Universidade do Minho
Escola de Ciencias da Saúde

Ana Margarida Ferreira da Cunha

Pain, emotion and cognition in left and right sided peripheral neuropathies: hemispheric-specific involvement of dopamine

Outubro de 2014

UMinho | 2014 Ana Margarida Ferreira da Cunha **Pain, emotion and cognition in left and right sided peripheral neuropathies: hemispheric-specific involvement of dopamine**

Universidade do Minho
Escola de Ciencias da Saúde

Ana Margarida Ferreira da Cunha

Pain, emotion and cognition in left and right sided peripheral neuropathies: hemispheric-specific involvement of dopamine

Dissertação de Mestrado
Mestrado em Ciências da Saúde

Trabalho Efetuado sob a orientação do
Professor Doutor Hugo Almeida

Outubro de 2014

DECLARAÇÃO

Nome: Ana Margarida Ferreira da Cunha

Endereço electrónico: anacunha@ecsaude.uminho.pt

Número do Bilhete de Identidade: 13830531

Título da dissertação: Pain, emotion and cognition in left and right sided peripheral neuropathies: hemispheric-specific involvement of dopamine

Orientador: Professor Doutor Hugo Almeida

Ano de conclusão: 2014

Ramo de conhecimento do mestrado: Mestrado em Ciências da Saúde

Universidade do Minho, Outubro de 2014

Assinatura:

_____

Para a minha avó

que me ensinou mais sobre dor e dopamina do que qualquer livro ou experiência...

Agradecimentos

Ao Hugo, pelo entusiasmo contagiante, por me incentivar a pensar e a olhar criticamente para todos os factos, resultados e teorias, pelas nossas discussões. Por acreditar em mim (e particularmente na minha capacidade de escrever a dita cuja revisão) muitas das vezes mais do que eu própria e por fazer questão de me ir lembrando para ser uma voz e não um eco.

Ao Marco, pelo companheirismo, pelo apoio, por partilhares comigo os altos e baixos deste ano. Pela tua alegria constante e pelas tuas mentirinhas irritantes, sem as quais já não te conheço.

Aos dois, pela ajuda, pelas horas partilhadas no biotério ou fora dele, por tudo o que me ensinaram. Esta tese, muito mais do que minha, é nossa.

À Ana e à Madalena também pela ajuda, pelo trabalho e pelas palavras de força.

A todos os NeRD's que direta ou indiretamente me ajudaram com este trabalho, por estarem sempre disponíveis a partilhar o vosso tempo, conhecimento e reagentes e materiais comigo, pelas discussões e colaborações, pelo apoio, pelas palavras amigas, pela animação constante.
Ao *gang da cantina*, cuja companhia tornou a hora de almoço num tempo de alegria e recuperação de forças.

Às tratadoras, principalmente à Susana e à Celina pela paciência, por me ajudarem com tudo o que precisava, mesmo quando lhes pedia em cima da hora, por fazerem o seu trabalho com simpatia e prontidão.

Aos meus pais, por estarem sempre lá para mim, por compreenderem as minhas ausências, pelo orgulho, pelo amor e pela dedicação. Ao meu irmão, pelo carinho e pela

companhia apesar dos jogos perdidos, apesar dos fins de semana que não pude estar com ele, apesar de toda a falta de atenção que lhe dei este ano.

Ao tio Domingos por assegurar a minha formação, pelo apoio e confiança constantes.

À Ana, por ter sido um apoio incontornável ao longo deste ano, pelos desabafos, pelas partilhas, pela amizade.

Ao Nélson, por me ouvir constantemente a falar de dopamina, das minhas experiências ou de ciência em geral, por ter sempre as palavras (e as músicas) certas para cada ocasião e por me ensinar que tudo, incluindo o meu trabalho, pode (e deve) ser uma expressão de amor.

Ao Fábio, ao Miguel Ângelo, ao Mile, à Fabiana, ao Carlos, à Ana Luísa, à Tatiana e a todos os meus amigos pelas conversas, pelos lanches e jantares, por estarem lá para mim.

À minha avó.

Pain, emotion and cognition in left and right sided peripheral neuropathies: hemispheric-specific involvement of dopamine

Pain is a complex sensation with a physiological protective role. However, in some poorly understood and individual-specific conditions, pain can chronify becoming highly debilitating and comorbid with emotional and cognitive impairments. Studies in animal models of chronic pain indicate that pain duration, left/right location and age are critically associated with the manifestation of emotional and cognitive behavioral outcomes. Paradoxically, these studies also suggest some degree of dissociation between pain and its comorbidities. Dopamine (DA) is in this context an interesting research target as it is a neurotransmitter associated with emotion, cognition and pain and its hemispheric asymmetries and age-related variations have been thoroughly demonstrated.

This experimental work aims to elucidate the relation between pain and its comorbidities and to study the involvement of DA in these lateralized phenomena. First, the impact of left- and right-sided peripheral neuropathies on behavior was studied in painful and non-painful conditions. The spared nerve injury (SNI) model was installed in the left (SNI-L) or right (SNI-R) side and, 1 month later, behavior was analyzed in a battery of behavioral paradigms. SNI-L rats presented an anxiety-like phenotype in the dark/light and spontaneous burrowing behavior paradigms. Also, SNI-R rats presented increased impulsivity in variable delay-to-signal test. In both cases, side-specific effects were in accordance with previous observations of the group. Moreover, these behaviors manifested independently of the presence of allodynia, a hallmark of neuropathic pain. Secondly, D1 and D2 DA receptors' mRNA was quantified by qPCR in the medial prefrontal cortex, orbitofrontal cortex, dorsal striatum and nucleus accumbens in both hemispheres. Results revealed an increase in D1 and D2 expression in the nucleus accumbens contralateral to the SNI lesion. Furthermore, ablation of cortical and subcortical DA efferences resulted in heightened impulsivity in right-sided lesions suggesting a causal relation between the side of the lesion and the behavioral impairments.

In conclusion, we demonstrated that the impact of a peripheral neuropathic lesion on emotional and cognitive behavior is to a certain extent independent of pain manifestations, suggesting that the nerve injury alone (i.e. in the absence of pain) can trigger central plastic events. Further studies should nevertheless be performed to clarify

the relation between the peripheral nerve injury and the central events in the lateralized bias observed.

Dor, emoção e cognição em neuropatias periféricas esquerdas e direitas: envolvimento lateralizado da dopamina

A dor é uma sensação complexa que possui uma função protetora. No entanto, em alguns indivíduos, por razões ainda não entendidas, esta pode tornar-se crónica sendo frequentemente acompanhada por problemas cognitivos e emocionais. Estudos em modelos animais de dor crónica indicam que a duração da dor, a localização à esquerda/direita e a idade estão intimamente associados com a manifestação de comportamentos emocionais e cognitivos. Paradoxalmente, estes estudos também sugerem um certo grau de dissociação entre a dor e as suas comorbilidades. A Dopamina (DA) é neste contexto um alvo de interesse, uma vez que é um neurotransmissor associado com emoção, cognição e dor. Além disso, assimetrias hemisféricas e variações relacionadas com a idade também já foram demonstradas.

Este trabalho experimental tem como objetivo elucidar a relação entre a dor e as suas comorbilidades e estudar o envolvimento da DA neste fenómeno lateralizado. Inicialmente foi estudado o impacto de neuropatias periféricas esquerdas e direitas nos comportamentos emocionais e cognitivos em condições de dor e não dor. O modelo *Spared Nerve Injury* (SNI) foi realizado no lado esquerdo (SNI-L) ou direito (SNI-R) e 1 mês mais tarde, o comportamento foi analisado numa bateria de paradigmas comportamentais. Os ratos SNI-L demostraram um fenótipo ansioso nos testes *dark/light* e *spontaneous burrowing*. Além disso, os ratos SNI-R apresentaram um aumento de impulsividade. Nos dois casos, os efeitos do lado estão de acordo com as observações prévias do grupo. Mais ainda, estes efeitos manifestam-se independentemente da presença ou não de alodinia, uma característica da dor crónica. No segundo conjunto de experiências foi realizada a quantificação do mRNA dos receptores de DA D1 e D2 no córtex prefrontal medial, no córtex orbitofrontal, no estriado dorsal e no núcleo accumbens dos dois hemisférios. Os resultados revelaram uma expressão aumentada dos receptores no lado contralateral à lesão SNI no núcleo accumbens. Para além disso, a ablação dos eferentes corticais e subcorticais de DA resultou numa impulsividade acrescida nos animais com lesão direita sugerindo uma relação causal entre o lado da lesão e os défices comportamentais.

Em conclusão, demonstramos que o impacto de uma lesão neuropática periférica é, até certa extensão, independente da manifestação da dor, sugerindo que a lesão do nervo por si só (i.e. na ausência de dor) pode despoletar eventos plásticos centrais. Estudos futuros deverão no entanto ser realizados para esclarecer a relação entre lesões periféricas do nervo, os eventos centrais e o viés de lateralidade observado.

TABLE OF CONTENTS

Abbreviations

5-HT- 5- hydroxytryptamine (5-HTa)

6-OHDA- 6-Hydroxydopamine

ACC- Anterior Cingulated Cortex

ADHD- Attention Deficit and Hyperactivity Disorder

ALL-L- Allodynic-left

ALL-R- Allodynic-right

AMY- Amygdala

BLA- Basolateral Nucleus of the Amygadala

BP- Binding Potential

COMT- Catechol-O-methyl transferase

CPP- Conditioned Place Preference

CRF- Continuous Reinforcement Schedule

CRPS- Complex Regional Pain Syndrome

D/L- Dark/Light Test

D_{1-5}R- Dopamine receptor 1 to 5

DA- Dopamine

DH- Dorsal Horn

DNA- Deoxyribonucleic acid

DRG- Dorsal Root Ganglia

EDTA- Ethylenediamine Tetraacetic acid

EPM- Elevated Plus Maze

ERK- Neuronal Extracellular Signal-regulated Kinase

FC- Fear Conditioning

fMRI- functional Magnetic Resonance Image

FST- Forced Swimming test

IASP- International Association for Study of Pain

DAT-1- Dopamine transporter 1

IL- Interleukin

L-DOPA - L-3,4-dihydroxyphenylalanine

MAO-A- Monoamine Oxidase-A

mPFC- medial Prefrontal Cortex

NAcc- Nucleus Accumbens

N-All-L- Non-Allodynic-Left

N-All-R- Non-Allodynic.Right

NA- Noradrenaline

OFC- Orbitofrontal Cortex

PAG– Periaqueductal Gray

PD- Parkinson's Disease

PET- Positron Emission Tomography

PFA- Paraformaldehyde

PFC- Prefrontal Cortex

qPCR- quantification Polymerase Chain Reaction

RAIC- Rostral Agranular Insular Cortex

RNA- Ribonucleic acid

Rpm- Rotations per minute

RR- Random Ratio

RT- Room Temperature

RVM- Rostral Ventromedial Medulla

SBB-Spontaneous Burrowing Behavior

SNI-L- Spared Nerve Injury-Left

SNI-R- Spared Nerve Injury-Left

SNI- Spared Nerve Injury

SNL- Spinal Nerve Ligation

SN- Substantia Nigra

SPF- Spontaneous Paw Flicks

SS- Somatosensory

STR- Striatum

STT- Spinothalamic Tract

TH- Tyrosine Hydroxylase

TNF- Tumor Necrosis Factor

VDS- Variable Delay-to-Signal

VF- Von Frey monofilaments test

VO- Ventral Orbitofrontal Cortex

VTA- Ventral Tegmental Area

WM- Working Memory

Figures Index

1. INTRODUCTION

1.1 Pain

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey & Bogduk, 1994) It is a complex experience that involves a sensory/discriminative dimension related with pain intensity and localization, an affective/motivational dimension associated with our capacity to interpret pain as an unlikable sensation and to modulate it according to our current status and a cognitive/evaluative dimension mostly related with our culture, education and memories (Casey, 1968).

