RT).

Differences in motor performance and motivation were assessed using simple group comparison. As normality could not be confirmed, non-parametric tests were used.

RESULTS

Pawedness (Inter-Rater Agreement)

Four observers (two experienced and two inexperienced) rated the first pawedness evaluation (**Figure 2A**). Inter-rater

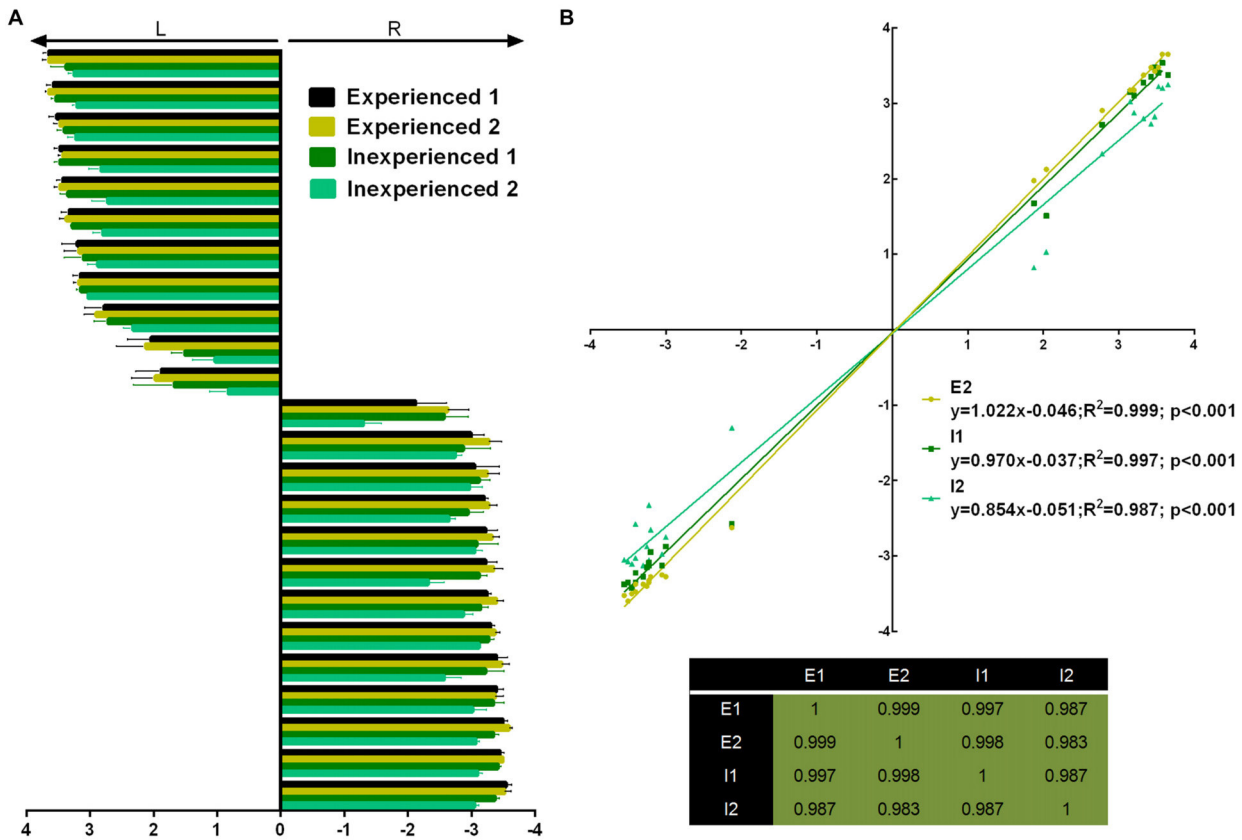

FIGURE 2 | Pawdeness trait test (PaTRaT) rater comparison. Two experienced and two inexperienced raters scored the first pawedness evaluation. **(A)** Representation of the score attributed by each rater for each animal shown as mean ± SEM. **(B)** Experienced rater 1's scores correlated with scores attributed by all other raters (graph) and all raters show high correlation between them (table of R^2s). L, Left; R, Right; E, experienced; I, inexperienced.

agreement was assessed and rendered a "substantial agreement" (Fleiss' Kappa = 0.615; $p < 0.001$; 95% CI = 0.590–0.640). Additionally, individual raters' scores showed to be linearly correlated among them (**Figure 2B**).

Pawedness (Temporal Stability)

As the two experienced raters presented very high correlation and inter-rater agreement (Cohen's kappa = 0.932, $p < 0.001$, 95% CI = 0.762–1.102) on the first evaluation, this data was averaged between them and subsequent evaluations were assessed by both (2 sessions each) and averaged. Repeated measures ANOVA showed no effect of moment (M) of evaluation (**Figure 3A**, $F_{(2,44)} = 0.641$, $p = 0.532$) and analysis of the logarithmic ratio between moments of evaluation showed no difference from 0 (**Figure 3B**, M2/M1: $Z = 0.400$, $r = 0.082$, $p = 0.689$; M3/M2: $Z = 1.156$, $r = 0.241$, $p = 0.248$; M3/M1: $Z = 1.247$, $r = 0.260$, $p = 0.212$).

Thus, from the total of 24 evaluated animals, all showed consistent paw preference across time. From these, 11 and 13, respectively showed left and right paw preference. Following veterinary decision one animal was excluded prior to M3. Up to this timepoint general aspects of well-being, weight and other behavioral measures were normal.

Variable Delay-to-Signal (Impulsivity)

During the learning phase, the evolution number of omissions across sessions (**Figure 4A**) showed an effect of session ($F_{(9,198)} = 40.428$, $p < 0.001$), but no effect of pawedness group ($F_{(1,22)} = 0.060$, $p = 0.809$) or interaction session/group ($F_{(9,198)} = 0.690$, $p = 0.718$) indicating that both groups learned equally well the task. Similar results were found for correct nosepokes (session: $F_{(9,198)} = 20.377$, $p < 0.001$; group: $F_{(1,22)} = 0.007$, $p = 0.935$; interaction: $F_{(9,198)} = 0.529$, $p = 0.852$) and premature responses (**Figure 4B**—session: $F_{(9,198)} = 38.350$, $p < 0.001$; group: $F_{(1,22)} = 0.005$, $p = 0.943$; interaction: $F_{(9,198)} = 0.415$, $p = 0.926$). Left and right pawed animals thus showed no differences in learning the task.

Regarding impulsivity (**Figure 4C**), which is evaluated by delay intolerance (premature responses per min), there was a significant effect of interval ($F_{(3,66)} = 12.617$, $p < 0.001$) but no effect of group ($F_{(1,22)} = 2.095$, $p = 0.162$) or interaction ($F_{(3,66)} = 2.371$, $p = 0.078$). Similarly, log (3sf/3si) showed no