The experience of pain starts at the peripheral primary afferent neurons - the nociceptor - that convey the signal to the spinal dorsal horn (DH) neurons, which, in turn, project supraspinally to multiple areas of the Brainstem, Thalamus and cortex. Activation of the nociceptor, i.e. nociception, and pain are autonomous, though related phenomena and can occur independently. For instance, tetraplegic patients do not feel evoked pain after a noxious stimulus in the limbs but reflexes are maintained.

Pain is an indispensable sensation since it activates withdrawal reflex, for instance in case of a burning, decreases the contact with the damaged parts, facilitating healing, and alert us that something is wrong. A dramatic evidence of this statement comes from individuals with congenital insensitivity to pain, which have no perception of the majority of painful stimuli without any other sensory deficit. These individuals present severe physical injuries including extensive burns and fractured bones and have a reduced lifespan when compared with the general population (Nagasako, et al., 2003).

On the opposite extreme, pain that persists for long periods of time without any evident biological gain is debilitating and frequently comorbid with increased anxiety, depression and cognitive impairments. The endogenous control pain system – which provides inhibitory and facilitatory control over the nociceptive stimulus – in a healthy individual has to be therefore under tight regulation at multiple stations of the neuroaxis.

1.1.1 From the nociceptive stimulus to pain

As stated in the previous section, pain is initiated by the stimulation of nociceptive afferent neurons. The cell-bodies of these neurons are located at the Dorsal Root Ganglia (DRG). From these, a single neurite ramifies projecting simultaneously to the periphery (skin, internal organs, bones and muscles) and to the DH of the spinal cord (Basbaum, et al., 2009) (Fig. 1). At the periphery, stimuli of different modalities (mechanical, thermal and chemical) can activate specific

afferent neurons, depending on the repertoire of the receptors expressed at their terminals (Harriott & Gold, 2009). Afferent neurons are categorized according to the caliber of their axons as Aβ, Aδ and C. Aβ are large myelinated fibers and are therefore the fastest conducting axons of the three. They are mostly implicated in proprioception and light touch but some reports also indicate nociceptive-related activity (Djouhri & Lawson, 2004). On the other hand, Aδ and C fibers are intrinsically involved in nociception. Small myelinated Aδ fibers have a faster conduct velocity than unmyelinated C fibers and are responsible for the first sharp pain sensation; C fibers, on the contrary, are associated with a dull, less precise, secondary pain (Marchand, 2008).

The central terminals of afferent neurons synapse with secondary neurons at the DH of the spinal cord (Fig. 1) with a precise topographical organization. Fibers from specific body parts project to specific levels of DH and consequently to precise supraspinal loci, which allows pain localization. Even at the same level, different afferent fibers synapse at diverse locations: Aδ and peptidergic C fibers terminate mostly in the exterior part of dorsal horn (laminae I and II_o of Rexed laminae), non peptidergic C fibers terminate at II_i and Aβ synapse even more deep in laminae III to V (Ossipov, 2012). Some studies show that this specific organization goes even further by revealing specific synapse loci for fiber subpopulations based on its expressed receptors (Braz, et al., 2014; Brown, et al., 1995). Secondary neurons project to supraspinal centers organized in different pathways, being the Spinothalamic tract (STT) one of the most characterized. The STT is classically divided into the lateral STT and the central STT based on their terminations on the

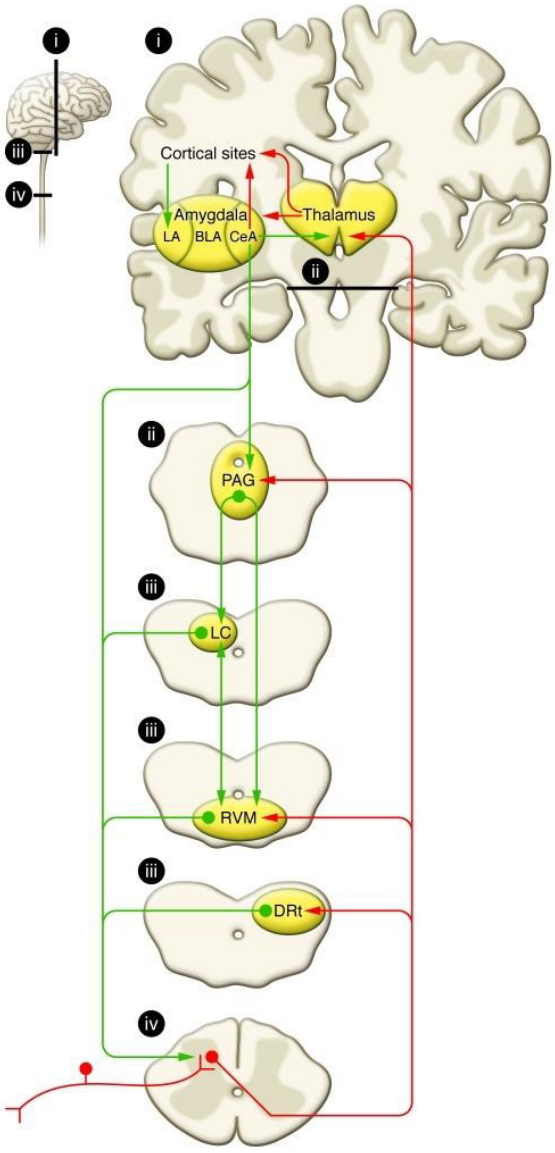

Figure 1- Ascending and descending pain circuits. Nociceptors stimulated in the periphery synapse in the dorsal horn (DH) of the spinal cord, which in turn sends inputs to supraspinal sites, namely to the thalamus. Pain modulation from brain nuclei goes through PAG and RVM, which project to the DH, facilitating or inhibiting pain. DRt=Dorsal Reticular nucleus, RVM= Rostral Ventromedial Medulla, LC= Locus coerulueus, PAG= Periaqueductal Gray área, LA=Lateral Amygdala, BLA=Basolateral Amygdala, CeA= Cental Nucleus of Amygdala

(Adapted from Ossipov et al., 2010)

lateral nuclei of Thalamus and in the medial nuclei of Thalamus, respectively. The lateral STT projects further to the primary and secondary somatosensory (SS) cortices. These projections and SS cortices maintain a high level of somatotopy (spatial body representation) and therefore lateral STT is associated with the sensory and discriminative dimension of pain (Ploner, et al., 1999). On the other hand, neurons from central STT project to limbic system nuclei, being therefore precluded as part of the affective and motivational components of pain (Ploner, et al., 1999; Rainville, et al., 1997). Other tracts project to areas like Brainstem, Amygdala (AMY) or Hypothalamus and are involved in the discriminative and motivational components of pain but also in pain modulation, autonomic responses, sleep alterations and endocrine responses (see (Almeida, et al., 2006; Almeida, et al., 2004; Millan, 1999) for revision).

While most of the tract definition studies were performed in animal models, the involvement of specific brain areas in human pain response starts to be more easily studied with the development of non-invasive image techniques. I and II SS cortices, Anterior Cingulate Cortex (ACC), Insula, Thalamus and Prefrontal cortex (PFC) are areas described to be activated after a nociceptive stimulus in most of the image studies and consequently accepted to be closely involved in pain. Some studies also report activation of the Brainstem, AMY, Cerebellum and Striatum (STR) (Apkarian, et al., 2005). As mentioned before, activation of these distinct areas will give rise to the complex experience of pain in all its dimensions.

1.1.2 Pain Modulation

Pain is a subjective and individual experience. The amount of stimulation upon the nociceptors is not proportional to the amount of pain experienced and neither is the activation of the different brain nuclei (Bingel & Tracey, 2008). Between different individuals and even in the same person similar lesions can elicit different pain perceptions depending on the physiological condition and context. As stated previously, in the most extreme cases pain can even be experienced in the absence of any detectable lesion. For instance, in a functional Magnetic Resonance Image (fMRI) study by Osborne and Derbyshire (2010) it was reported that the observation of images containing painful conditions elicited similar patterns of activity as evoked by actual nociceptive experiences; these individuals also reported somatization upon the observation of the images. Modulation of pain is an important feature of the system because it enables a decrease or even a suppression of pain in survival threatening situations (Beecher, 1946). It also explains why anxiety, expectation and attention can result in an increase of

perceived pain or, on the other hand, why distraction and excitation can have the opposite result (Bingel, et al., 2011; Villemure & Bushnell, 2002).

Pain modulation operates via descending pathways, which act upon the afferent pathways in the DH of the spinal cord. The Periaqueductal Gray (PAG) area in the Mesencephalon was the first nuclei described to be involved in pain modulation. In a seminal study, Reynolds stimulated the PAG with a small current and the powerful anesthetic effect elicited allowed the execution of an invasive abdominal surgery in rats (Reynolds, 1969). Similar effects were also observed in humans (Richardson & Akil, 1977). PAG anesthetic effect is mediated by the Rostral Ventromedial Medulla (RVM), which in turn projects to the DH (Fig. 1). RVM receives inputs from the PAG but also from other areas that can modulate pain negatively like ACC and PFC, positively as Hypothalamus and Dorsal Reticular nucleus (DRt) or in both ways like AMY (Hardy & Haigler, 1985; Heinricher, et al., 2009; Lima & Almeida, 2002; Lorenz, et al., 2003; Martenson, et al., 2009; Strobel, et al., 2014). In that way, RVM is considered a critical relay in pain modulation. Studies in rodents demonstrated that the RVM presents bifunctional effects in pain sensation: it can inhibit pain when electrically stimulated with high intensity current (50-200 μA) or glutamate quantity (2.5 nmol) and facilitate when low current (5-25 μA) or glutamate quantity (0.25 nmol) is applied (Calejesan, et al., 2000; Gebhart, 2004; Zhuo & Gebhart, 1997). Very likely, this is possibly due to the presence of three different types of cells, not anatomically independent: On, Off and Neutral cells. In electrophysiological recordings On-cells increase firing upon the application of a noxious stimulus, just before a retraction movement is elicited (e.g. tail flick). Off-cells, on the other hand, maintain in normal conditions a tonic activity, which is interrupted upon the application of a noxious stimulus. These cells have been hypothesized to be involved in pain facilitation and inhibition, respectively. Finally Neutral cells present no alterations in their firing rate elicited by noxious stimulation (Fields, et al., 1983).