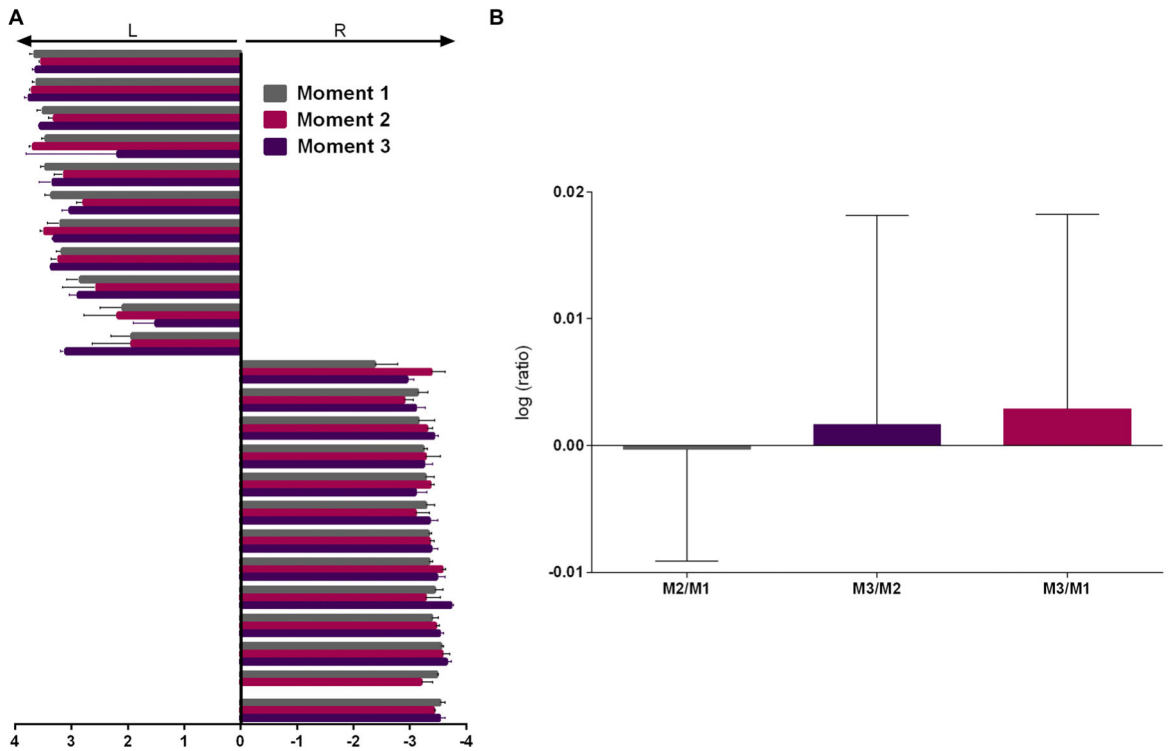

FIGURE 3 | PaTRaT time-related stability. Pawedness was evaluated at three separate moments. **(A)** Representation of the score attributed at each moment. 11/13 animals showed left/right preference, respectively. **(B)** Graph shows the logarithmic ratios between the three moments of evaluation. Data is shown as mean ± SEM. L, Left; R, Right; M, moment.

differences between animals with left and right paw preference (**Figure 4D**, $Z = 0.758$ Cohen's $d = 0.377$, $p = 0.448$).

Water Maze (Memory)

The WM part of the water maze (**Figure 5A**) trial showed a significant effect on time to reach the platform ($F_{(3,66)} = 8.487$, $p < 0.001$), but no influences of pawedness group ($F_{(1,22)} = 1.104$, $p = 0.305$) or interaction ($F_{(3,66)} = 0.373$, $p = 0.773$) were found. Similar data was found regarding MWM (**Figure 5B**—day: $F_{(3,66)} = 1.075$, $p = 0.001$; group: $F_{(1,22)} = 0.001$, $p = 0.974$; interaction: $F_{(3,66)} = 1.075$, $p = 0.366$). No effects were found on the RT component of the test (**Figure 5C**—quadrant: $F_{(1,22)} = 1.291$, $p = 0.268$; group: $F_{(1,22)} = 0.387$, $p = 0.540$; interaction: $F_{(1,22)} = 1.932$, $p = 0.178$). In all cases, similar results were found when analyzing distance traveled rather than time to reach the platform (data not shown).

Fine Motor and Locomotor Performance

The staircase test showed no group differences regarding fine motor coordination for both right (level normal: $Z = 0.434$ Cohen's $d = 0.105$, $p = 0.664$; level forced: $Z = 1.074$ Cohen's $d = 0.110$, $p = 0.283$; success rate normal: $Z = 0.838$ Cohen's $d = 0.334$, $p = 0.402$; success rate forced: $Z = 1.131$ Cohen's $d = 0.455$, $p = 0.258$) or left (level normal: $Z = 0.203$ Cohen's $d = 0.073$, $p = 0.839$; level forced: $Z = 0.602$ Cohen's $d = 0.117$, $p = 0.548$; success rate normal: $Z = 0.260$ Cohen's $d = 0.177$, $p = 0.795$; success rate forced: $Z = 1.389$ Cohen's $d = 0.176$, $p = 0.165$) paws (**Figure 6A**).

Regarding latency to reward retrieval in the VDS no differences between animals with left or right paw preference were found ($Z = 0.513$ Cohen's $d = 0.124$, $p = 0.608$), as seen through the time to retrieve the reward during the VDS test (**Figure 6B**). Additionally, no differences were found in the water maze average swimming velocity ($Z = 0.051$ Cohen's $d = 0.486$, $p = 0.959$; **Figure 6C**).