It is believed that overall pain perception results from the balance between facilitatory and inhibitory supraspinal stimulus from the RVM and other areas. These stimuli reach DH by different pathways including dorsolateral and ventrolateral funiculi. The interruption of these pathways has been show to block supraspinal inhibitory and facilitatory pain influences, respectively (Porreca, 2002; Zhuo & Gebhart, 2002). The involvement of different neurotransmitters and receptors in the process has also been demonstrated (Millan, 2002; Ossipov, et al., 2010).

Opioid receptors are one of the most studied. Their stimulation by opioids like morphine or endogenous opioid peptides like endorphins has a potent inhibitory effect on pain. Injection of opioids in spinal cord, AMY, PAG and RVM decrease pain perception (Fang, et al., 1989; Heinricher, et al., 1994; Helmstetter, et al, 1998; Sohn, et al., 2000) and administration of opioid' antagonists systemically, in the PAG or in the RVM, has the opposite effect (Oliveras, et al., 1977; Sohn, et al., 2000). Opioids analgesic effect results in part from activation of Off-cells and inhibition of On-cells in RVM (Fang, et al., 1989; Fields, et al., 1983). Despite its side effects (constipation, nausea, respiratory depression, addiction) opioids are still very used in the clinic, especially in cancer-related pain and some hard to treat neuropathies (Ahlbeck, 2011; Ashburn & Staats, 1999).

Monoaminergic neurotransmitters like Noradrenaline (NA), a catecholamine involved in autonomic responses responsible for increased alertness, and Serotonin (5-HT:5-hydroxytryptamine), usually associated with feelings of happiness and well being, also play a role in pain modulation (Ossipov, et al., 2010; Pertovaara, 2006; Sommer, 2004). Locus Coerulueus, which is connected with PAG and RVM, and nucleus Raphe Magnus, a RVM's nuclus, are the main afferent brain nucleus of NA and 5-HT, respectively, and both project to DH. Stimulation of PAG and RVM, which leads to antinociception, induces the release of NA and 5-HT in the spinal cord and the antinociceptive effect of PAG stimulation is blocked by serotonergic and NAergic antagonists administration, directly implicating these neurotransmitters in pain blockage (Cui, et al., 1999; Hammond & Yaksh, 1984; Hentall, et al., 2006). Central NA release has an antinociceptive effect, mostly mediated by $\alpha 2$ receptors (Pertovaara, 2006) and 5-HT induces pain facilitation and inhibition, depending of the stimulated receptors. Stimulation of 5-HT_3, 5-HT_4 and 5-HT_{2a} receptors increases pain perception while stimulation of 5-HT_{1a}, 5-HT_{1b}, 5-HT_{2c} and 5-HT_7 is inhibitory (Dogrul, et al., 2009; Viguier, et al., 2013). Dopamine (DA), other monoaminergic neurotransmitter, is also involved in pain modulation, and its role will be discussed further.

1.1.3 Categorization of pain

1.1.3.1 Stimulus

Pain can be classified according to the triggering stimulus as nociceptive, inflammatory and neuropathic (Treede, et al., 2008; Woolf, 2010, Merskey & Bogduk, 1994).

Nociceptive pain results from the activation of the nociceptors by noxious stimuli. This type of pain is short-lasting and it is not associated with lesions in the tissues (Woolf, 2010). On the

other hand, inflammatory pain can be both the result of a continued inflammation, like in the case of appendicitis, or be involved in the healing of damage tissues by discouraging movement and/or physical contact (Woolf, 2010; Xu & Yaksh, 2011). Inflammatory pain involves the presence of inflammatory modulators, like cytokines, which induce tissue sensitization that leads to hyperalgesia (increased pain response due to a usually painful stimulus) and allodynia (pain due to a usually innocuous stimulus) (Xu & Yaksh, 2011).

Finally, neuropathic pain arises from damage to the somatosensory system caused by a lesion or disease (Treede, et al., 2008). Neuropathic pain is the most frequent pathological pain and is associated with a number of clinical conditions such as diabetic neuropathy, trigeminal neuralgia, postherpetic neuralgia, fibromyalgia, neuropathic back pain and complex regional pain syndrome (CRPS) (Jay & Barkin, 2014; Taylor, 2006). Neuropathic pain patients complain about intense continuous or episodic pain sensations as stabbing, burning, shocking, itching and numbness, which are much of the times debilitating (Jay & Barkin, 2014; Woolf & Mannion, 1999). Moreover, treatment efficiency varies drastically from person to person (the best drugs benefit only 30 to 60% of the patients) and between chronic pain types, which implies that prescriptions are based in trial and error and even efficient therapies do not eliminate pain permanently (Finnerup, et al., 2005; Sindrup & Jensen, 1999).

1.1.3.1 Duration

Pain can also be classified according to its duration as acute - lasts from days to weeks (usually no more than 1 month) -or chronic- lasts more than 3 months and can last all life. Acute pain can refer to the first pain response associated with nociceptive pain and which does not last more than a few hours or to a secondary pain related with inflammatory pain and the healing process and that last from days to weeks.

This classification is based on pain duration, however, in fact, acute and chronic pain distinguish protective from pathological pain. Acute pain does not last more than a few weeks because it disappears as soon as the cause is gone or even before the healing process terminates (Xu & Yaksh, 2011). On the contrary, chronic pain remains long before the cause is gone and can even appear without any apparent reason. Nowadays, chronic pain is considered itself a disease and although it can have different etiologies, affect different body parts and respond differently to treatment, constant pain is the element in common.

A study from World Health Organization in 15 countries all over the world reveal that chronic pain affects in average 22% of people and Azevedo and colleagues show that it reaches 37% of Portuguese population (Azevedo, et al., 2012; Gureje & Korff, 1998). It is also important to consider that it not only impacts patients' daily life but also has a huge economic impact, directly by the amount of money spent in the treatments and health care and indirectly by the costs of lost productivity since the permanent pain precludes patients to work. A recent study by Rasu and colleagues reports that only in United States $ 17.8 spent per year in medication prescribed for pain (Rasu, et al., 2014).

The mechanisms underlying arise and maintenance of chronic pain are not fully understood. However, studies in chronic pain patients and in experimental models, particularly concerning neuropathic pain, have been revealing important information at anatomical, functional and biochemical levels.

First, it is well accepted that chronic pain patients and neuropathic pain animal models show spontaneous ectopic activity generated in sensory nerve axons or in their cell bodies in DRG (Han, et al., 2000; Study & Kral, 1996), which arises between 12h and 2 days after injury and decreases over time (Han, et al., 2000; Sun, et al., 2005). Suppression of spontaneous activity, by nerve blockage, before or 3 to 5 days after injury reduces or totally eliminates pain behavior (Chaplan, et al., 2003; Xie, et al., 2005), implicating this mechanism in chronic pain initiation.

Other explored mechanism for chronic pain is central glia activation and releasing of proinflammatory cytokines. In animal models of neuropathic pain, microglia inhibition by administration of minocycline decreased mechanical hyperalgesia and allodynia (Hains & Waxman, 2006; Raghavendra, et al., 2003), directly implicating inflammation in chronic pain maintenance. This effect seems to be through the pro-inflammatory cytokines Tumor Necrosis Factor (TNF)-α and Interleukin 1(IL-1) release (Sommer, et al., 1999; Sorkin & Doom, 2000, Quintão, et al., 2006; Xu, et al., 2006).

Also, it was observed in humans and monkeys that after a nerve injury, cortical space formerly represented by the lesioned body part was functionally occupied by neighbor body parts (Elbert, et al., 1994; Merzenich, et al., 1984; Pons, et al., 1991; Wrigley, et al., 2009). Increased cortical reorganization is related with increased magnitude of pain in chronic back pain, complex regional pain syndrome and spinal cord injury patients (Flor, et al., 1997; Maihöfner, et al., 2003; Wrigley, et al., 2009). Nevertheless, it remains unknown if cortical reorganization is behind chronic pain appearance or if it is a response to pain maintenance.

Brain imaging studies showed also alterations in the grey matter volumes in several chronic pain conditions including chronic back pain (Apkarian, et al., 2004), fibromyalgia (Kuchinad, et al., 2007; Lutz, et al., 2008), trigeminal neuropathy (Obermann et al., 2013), CRPS pain (Geha et al., 2008) and chronic tension type headache (Schmidt-Wilcke et al., 2006) (see for review (May, 2008)). Cingulate, Orbitofrontal (OFC) and Insular cortices repeatedly present decreased grey matter volumes (Apkarian, et al., 2004; Emerson et al., 2014) which have been shown to revert upon analgesic treatment (Seminowicz et al., 2011). Similar observations were also made in an experimental model of neuropathic pain (Seminowicz et al., 2009). Of notice, these frontal brain areas, particularly the medial PFC (mPFC) and the OFC are critical elements of the networks involved in emotional and executive function (Kesner & Churchwell, 2011; Kouneiher, et al., 2009), which suggests a relation between chronic pain and the associated comorbidities.

1.1.4 Chronic pain comorbidities

Chronic pain is frequently comorbid with emotional and cognitive deficits (Bair & Robinson, 2003; Hart, et al., 2000; Moriarty, et al., 2011; Simons, et al., 2014). Chronic pain patients are 3 to 5 times more likely to develop depression than healthy subjects and its prevalence increases with pain severity (Bair & Robinson, 2003; DeVeaugh-Geiss et al., 2010). On the other hand, depressive patients complain more about pain and it has been shown that depressive symptoms can predict chronic pain appearance (Bair & Robinson, 2003). Chronic pain patients also have higher prevalence of anxiety (Jordan & Okifuji, 2011) and sleep disorders than healthy people (Menefee, et al., 2000; "Self-reported sleep and mood disturbance in chronic pain patients.," n.d.) and present a suicide risk two times higher than healthy subjects (Tang & Crane, 2006).

In animal models of chronic pain, albeit some contradictory results, depression and anxiety-like behaviors have also been demonstrated ((Gonçalves, et al., 2008; Matsuzawa-Yanagida, et al., 2008; Wang, et al., 2011) confront with (Gonçalves, et al., 2008; Hasnie, et al., 2007; Kontinen, et also., 1999)). Interestingly, it has also been shown that basal anxiety traits are predictors of heightened pain after the installation of a neuropathy (Geerse, et al., 2006) suggesting, like in humans, a bidirectional relation between emotional factors and the sensory abnormalities.