DISCUSSION

PaTRaT is a grading system to evaluate pawedness in rats. It is of simple implementation, requiring minimal equipment. Also, it has high inter-rater reliability and temporal stability of the outcomes. The PaTRaT displays several advantages over the most used tests—the Collins' (Collins, 1968) and LPP (Waters and Denenberg, 1991) tests, namely: (i) the reward can be readily seen, smelled and touched by the animals, increasing motivation. Indeed, we observed in preliminary assays that animals were more prone to perform the test when a greater amount of reward (≈50 vs. ≈20 cheerios®) was available (data not shown); (ii) the apparatus imposes no constraints to the simultaneous use of both paws for reward manipulation,

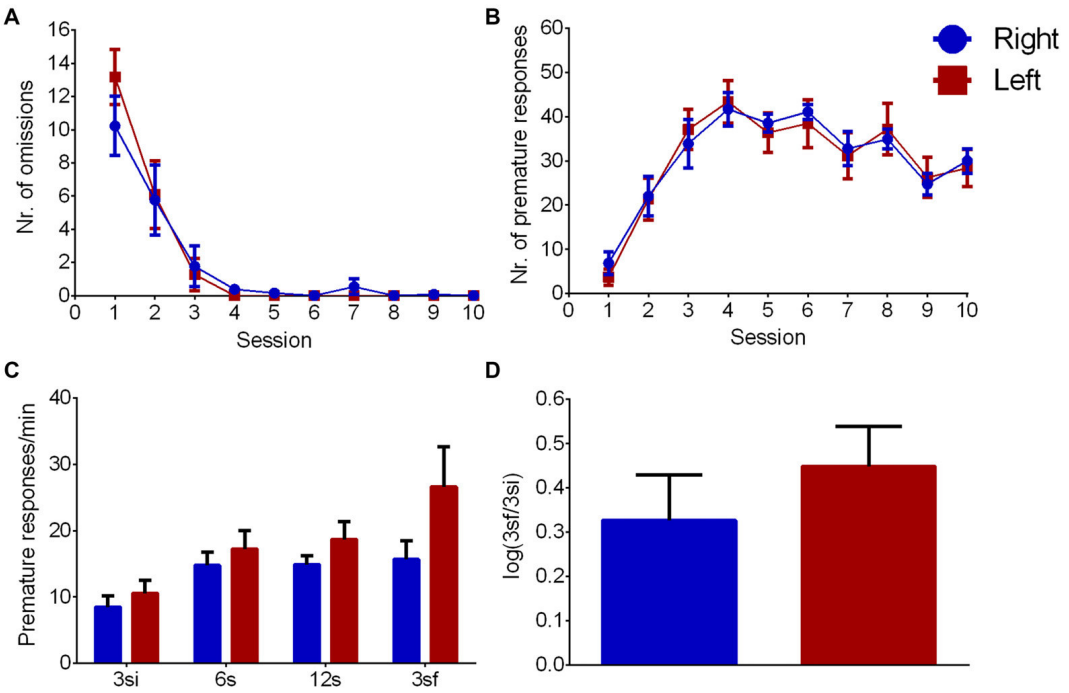

FIGURE 4 | Left/Right differences in impulsivity. Group comparison during VDS training **(A,B)** and test **(C,D)**. **(A)** Number of omissions, **(B)** number of premature responses, **(C)** premature responses per minute per interval and **(D)** logarithmic ratio of the number of premature responses in 3sf and 3si intervals. Data is shown as mean ± SEM. Blue—animals with right paw preference; Red—animals with left paw preference.