The emergence of anxiety- and depression-like behaviors in the animal model is intrinsically related with the temporal factor, the former emerging 2-4 weeks after pain onset and the later 6-8 weeks (Suzuki, et al., 2007; Yalcin, et al., 2011); such suggests a well defined

kinetic of events between the onset of pain and the manifestation of behavioral abnormalities, which are not entirely understood. In addition, it has been demonstrated that the age of the individual plays a critical role as anxiety-like behavior in Spared Nerve Injury (SNI) rats increases with age and depressive-like behavior is only potentiated by the lesion in mid-aged animals (Leite-Almeida, et al., 2009). Furthermore, lesion side also impacts behavioral outcomes as rats lesioned in the left side spend less time in open arms when compared with Sham and right injured animals, in Elevated Plus Maze (EPM) test (Leite-Almeida, et al., 2012).

Concerning cognition, studies show that chronic pain patients have impairments in memory, particularly working-memory (WM) (Berryman, et al., 2013; Dick & Rashiq, 2007; Ling, et al., 2007; Luerding, et al., 2008), attention (Grisart & Plaghki, 1999) and decision-making in a Iowa Gambling Task (Apkarian, et al., 2004; Walteros, et al., 2011). Also, these cognitive deficits seem to be independent of emotional impairments (Grisart & Plaghki, 1999; Reyes Del Paso, et al., 2012).

Once again, similar deficits have been observed in animal models. For instance, Low and colleagues demonstrate that chronic neuropathic pain rats spend less time exploring a new object than Sham animals, revealing attention or memory impairments (Low, et al., 2012). In a chronic inflammatory pain rat model (intra-articular injection of Complete Freund's Adjuvant), impaired performance in a test analogous to Iowa Gambling Task revealed deficits in emotional decision-making as these showed a preference for high-risk options (Pais –Vieira, et al., 2009). In contrast, Grégoire and colleagues did not find differences in spatial recognition when comparing neuropathic and Sham rats (Grégoire, et al., 2012). Thus, as in the emotional studies, cognitive impairments depend of experimental conditions. Recent studies demonstrate the influence of lesion side and animals' age in the effect of chronic pain in cognition. Right-side injured animals, but not left-side, show impairments in attentional set-shifting, WM and delay-to-signal impulsivity tasks in young animals (Leite-Almeida, et al., 2012, 2014). Left-sided pain, in turn, resulted in WM and reversal learning deficits but only in mid-aged rats (Leite-Almeida, et al., 2009).

Altogether, the animal studies suggest that the emergence of behavioural impairments in chronic pain conditions results from the interplay of numerous factors like pain duration, side and the age of the individual. Such most probably explains the apparent discrepancy observed between groups in the behavioural studies.

1.2. Dopamine

1.2.1 Dopamine as a neurotransmitter

Catecholamines are Tyrosine-derived chemicals which function both as hormones and neurotransmitters and include DA and NA (Eisenhofer, et al., 2004). L –tyrosine is converted into L-3,4-dihydroxyphenylalanine (L-DOPA) by Tyrosine Hydroxylase (TH), from which L-DOPA decarboxylase originates DA (3-hydroxytyramine). DA can further be converted in to NA by DA β-hydroxylase. Catecholaminergic neurons were identified by histochemical fluorescence and classified into groups according to their localization. Areas A1 to A7 represent groups of noradrenergic neurons while areas A8 to A14 correspond to dopaminergic neurons (Dahlstroem & Fuxe, 1964; Felten & Sladek, 1983). Areas A9 and A10 correspond respectively to the Substantia Nigra (SN) and Ventral Tegmental Area (VTA), the major dopaminergic efferences to the cortical and subcortical areas. Though corresponding to only 1% of the total neurons in the human brain, this system is of critical importance for motor and cognitive processes (Chinta & Andersen, 2005; Iversen & Iversen, 2007; Robbins & Roberts, 2007). Neurons from SN project to the STR via the Nigrostriatal pathway (Fig. 2), which is involved in motor control and coordination. Degeneration of these neurons, which happens for instance in Parkinson's disease (PD), leads to motor impairments and other disabilities (Mazzoni, et al., 2012; Pavese, 2012). On the other hand, neurons from VTA project to the frontal cortex, as part of the Mesocortical pathway (Fig. 2), or to Nucleus Accumbens (NAcc), AMY and Hippocampus through Mesolimbic pathway. These pathways are involved in more emotional functions like motivation, reward and attention (Arias-Carrión, et al., 2010; Landau, et al., 2009; Wise, 2004). There are evidences that schizophrenia, a mental disorder characterized by the presence of hallucinations, impaired social behavior and paranoia, and attention deficit and hyperactivity disorder (ADHD),

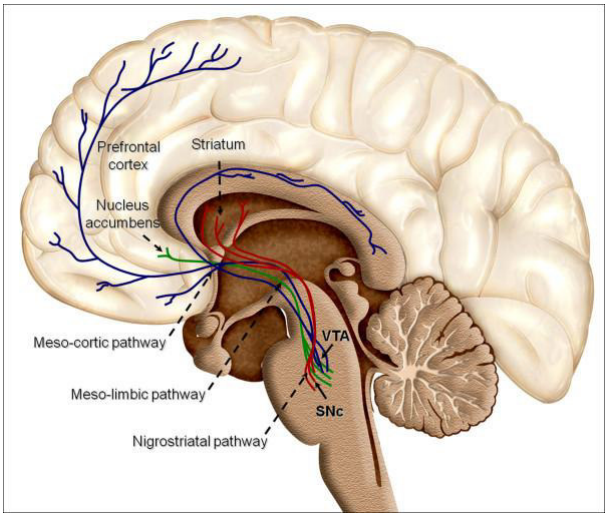

Figure 2- Dopaminergic Pathways. Nigrostriatal pathway projects from SN to Sriatum. Mesolimbic and Mesocortic pathways project from VTA to Nucleus Accumbens and Prefrontal cortex, respectively. The first is more involved in motor coordination while the last two are related with motivation and reward. VTA=Ventral Tegmental Area, SNc= Substantia Nigra.

(Adapted from (Arias-Carrión, et al., 2010)

a disorder which symptoms are attention deficits, higher impulsivity and hyperactivity, are the result of alterations in these pathways (Genro, et al., 2010; Wu, et al., 2012).

Not long after the discovery of DA as a neurotransmitter, two G protein-coupled receptors were identified as DA receptors. Their distinction, D_1R and D_2R, was based, respectively, in the stimulation or not of adenylyl cyclase. Three different receptors -D_3R to D_5R- were later identified. Considering their similarities with D_1R or D_2R receptors, now D_1R and D_5R are known as the D_1R-like family and D_2R, D_3R and D_4R as D_2R-like family. Both families show differences in structure, pharmacological properties, distribution and function (Missale, et al., 1998).

1.2.2 Dopamine and Acute Pain

A significant number of studies have been exploring the importance of DA in the context of pain. Pathologies characterized by substantial alterations of dopamine availability and/or processing have been providing interesting information in this respect. PD patients, for instance, although having normal ascending conduction of the nociceptive signal, have inferior pain thresholds and considerably more pain than general population (Beiske, et al., 2009) (Schestatsky, et al., 2007), which is diminished by administration of L-DOPA (Brefel-Courbon et al., 2005; Schestatsky et al., 2007). The alterations in muscular tonus might be a contributing factor for painful manifestations in these patients. However, the decreased availability of DA alone seems to be detrimental as PD patients with pain have a lack of habituation of sympathetic sudomotor responses to repetitive laser-induced pain stimuli, which reveals an abnormal control of the effects of pain inputs on autonomic centers.

In the opposite side, schizophrenia patients, which according to DA hypothesis have an overactivation of D_2R (Seeman, 1987), present a diminished response to pain (Dworkin, 1994; Potvin & Marchand, 2008), which is independent of their medication (Potvin & Marchand, 2008) and disease side-effects, since it is also present in schizophrenia patients' families (Hooley & Delgado, 2001).

The role of DA in pain modulation and/or perception has been confirmed by other studies in healthy human subjects. For instance, the injection of the DA receptors agonist apomorphine, decrease the amount of pain experience during a noxious cold stimuli when applied simultaneously with a heat noxious stimulus in other body part, implicating DA in pain inhibition (Treister, et al., 2013). Moreover, Positron Emission Tomography (PET) studies show that D2/D3 Binding Potential (BP), in STR, decreases after pain induction (Scott, et al., 2007) and that this decrease correlates with pain threshold and low modulatory capacity (Hagelberg, et al., 2002;

Pertovaara, et al., 2004; Scott, et al., 2007). Which suggests i) an increase in DA release after pain stimulation and ii) that DA plays an inhibitory role in pain modulation. Also, genetic studies reveal that pain sensitivity is related with specific polymorphisms in the DA transporter 1 (DAT-1), DA degradation enzymes Monoamine Oxidase-A (MAO-A) and Catechol-O-methyl transferase (COMT) and DA receptors D_3R and D_4R genes (Diatchenko, et al., 2005; Potvin, et al., 2009; Treister, et al., 2009). More specifically, it appears that people with polymorphisms which lead to lower DA levels, present higher sensitivity to pain.

Animal studies have also helped clearing the role of DA in pain modulation. Prolonged tail pinch stimulation in rats induce an increase of at least 25% in NAcc DA (Louilot, et al., 1986) indicating, like in humans, a DA release after pain stimuli. Similarly, apomorphine intrathecal administration in rats lead to a dose-dependent increase of nociceptive behavior responses latency in the hot plate and acetic acid tests, which was inhibited by the previous administration of DA but not NA, 5-HT or opioid antagonists (Jensen & Yaksh, 1984). The same authors, however, observed no differences in tail flick latencies in any of the tested doses, which suggest that DA antinociceptive effect bares some specificity.

Antinociception in formalin-induced responses was produced after chemical inhibition of DA (and NA) reuptake (i.e. increased synaptic DA availability) either systemically (Basile & Janowsky, 2007) or directly in the Rostral Agranular Insular cortex (RAIC) (Burkey, et al., 1999), reinforcing once again the importance of dopamine release in nociceptive modulation. Finally, 6-Hydroxydopamine (6-OHDA) lesions in SN and/or VTA, which completely depletes DAergic (and NAergic) inputs to the frontal circuitry, decrease latency to nociceptive behavior in rats and increase mechanical and thermal allodynia (Chudler & Lu, 2008; Dieb, et al., 2014; Saadé, et al., 1997; Zengin-Toktas, et al., 2013).

Both, human and animals studies, reveal that DA involvement in pain modulation is dependent of its receptors as the observed antinociceptive effects are mainly mediated via D_2R-like receptors and D_1R-like agonists and antagonists apparently do not have an impact in the animals nociceptive responses (Magnusson & Fisher, 2000; Mansikka, et al., 2005; Sheng, et al., 2009). DA receptors distribution in the brain can, for that reason, help clear the incongruities found in respect to the antinociceptive role of DA.

1.2.3 Dopamine and Chronic Pain

Besides its clear involvement in acute pain modulation, there are evidences of dysregulation in DAergic system in chronic pain patients and animals models. PET studies in

chronic pain patients show decreased presynaptic uptake of 6-[18F] fluorodopa and higher D_2R BP suggesting therefore reduced levels of DA (Jääskeläinen et al., 2001; Wood et al., 2007). Indeed, DA or its metabolites are diminished in the urine and Cerebrospinal Fluid (CSF) of chronic pain patients when compared with healthy controls (Legangneux et al., 2001; Riva, et al., 2012).

DA release after a painful stimulus, which correlates with perceived pain in healthy subjects, is altered in chronic pain conditions (Wood, et al., 2007) revealing impairments in DAergic reaction to pain stimuli. Furthermore, administration of Bupropion, a NA-DA reuptake inhibitor with antidepressive effects, decreased pain in chronic neuropathic pain patients (Moreira, 2011; Shah & Moradimehr, 2010).

In animals, injections of DA in ACC lead to a dose-dependent decrease in autotomy behavior in a rat model of chronic pain (López-Avila, et al., 2004). As in the acute pain, D_2R seem to play a major role in chronic pain since activation of this receptors in RAIC and STR induce antinociception in a neuropathic model, and in contrast, its inhibition was nociceptive (Ansah, et al., 2007; Coffeen, et al., 2008). In inflammatory and neuropathic pain animal models, a decrease in the nociceptive threshold was, just like in humans, observed after administration of Bupropion (Basile & Janowsky, 2007).

1.3 Rational and aims

Nowadays, it is accepted that the emergence of emotional and cognitive alterations in chronic pain patients and animal models is the result of continued pain. However, the literature suggests that pain alone, might not entirely explain the appearance of these comordibities. For instance, Dimitrov and colleagues recently reported the presence of emotional deficits in mice in the absence of pain manifestations. In this study, the cuff model was applied in the left sciatic nerve of C57BL/6J mice. When the cuff was removed 3 weeks later in a subgroup of animals, pain resolved but anxiety and depressive-like behaviors manifested at the same levels as the cuff group (Dimitrov, et al., 2014). Also, Ren and colleagues reported that right-sided SNI animals presented WM deficits whether or not allodynia manifested (Ren, et al., 2011). Secondly, as stated before, there is a temporal mismatch between the manifestation of pain, which starts immediately after the neuropathic lesion and the behavioral alterations, which take at least 2 weeks to emerge, suggesting a segregation between the sensory and the emotional/cognitive deficits (Suzuki, et al., 2007; Yalcin, et al., 2011). Furthermore, chronic pain in the right or in the left side of the body is associated with different emotional outcomes, although pain scores do not

differ. Men with left-sided pain show higher levels of anxiety and depression and lower quality of life than men with right-sided pain (Wasan, et al., 2010) and in an animal model of neuropathic pain, rats with lesion in the left paw show emotional deficits but no alterations in cognition and animals with lesion in the right paw display only cognitive problems (Leite-Almeida, et al., 2012). To test the hypothesis that pain is not needed to the establishment of emotional and cognitive deficits we decided, in a first moment, to study the behavior of SNI animals with and without pain.

The second part of the thesis, in turn, will focus on the biochemical alterations underlying the initiation and maintenance of chronic pain and its comorbidities. DA appears here as an appealing target since, as explained in the previous section, DAergic system is dysregulated in chronic pain conditions. Besides that, it has a pivotal role in WM, attention and decision-making, i.e. in behavioral domains affected by chronic pain, and it is involved in depression (Brown & Gershon, 1993; Cools & D'Esposito, 2011; de Wit, et al., 2012; Rogers, 2011; Savitz, et al., 2006). Finally, there are evidences of lateralization of the DAergic system in humans and rats (Larisch, et al., 1998; Molochnikov & Cohen, 2014; Nowak, 1989.; Schneider, et al., 1982; Tomer, et al., 2014; Vernaleken, et al., 2007).

Previous unpublished results from our lab showed that after habit induction rats lesioned in the left side are incapable of recognizing reward devaluation, indicating an impaired transition from habit-based to goal-directed behaviors in these animals. Once again, it is very likely that DA plays a role in this behavior.

Considering all of these, this master thesis intends to:

- Evaluate the emotional and cognitive behavior of right- and left-sided injured rats in painful and non-painful conditions

- Assess the state of the DAergic system in chronic pain conditions, considering brain hemisphere and lesion side.

- Study the impact of lateralized DA depletion in decision-making and impulsivity.

2. MATERIALS AND METHODS

2.1 Behavioral performance in painful and non-painful neuropathic conditions

2.1.1 Study-design

Two independent experiments were executed according to the study-design present in Fig. 3. A battery of behavioral tests (see 2.1.5) was performed 30 days after the installation of the SNI neuropathic lesion (see 2.1.3) and pain was measured with Von Frey (VF) monofilaments in three time points. The order of the behavioral tests was design to minimize possible cross negative impacts between tests on subsequent tests. The VDS was performed at the end of the battery as it requires food deprivation.

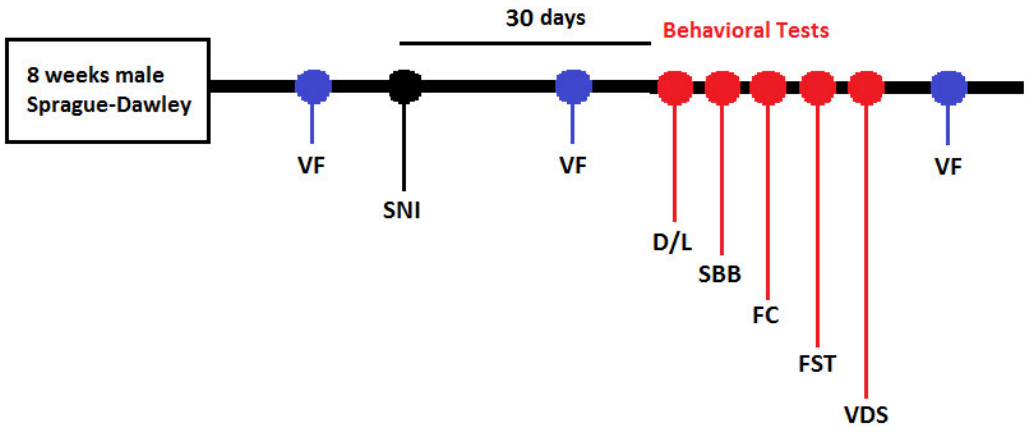

Figure 3. Schematic organization of the study 1 timeline. Behavioral tests started 30 days after SNI installation. The manifestation of allodynia was assessed 3 times: prior to SNI; after SNI pre- and post-behavior. VF=Von frey test, SNI= Spared Nerve Injury, D/L= Dark/Light test, SBB= Spontaneous Burrowing Behavior, FC= Fear conditioned, FST=Forced Swimming Test, VDS= Variable Delay-to-Signal

2.1.2 Animals

Two months-old male Sprague-Dawley rats were used. All of the procedures were made according to the guidelines of the European Communities Council Directive 2010/63/EU. Animals were housed in groups of three in a 12h light/dark cycle (lights on at 8 am) with controlled temperature (21±1°C) and humidity (50-60%). Rats had food (4RF21; Mucedola SRL, Settimo Milanese, Italy) and water *ad libitum*, except during the Variable Delay-to-Signal (VDS) test, in which food availability/consumption was restricted to the first hour of the circadian light cycle. Handling was done at least once a week until the start of behavior tests, beginning 2 weeks before surgery. Body weight was controlled along the experience.

2.1.3 Spared Nerve Injury (SNI)

Chronic neuropathic pain was induced using the SNI model, in the right (SNI-R) or left (SNI-L) hind paw, as described by Decosterd and Woolf (Decosterd & Woolf, 2000). Rats were anesthetized via intraperitoneal administration of 1:1.5 mix (1 ml/kg) of Dorbene® Medetomidine Hydrochloride) and Imalgene® (Ketamine), respectively. A blunt incision was then performed to expose the three branches of sciatic nerve: common peroneal, tibial and sural nerves. The first two nerves were individually ligated with a 4-0 suture (Silk Suture Thread, FST, Heidelberg, Germany) and distally cut; great care was taken to avoid lesions to sural nerve (spared nerve). In sham animals the nerves of right or left paw were exposed but left intact. After surgery muscle and skin were sutured separately in two layers, with 5-0 and 2-0 sutures (Coated vicryl braided absorbable suture, Johnson & Johnson, Inc., New Jersey, USA), respectively. 150µl of Antisedan® (Atipamezole Hydrochloride) were injected subcutaneously to reverse anesthesia and animals were then left to recover in their cages. One animal of each group (SNI-L, SNI-R and Sham) was present in all cages. Animals were monitored in the following days for open wounds and signs of inflammation. No major problem was observed in the study.

2.1.4 Pain behavior assessment

Measurement of allodynia was done using the up-and-down method with VF monofilaments test (Chaplan, et al., 1994). On the day before the first measurement, animals were habituated to the experimental setting, which consisted in an elevated mesh wire (approximately 50 cm).During habituation rats were left in the mesh for 3 minutes with their movement restricted by a plastic box (25 L x 15 W x 15 H) perforated to allow air exchange. In the test days, each animal was left alone for at least 30 seconds in the mesh. After that, spontaneous paw flicks (SPF) were scored for 2 minutes, without any interference. In the end, VF was done, as described by Chaplan and colleagues (Chaplan, et al., 1994). In this experiment, 8 monofilaments ranging from 0.4 to 15 g were used. The test started with the stimulation of the lesioned paw with the central monofilament (2 g). If the animal withdrawed the paw, a weaker monofilament was used, if not, a stronger one was applied. Stimulation was done until the animal responded to the 0.4 g, did not respond to 15 g monofilaments or after a total of 6 measures around threshold first crossing. 50% threshold was calculated using the formula:

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

in which Xf is the value (in log units) of the final monofilament used, K is the tabular value for the pattern of positive/negative responses and δ is the mean difference between stimuli (in this case it is equal to 0.224).