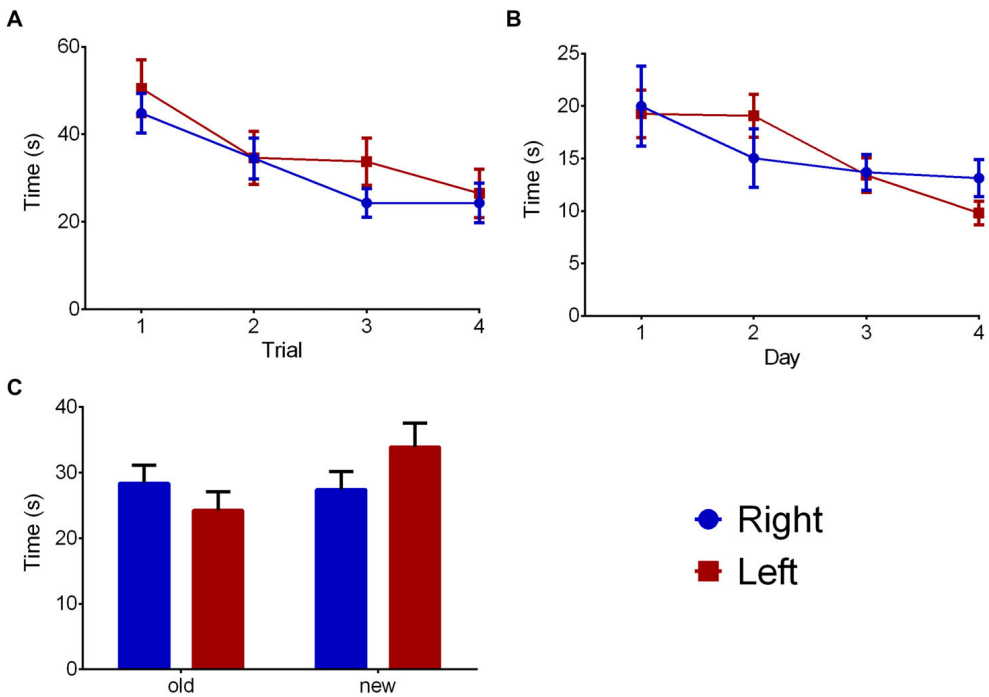

FIGURE 5 | Group effects on memory. Group comparison on the water maze test in WM **(A)**, classic Morris **(B)** and reversal **(C)** components. Data is shown as mean ± SEM. Blue—animals with right paw preference; Red—animals with left paw preference.

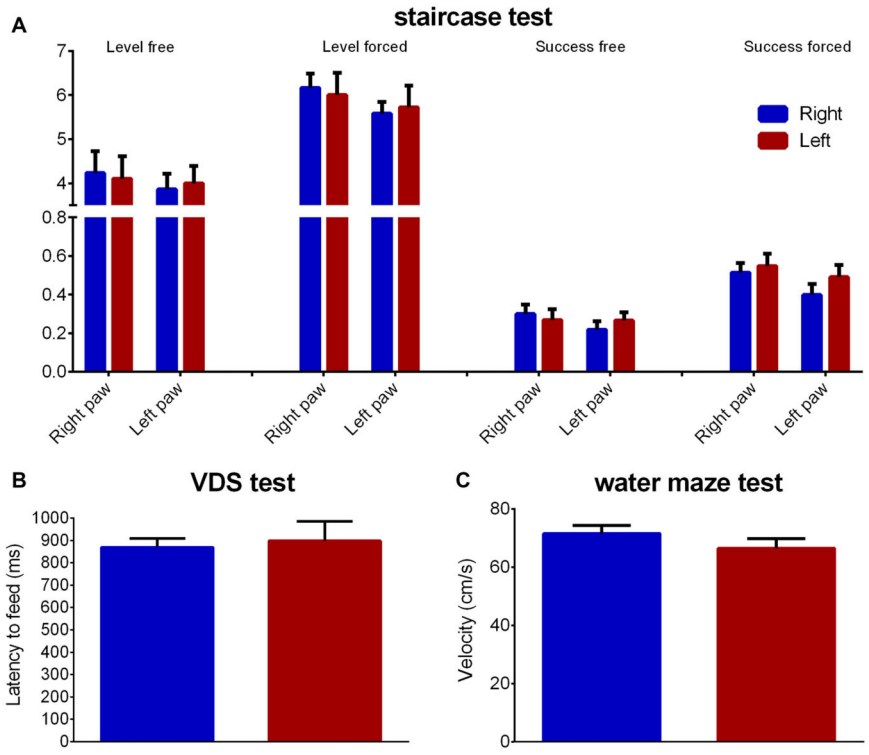

FIGURE 6 | Fine motor and locomotor performance. Group comparison regarding fine motor coordination (**A**—staircase test), motivation to eat (**B**—latency to feed in the VDS test) and motor performance (**C**—velocity in the water maze). Data is shown as mean ± SEM. VDS, Variable Delay-to-Signal; Blue—animals with right paw preference; Red—animals with left paw preference.

decreasing potential selection biases (Ribeiro et al., 2011) and providing a more ethological setting; (iii) the large reward size relative to the mesh grid imposes higher dexterity, i.e., a sequence of relatively complex movements is required for a successful retrieval of the reward; (iv) assessment is based on four independent sessions (10 trials each) avoiding potential confounders as limb alternation due to tiredness and satiation (Bulman-Fleming et al., 1997); (v) the grading system is of simple application and has a high inter-rater agreement even among inexperienced observers; and finally (vi) PaTRaT side preference index, contrary to the simple quantification of left/right paw retrievals, accounts for intermediate pawedness and is therefore more akin to human handedness assessment (Prichard et al., 2013). Additionally, two sessions were enough to achieve a sustained performance in 80% of the animals. In our study, the prevalence of right- and left-pawed rats was similar (54% vs. 46%, respectively), which is in accordance with a previous report (Stashkevich and Kulikov, 2001) but in opposition with others that report a rightward bias up to ≈82.4% (Pençe, 2002; Elalmis et al., 2003; Güven et al., 2003; Wu et al., 2010). Scores were stable across the three evaluations even when other manipulations/behavioral paradigms were interposed; potential differences in fine motricity (or even in general motor performance) were experimentally excluded. Importantly, we evaluated the PaTRaT in male Sprague-Dawley rats but strain, sex and species differences have been described in the context of paw preference protocols (see for example Betancur et al., 1991).