2.1.5 Behavioral tests

Behavioral tests started 4 weeks after SNI lesion and were done during the dark phase of the circadian cycle.

i) Dark/Light (D/L)

The D/L apparatus consists in a square arena (43.2x43.2 cm) in which half is brightly illuminated and the other half consists in a dark compartment (MedAssociates Inc. model ENV-515). Animals in the D/L face a conflict between staying in the close and safe compartment (dark) and the innate drive to explore a new and exposed environment (Crawley & Goodwin, 1980). Anxiety-like behavior in this paradigm manifests in higher D/L ratios. In the beginning of the test animals were placed in the illuminated area and left to explore the arena for 10 minutes. Infrared beams at the floor level allow to automatically monitor animal's position. Time and distance spent in each area were calculated using Activity Monitor software (MedAssociates, Inc.). After each animal the arena was cleaned with 10% ethanol.

ii) Spontaneous Burrowing Behavior (SBB)

SBB evaluates the general well-being of rodents and it was also described as a good correlate of ongoing pain (Andrews, et al., 2012). It is based on the assumption that burrow is an ethologically relevant behavior in rodents and therefore alterations in SBB could signal disease and/or pain. The SBB protocol employed was the same described by Deacon (Deacon, 2006), except for some minor alterations as described. The SBB test was done in rats housing cages (without bedding) reserved for this sole propose. Each rat performed the test in the same location and in the same apparatus along the testing days. In the first day of habituation, empty PVC tubes (diameter: 90 mm, length: 210 mm) with one end closed were left in the rats' home cages for a period of 24 hours. In the second day rats were putted in pairs in the SBB cages for 2 hours (1h with each cage mate); in this case the open end of the tubes were elevated 70 mm above the cage floor and each tube was filled with approximately 2 kg of gravel. On days 3, 4 and on test day each animal was placed alone in SBB cages for 2h after which amount of displaced (burrowed) gravel was weighted. Results are shown as the percentage of

initial gravel burrowed by the animals in test day. During the sessions no food or water was available in the SBB cages.

iii) Forced Swimming Test (FST)

To evaluate learned helplessness behavior the FST was done. Animals that spend more time immobile during the test are described as having a more depressive-like behavior (Porsolt, et al., 1977). Rats were placed in a glass cylinder filled with water (21-23°C) 30 cm deep, from which they cannot escape, and left there for 5 minutes. Two sessions in subsequent days were performed and the second was recorded for posterior behavioral scoring by a blind researcher using Etholog V 2.2 software (Ottoni, 2000). Immobility, struggling, swimming and latency times were calculated.

iv) Fear Conditioning (FC)

FC was done in a startle response box (SR-LAB™, San Diego Instruments, San Diego, CA, USA) with a non-restrictive Plexiglas cylinder (diameter 8.8 cm, length 22.2 cm) and a steel grid through which an electric current could be passed.

FC protocol lasted for three consecutive days. In the first day (habituation) each animal was placed in the FC box for 11 minutes. In the second day (conditioning) each rat was left in the box for 11 minutes divided in 3 minutes without the stimulus and 8 minutes with 6 pairs of light/ shock (0.4±0.1 mA), with an inter-stimulus interval of 60 seconds. Light was on for 20 seconds and the shock was given immediately after light was turned off. In the last day (test day) the protocol was equal to the day before but no shock was given to the animal. Videos were recorded and freezing time analyzed in Etholog V 2.2 software (Ottoni, 2000). Freezing was defined as the complete absence of voluntary movements, except for respiratory movements.

v) Variable Delay to Signal (VDS)

VDS was done in a 5-hole box (TSE-systems, Bad Homburg, Germany) as described by Leite-Almeida and colleagues (Leite-almeida, et al., 2013). The apparatus consists in a box with five holes in one side and a food magazine connected to a pellet dispenser on the opposite side. A total of 15 sessions were performed, two per night, including habituation, shaping and the VDS session, as explained below.

In the first night, rats were left exploring the box for 30 minutes. All the holes were closed and lights off. Sugared pellets (dustless precision pellets ® 45 mg, Bio-Serv) were left in the food magazine. In the second night of habituation, animals explored the box for 15 minutes. In these 2 sessions the central hole was open and 2-3 sugared pellets were also presented there. Food magazine, central hole and house lights were on during the entire session. 10 training sessions then followed. Each session started with the delivery of a sugared pellet in the food magazine and with the house-light on. Then, after a 3 seconds delay, the light in the central hole was switched on (for 60 seconds). If the animal nosepoked in that time (correct nosepoke) it was rewarded with a pellet. But if the animal nosepoked during the 3 seconds delay period when the light was off (premature response) or if it did not nosepok (omission) it was punished with 5 seconds in complete darkness and no reward was delivered. Each session ended after 100 complete trials or 30 minutes. Premature responses, correct nosepokes, omissions and perseverant responses were recorded. Correct nosepokes during the shaping protocol allow the study of learning capacity. In the VDS test proper (15th session), a total of 120 trials were performed, consisting of 25 trials of 3 seconds delay in the beginning and in the end flanking 70 trials of 6 or 12 seconds delay (randomly distributed by the computer). Contrary to the shaping session, during the VDS animals are allowed to perform premature responses; these are registered but not punished. Prematurity rate – number of impulsive responses per time of available delay – was used to measure impulsivity.

2.1.6 Euthanasia

After behavior tests rats were euthanized. First they were anesthetized with an intraperotoneal injection of Eutasil ® (Pentobarbital). Then the sciatic nerve from both back paws was removed and stored at -80°C. Following perfusion with paraformaldeyde (PFA) 3% in PBS, brain and spinal medulla were removed, submersed in Richard-Allan Scientific™ Neg-50™ Frozen Section Medium (Thermo Fisher Scientific, Inc., USA) and stored for posterior analyzes.

2.2 Dopaminergic system in chronic pain conditions

2.2.1 Animals

Two months-old male Wistar-Han rats were used in this experimental set. Husbandry conditions were as described in 2.1.1.

2.2.2 SNI lesion and pain assessment

SNI lesion and pain assessment were performed following the procedures described in sections 2.1.3 and 2.1.4, respectively. Except for handling 1-2 times a week animals were left undisturbed during 1 month starting at SNI installation.

2.2.5 Euthanasia

Thirty days after SNI animals were euthanized. Euthanasia was done in a room of the animal facilities to decrease the time required for the procedure and minimize possible stress-related neurotransmitter fluctuations. A single cage at a time was brought to the room and between cages, sham, SNI-L and SNI-R animals were sacrificed by decapitation in alternate sequences to avoid biases, in a total time not superior to 2 minutes. The skull was then submerged in liquid nitrogen during 8 seconds and the brain immediately removed and placed in a cold brain slicer. 1 or 2 mm slices, depending in the areas of interest, were obtained. These were placed in a clean and cold Petri dish and mPFC, OFC, STR and NAcc from both hemispheres were macrodissected and immediately stored at -80°C.

2.2.6 D_1R and D_2R mRNA quantification

i) Ribonucleic acid (RNA) extraction and quantification

RNA extraction was done using Trizol® Reagent (Life technologies) according to the manufacturer instructions. 1 mL of Trizol reagent was added to the samples (in ice) and homogenization was done with a 23G needle. After homogenization, 200 µl of clorophorm was added, the tubes were manually shaken for 15 seconds and the mixture was kept 2-3 minutes at room temperature (RT). After that period, samples were centrifuged 15 minutes at 8000 rotation per minute (rpm) at 4°C. The upper phase containing the RNA was then removed to a new eppendorf and RNA was precipitated with 500 µl of isopropanol. After 10 minutes of incubation at RT, samples were centrifuged at 9000 rpm during 10 minutes at 4°C. Then, the supernatant was removed and the pellet was washed with 100 µl of ethanol 70%. Samples were centrifuged for 7 minutes at 5000 rpm at 4°C. Finally, ethanol was removed and the pellet was eluted in milliQ H_2O. After RNA extraction, RNA in each sample was quantified using a nanodrop spectrometer (Nanodrop Technologies, Inc., ThermoScientific, Wilmington, USA). 260/280 ratio of all the samples was higher than 1.8, ensuring its purity.

ii) DNase treatment and cDNA synthesis

In order to eliminate genomic DNA from the samples a treatment with DNase was done before cDNA synthesis. 1 µl of DNase and other of DNase buffer were added to 1 µg of RNA from each sample diluted in 10 µl of RNase free water. Samples were left 30 minutes at 37°C, the ideal temperature for DNase activity. 1 µl of EDTA was then added to stabilize RNA and samples were incubated for 10 minutes at 65°C, to inactivate DNase. cDNA was synthesized with iScript ® kit (Biorad) according to the manufacturer instructions. 4 µl of 5x iScript ® reaction mix and 1 µl of iScript ® Reverse Transcriptase were added to each sample and submitted 5 minutes at 25°C, 60 minutes at 42°C and 5 minutes at 85°C in a thermal cycler (MWG Biotech Inc. Primus 96 Thermal Cycler). cDNA was stored at -20°C.

iii) Quantification Polymerase Chain Reaction (qPCR)

D_1R (Primers: Forward- 5- TCC TTC AAG AGG GAG ACG AA -3; Reverse- 5- CCA CAC AAA CAC ATC GAA GG -3) and D_2R (Primers: Forward- 5- CAT TGT CTG GGT CCT GTC CT-3; Reverse- 5- GAC CAG CAG AGT GAC GAT GA-3) gene expression was assessed in qPCR using Evagreen ® (Bio-Rad) reagent. GAPDH (Primers: Forward- 5- AGC CTC GTC TCA TAG ACA AGA TGG T -3; Reverse- 5- AGG TGA GCC CCA GCC TTC TCC -3) was used as the control gene. qPCR reaction (1 minutes 95°C and 40 cycles of 15 seconds at 95°C, 20 seconds at 60°C and 20s at 72°C) was done using the CFX96™Real-Time System (Bio-Rad). Transcript levels were calculated, by comparison, using the formula $2^{(-\Delta\Delta_{Ct})}$. A laterality index ($2^{(-\Delta\Delta_{Ct})}$ left - $2^{(-\Delta\Delta_{Ct})}$ right/ ($2^{(-\Delta\Delta_{Ct})}$ left + $2^{(-\Delta\Delta_{Ct})}$ right) was used to evaluate the relative expression of the receptors.

2.3 Habit and impulsivity in 6-OHDA lesioned animals

2.3.1 Animals

A pilot study was conducted using 8 male Sprague-Dawley rats with 6 months. Husbandry conditions were as described in 2.1.1 except for food that was only available in the last hour of the light phase of the cycle. Body weight was controlled along the experience.

2.3.2 6-OHDA Lesions

Rats were anesthetized by intraperitoneal administration of a 2:3 mix (1 ml/kg) of Dorbene® (Medetomidine Hydrochloride) and Imalgene® (Ketamine), respectively. Then they were fixed in a stereotaxic frame and 2 µl of 6-OHDA (at 2 or 4 µg/µl) were unilaterally injected

in the medial forebrain bundle (AP=-4.4; ML= 1 ou –1; DV=-7.8). After surgery, animals were sutured, injected subcutaneously with 150 µl of Antisedan® (Atipamezole Hydrochloride) to reverse anesthesia and left to recover in their home cages for 3 weeks.

2.3.3 Habit behavior

Decision-making study was done in a skinner box (MedAssociates Inc.; ENV-467). During the test only one lever (side was randomized across experimental groups) was available and reinforcement – a sugar pellet (dustless precision pellets ® 45 mg, PHYMEP, Paris) or 0.1 ml of 20% sucrose – was delivered into a food magazine localized between the two levers. After 2 days of habituation to the operant box, animals were subjected to 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 13 day of RR-20, where the probability of receiving a reward was 20, 10 and 5% per lever press, respectively (Fig 4). Each session ended after 30 reinforcements or 30 minutes. Sucrose 20% was the reinforcement in all sessions, except in the last 6 RR20, where sugar pellets were used.

Animals were tested in a reversal devalue paradigm after the 1st, 7th and 13th RR20 session (Fig 4). On the first day of test (devalue day), the animals were given *ad libitium* access to the reinforcement learned in the previous days during 1 h, after which rats were subjected to a 5 minutes session in extinction (i.e. the lever was presented but no reward was delivered). On the the second day, the same procedures were executed except that the reinforcement available was the opposite of the 1st day (valuation). Number of lever presses, nosepokes and session time were recorded by MED-IV software.

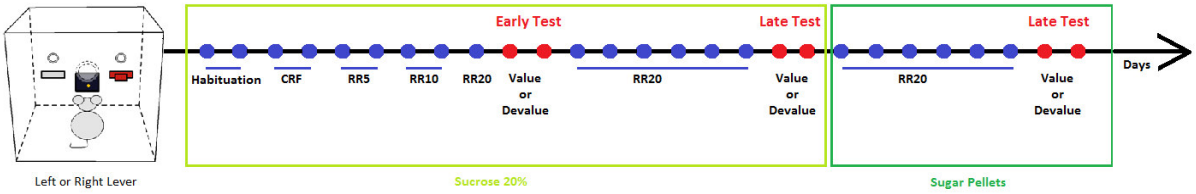

Figure 4- Habit-behavior paradigm schematics. Early (before habit induction) and late (after habit induction) devaluation tests evaluate if animal behavior is goal direct or habit based. CRF= Continuous reinforcement schedule; RR=Random Ratio.

2.3.4 VDS

VDS test was done as described in section 2.1.5 vi.

2.3.5 Euthanasia

Euthanasia was performed as described in section 2.1.6, except neither nerves nor spinal medulla were removed.

2.4 Statistical analysis

All statistical analyses were done in IBM SPSS Statistics 22 (IBM software, Inc., New York, USA) and graphs in GraphPad prism 5.0 (GraphPad software, Inc., La Jolla, CA, USA). Extreme outliers (lower than Q1-3x (Q3-Q1) and higher than Q3-3x (Q3-Q1); being Q1 and Q3, 1st and 3rd quartile, respectively) were excluded, at a maximum of two by group, in all the analyzed parameters. To assess if data followed a normal distribution Shapiro-Wilk test was used. Equality of variances was tested with Levene's test. Comparisons between groups were done using one-way ANOVA in groups in which normality could not be rejected and Kruskal-Wallis in groups in which normality was rejected. Bonferroni or Games-Howell' tests were used as multiple comparison post hoc tests when equality of variance was assumed or not, respectively. Comparisons between groups of more than one level were done using two-way ANOVA if normal distribution was not rejected. On the contrary, Mann-Whitney's test was done considering each factor individually. Learning and weight evolution was compared recurring to repeated-measures test and correlation analysis was done by Pearson's test. Data were considered significant if $p < 0.05$. All results are represented as mean ± SD.

.

3. RESULTS

3.1 Behavioral performance in painful and non-painful neuropathic conditions

The intention of this first task was to study and compare the behavior of neuropathic allodynic and non-allodynic rats in a battery of emotional and cognitive paradigms. Accurate pain assessment and posterior groups definition was therefore essential to the work. Previous studies from the group demonstrated a high correlation ($r=0.929$, $p<0.001$) between the measures of mechanical allodynia in the VF test performed by two independent investigators. Therefore, to avoid unnecessary distress to the experimental subjects caused by repetitive probing with VF monofilaments, this test was done by a single investigator. In addition to evoked pain, SPF were also monitored. Prior to the SNI surgery none of the animals presented allodynia. After SNI surgery, VF 50% threshold and SPF correlated significantly in the two moments of observation prior and after the behavioral battery ($r=-0.474$, $p<0.001$; $r=-0.467$, $p<0.001$; respectively) (Fig 6 A and B). SNI animals were then divided in left-lesioned allodynic (All-L), right-lesioned allodynic (All-R), left non-allodynic (N-All-L) and right non-allodynic (N-All-R) considering low and high threshold groups – 50% threshold lower and higher than 5 g, respectively. However, it was observed that in a small number of individuals the phenotype was not stable between the two observations and therefore the low and high threshold groups presented minor variations (Fig 6 C-F). Such was an unexpected result based on the present literature (De Felice, et al., 2011; Ren, et al., 2011) and will be discussed in the following section. For this reason, behavioral results in the D/L, SBB, FST, FC and VDS will be presented considering both the VF measurements obtained pre- and post-behavior.

From the initial 87 SNI-operated rats, 26% and 18%, based on pre and post-behavior VF, respectively, were considered non-allodynic (Fig 5). No side susceptibilities were observed considering allodynia, since non-allodynic animals are equally distributed by N-All-L and N-All-R.

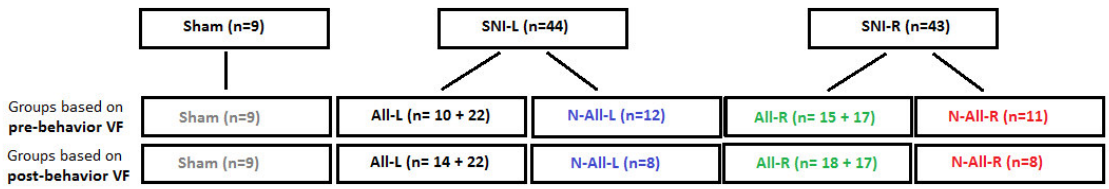

Figure 5. Animals distribution by experiemtnal group. From the 87 animals lesioned 64 were allodynic (32 left+32right) and 23 non-allodynic (12 left + 11 right) considering pre-behavior based groups and 71 were allodynic (36 left+35right) and 16 non-allodynic (8 left + 8 right) considering post-behavior based groups. 39 allodynic animals were excluded from the behavioral analysis.

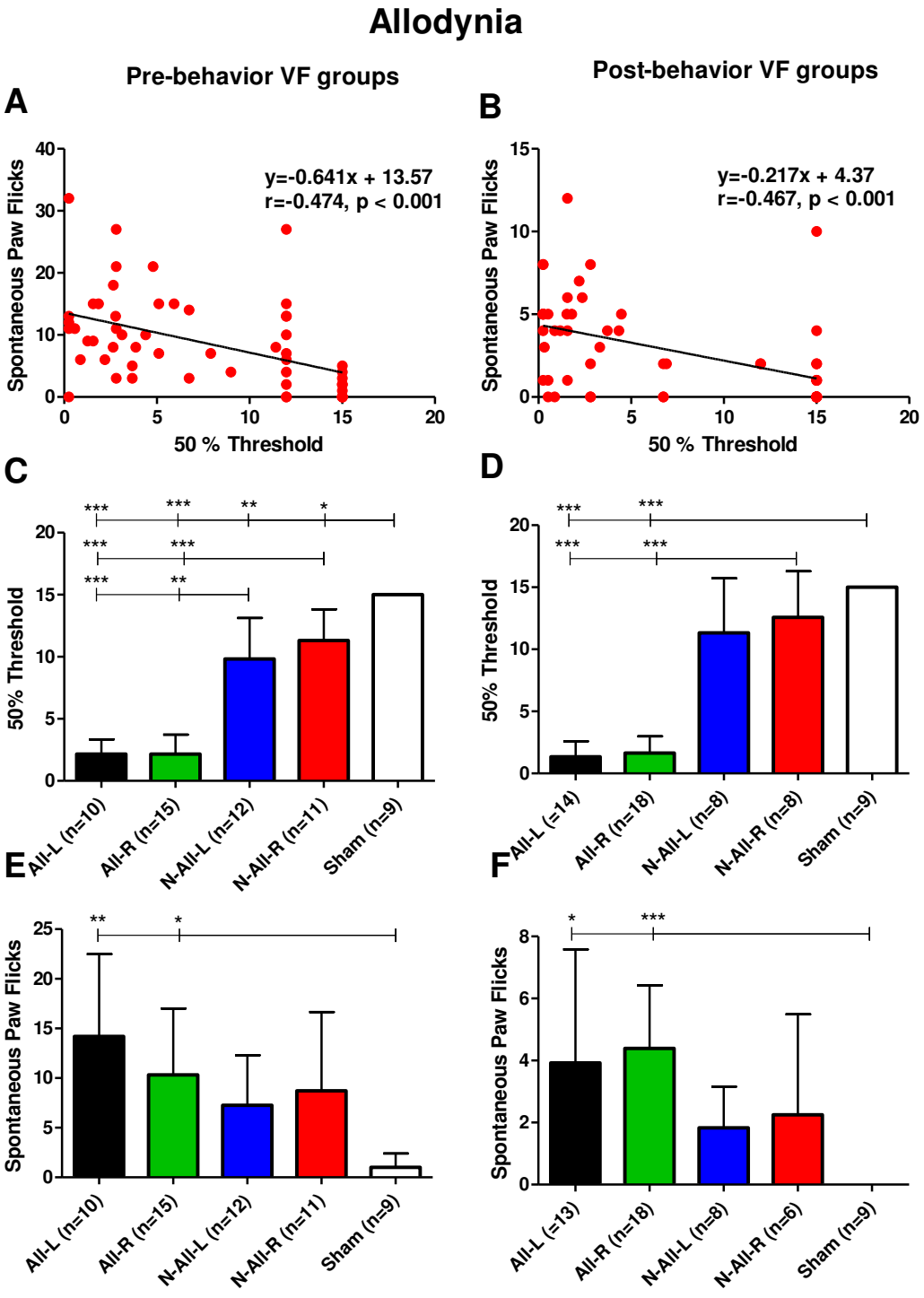

Figure 6 Mechanical and spontaneous allodynia. Correlation between the number of spontaneous paw flicks and mechanical allodynia measured by the Von Frey test pre (A) and post (B) behavior. Considering this, the groups were divided in allodynic (50% threshold <5) and non-allodynic (50% threshold >5) based on VF measurements. Mechanical allodynia of groups in pre (C) and post (D) behavior measures and spontaneous paw flick also pre (E) and post (F) behavior are shown. Data presented as mean ± SD, *p<0.05, **p<0.01, ***p<0.001

Two-way ANOVA analysis in pre and post-behavior groups reveal an effect of pain, and not side, on VF (Pre ($Z_{1,45}$= 150.46, p<0.001); Post ($Z_{1,46}$= 274.34, p<0.001)) and SPF measures (Pre ($Z_{1,45}$= 7.37, p=0.008); Post ($Z_{1,46}$= 16.54, p<0.001)). Posterior post-hoc analysis show differences between Sham and the other groups (Sham vs All-L (2.15 ± 1.2), p<0.001; Sham vs All-R (2.15 ± 1.5), p<0.001; Sham vs N-All-L (9.83 ± 3.3), p<0.001; Sham vs N-All-R (11.31 ± 2.5), p=0.014) and between allodynic and non-allodynic animals (N-All-R vs All-L, p<0.001; N-All-R vs All-R, p<0.001; N-All-L vs All-L, p<0.001; N-All-L vs All-R, p=0.001) (Fig 6C).