It has been argued that a less marked lateralization in rodents (or other animals) in comparison with humans results from the fact that pawedness assessments rely on the evaluation of simple movements (e.g., grabbing food) instead of fine movements as in humans (see for review on nonhuman primates, Hopkins, 2013). Thus, the increased movement complexity of the PaTRaT assay may explain the absence of ambidextrous animals, normally reported as being 7.4%–23% (Stashkevich and Kulikov, 2001; Pençe, 2002; Elalmis et al., 2003; Güven et al., 2003; Wu et al., 2010), as it allows a better separation of left- and right-pawed animals (i.e., average scores close to the limits of the scale). In fact, decreases in the PaTRaT absolute scores were mostly related with poorer performances of the preferred paw and not with retrievals with the non-preferred paw (practically absent).

Pawedness in rats has been associated with central asymmetries in monoamines, notably dopamine and other molecular players (Barnéoud et al., 1990; Cabib et al., 1995; Budilin et al., 2008). These have been hypothesized to underlie associations between pawedness direction (and/or magnitude) and performance in several behavioral domains (Wu et al., 2010)—see also Prichard et al. (2013) for a comprehensive review in human studies. Specifically regarding impulsive behavior, several studies have demonstrated an influence of

the dopamine levels (see for review, Dalley and Roiser, 2012; D'Amour-Horvat and Leyton, 2014). We have nevertheless observed no differences between left- and right-biased animals on impulsive behavior both on the learning protocol or in the VDS test. Additionally, no differences were observed in long-term and working spatial reference memories and reversal learning.

In conclusion, the PaTRaT is a simple, inexpensive and reliable test for assessment of pawedness degree and direction in rats. It relies on a grading system for hind paw use and has high inter-rater agreement (even for inexperienced observers). At the population level, we observed a nearly equal distribution of left- and right-biased rats and individual preferences were stable across sessions.

AUTHOR CONTRIBUTIONS

AMC, ME and HL-A designed the experiment; AMC, ME and MRG performed the experiments, AMC, ME, SPN and SB rated the animals; ME and HL-A performed the statistical analysis; AMC, ME, NS, AA and HL-A analyzed and interpreted the data; AMC, ME and HL-A wrote the article's initial version. All authors revised and approved the final version of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX B

Impact of chronic pain on rodents' pawedness

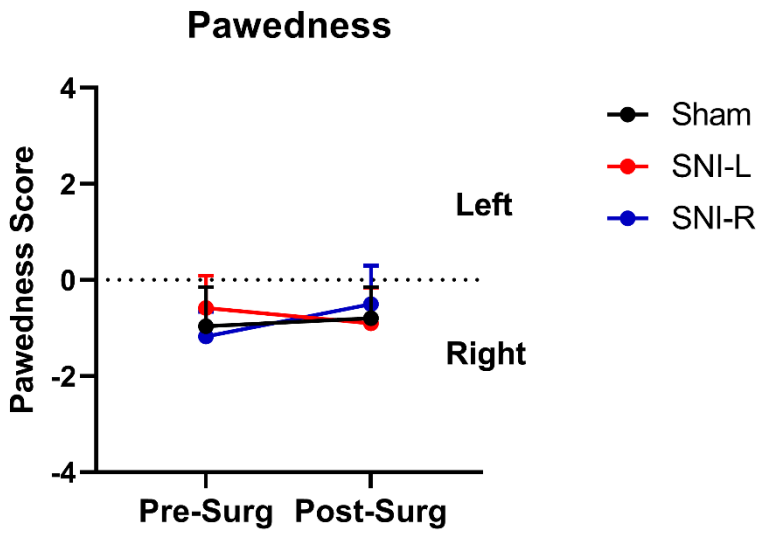

Figure 1. Impact of chronic pain on rodents' pawedness Adult male Wistar-Han rats performed the PaTRaT test 1 week before and 4 weeks after a neuropathy. Pawedness score was not affected by the spared nerve injury (SNI) (Mixed Design ANOVA- Group: $F_{(2,20)}$=0.013, p=0.987, η^2=0.001; Group*Time: $F_{(2,20)}$=0.514, p=0.606, η^2=0.013). Results presented as mean ± SEM. Each score represents a total of 20 reward retrievals e 2 different sessions. n=8

APPENDIX C

Impact of D1 and D2 antagonism of NAc neurons on impulsive behavior

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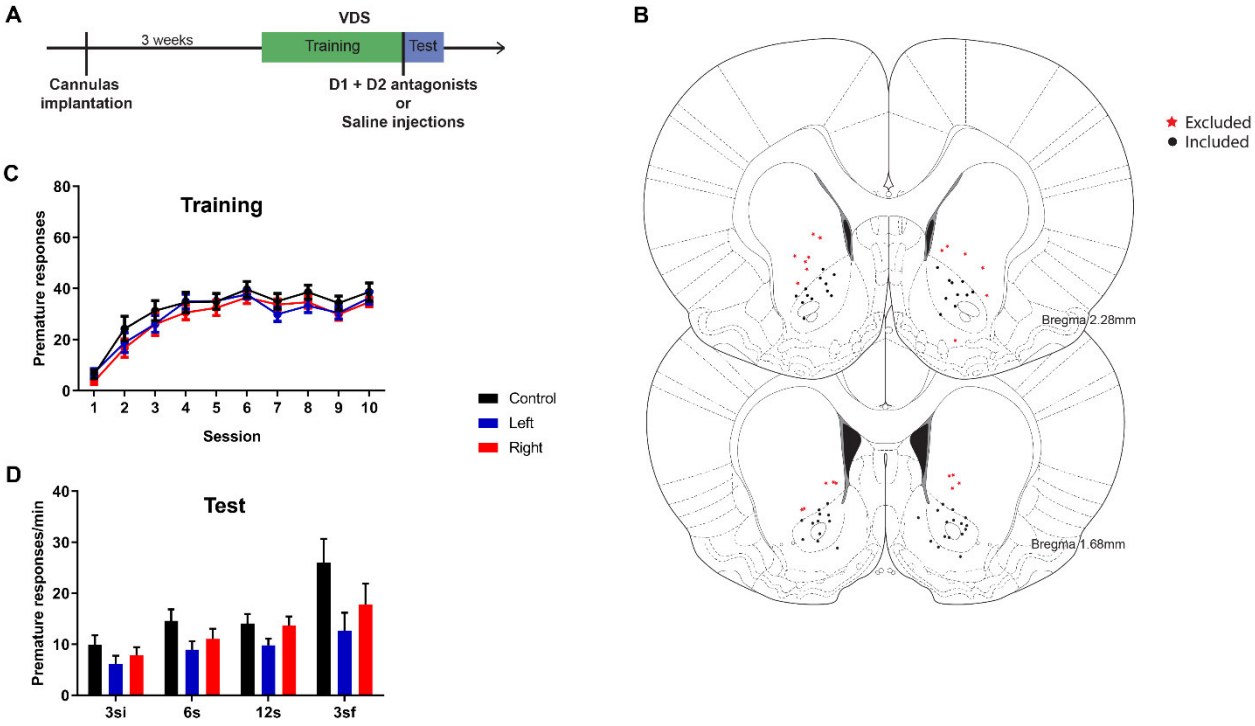

Figure 1. Impact of D1 and D2 antagonism of NAc neurons on impulsive behavior. Adult male Wistar-Han rats performed the VDS test for impulsive behavior (Leite-Almeida 2013) 3 weeks after cannula implantation. Before the test, the animals were injected with 1µl of D1 (SCH-23390 (0.5 µg/µl)) and D2 (Sulpiride (0.2 µg/µl)) antagonists or saline (controls) in the left or right NAc (AP:1.2; ML:±2.1; DV:-7) **(A)**. After confirmation of the localization of the cannulas in the NAc 13 Control, 18 Left and 16 Right rats were included **(B)**. No differences between the groups were observed during VDS training **(C)**. During the test, injected rats present higher tolerance to the delay than controls. Results presented as mean ± SEM.