Posterior post-hoc analysis show differences between Sham and the other groups (Sham vs All-L (2.15 ± 1.2), p<0.001; Sham vs All-R (2.15 ± 1.5), p<0.001; Sham vs N-All-L (9.83 ± 3.3), p<0.001; Sham vs N-All-R (11.31 ± 2.5), p=0.014) and between allodynic and non-allodynic animals (N-All-R vs All-L, p<0.001; N-All-R vs All-R, p<0.001; N-All-L vs All-L, p<0.001; N-All-L vs All-R, p=0.001) (Fig 6C). In post-behavior groups post hoc test reveal differences between Sham and N-All-R (11.32 ± 4.4) and allodynic groups (Sham vs All-L (1.34 ± 1.2, n=14), p<0.001; Sham vs All-R (1.64 ± 1.4), p<0.001; N-All-R vs All-L, p<0.001; N-All-R vs All-R, p<0.001). No statistically significant differences were found between N-All-L (11.32 ± 4.4) and the other groups (Fig 6D).

Considering SPF, although non-allodynic animals have lower values than allodynic, there are only statistically significant differences between Sham and allodynic animals both in pre (Sham (1.00 ± 1.4) vs All-L (14.20 ± 8.3), p=0.003; Sham vs All-R (10.33 ± 6.7), p=0.013) (Fig 6E) and post-behavior groups (Sham vs All-L (3.92 ± 3.7), p=0.028; Sham vs All-R (4.39 ± 2.0), p<0.001) (Fig 6F).

Regarding animals body weight there are no differences between neither of the group sets along the experiment. Also, during FD none of the animals lost more than 15% of its body weight.

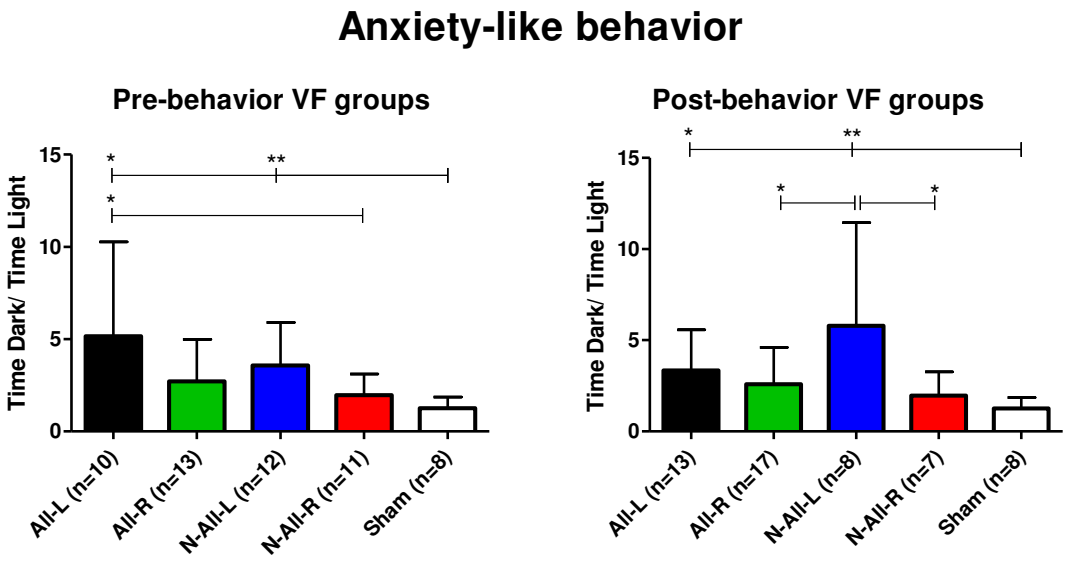

Figure 7- Anxious-like behavior in the Dark/Light test. All-L and N-All animals spend more time in the dark side than sham and right lesioned animals considering pre-behavior (A) and post-behavior (B) based groups, which reflects a more anxious-like behavior. All= Allodynic, N-All= Non-allodynic, L= lesion in the left paw, R= lesion in the right paw. Data presented as mean ± SD, *p<0.05, **p<0.01,

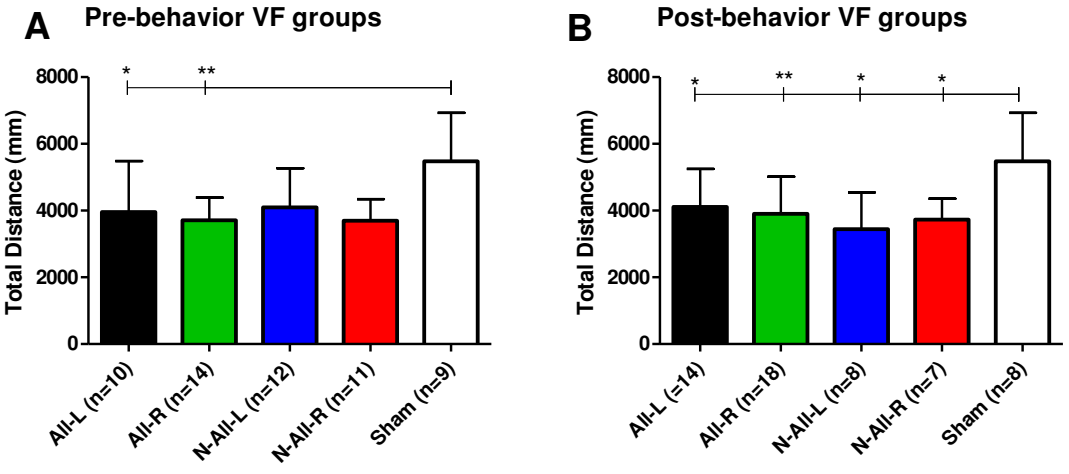

Figure 8- Total distance walked during Dark/Light test. Spared Nerve Injury animals walked less than sham animals considering pre-behavior (A) anc post-behavior (B) based groups, which could indicate motor impairments. All= Allodynic, N-All= Non-allodynic, L= lesion in the left paw, R= lesion ir the right paw. Data presented as mean ± SD, *p<0.05, **p<0.01,

Concerning D/L test, which evaluates anxiety-like behavior, a considerable influence of the lesion side and not of the pain was observed in pre (U_1 = 226.00, p=0.018) and post-behavior groups (U_1 = 219.00, p=0.024). Left lesioned animals are more anxious-like than sham and right-lesioned animals, independently of allodynia, in pre (Sham (1.26 ± 0.6) vs All-L (5.16 ± 5.1,), p=0.010; Sham vs N-All-L (3.57 ± 2.3), p=0.007; All-L vs N-All-R (1.96 ± 1.2), p= 0.041) (Fig. 7A) and post-behavior groups (Sham vs All-L (3.34 ± 2.2), p=0.014; Sham vs N-All-L (5.79 ±

5.6), p=0.003; All-R (2.59 ± 2.0) vs N-All-L, p= 0.045; N-All-L vs N-All-R (1.95 ± 1.3), p=0.049) (Fig 7B).

Total distance walked during D/L test was also analyzed. No differences were found between SNI groups but Sham walked more than SNI animals, which could indicate motor impairments. Games-Howell' test show difference between Sham and all the others post-behavior groups (Sham (5476.26 ± 1456.8) vs All-L (4109.21 ± 1143.4), p=0.023; Sham vs All-R (3901.68 ± 1112.1), p=0.006; Sham vs N-All-L (3445.97 ± 1097.2), p=0.013; Sham vs N-All-R (3728.48 ± 626.9), p=0.021) (Fig. 8B)but only for pre-behavior allodynic groups (Sham vs All-L (5476.26 ± 1456.8), p=0.034; Sham vs All-R (3710.51 ± 677.2), p=0.004) (Fig.8A).

The percentage of gravel burrowed during SBB was not, as described by others (Andrews et al., 2012), a good test to access pain, since there are no differences between allodynic and non-allodynic animals (Fig. 9). Instead an effect from lesion side appears to exist; SNI-L animals burrow less than SNI-R animals, independently of mechanical allodynia.

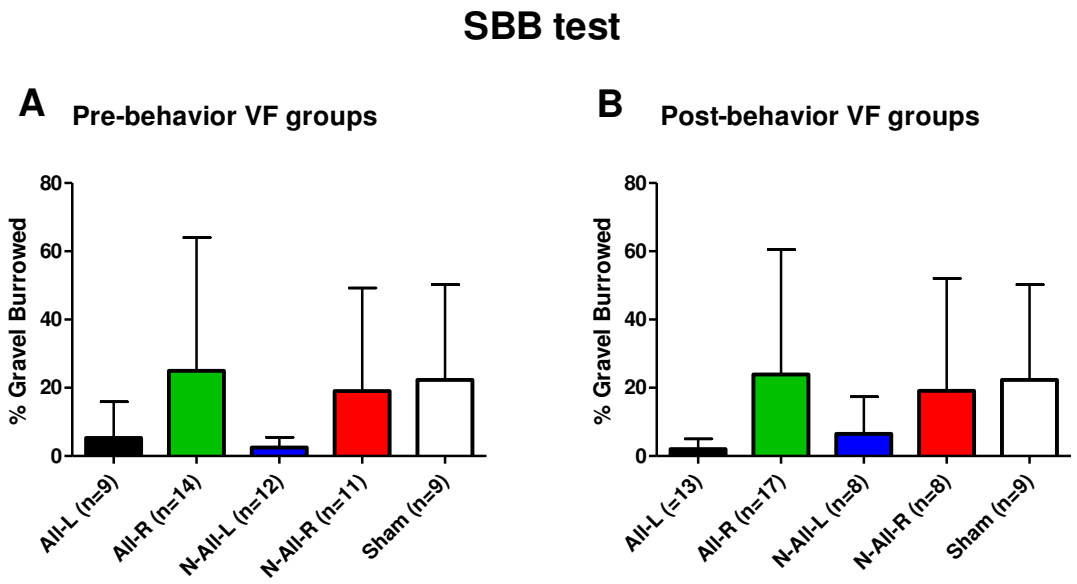

Figure 7- Percentage of gravel burrowed in Spontaneous Burrowing Behavior test. Allodynic and non-allodynic left lesioned animals show a tendency to burrow less gravel than sham and right lesioned animals considering pre-behavior (A) and post-behavior (B) based groups. All= Allodynic, N-All= Non-allodynic, L= lesion in the left paw, R= lesion in the right paw. Data presented as mean ± SD

Considering depressive-like behavior Sham animals (78.06 ± 32.1) spend more time struggling than All-L (45.25 ± 11.8), All-R (48.15 ± 18.5) and N-All-L (47.66 ± 22.3) pre-behavior groups. The same tendency is observed when we consider post-behavior groups (Fig.10). On the other hand, there are no observable differences between SNI groups, indicating an impact of lesion, and not pain or side, in depressive-like behavior establishment.

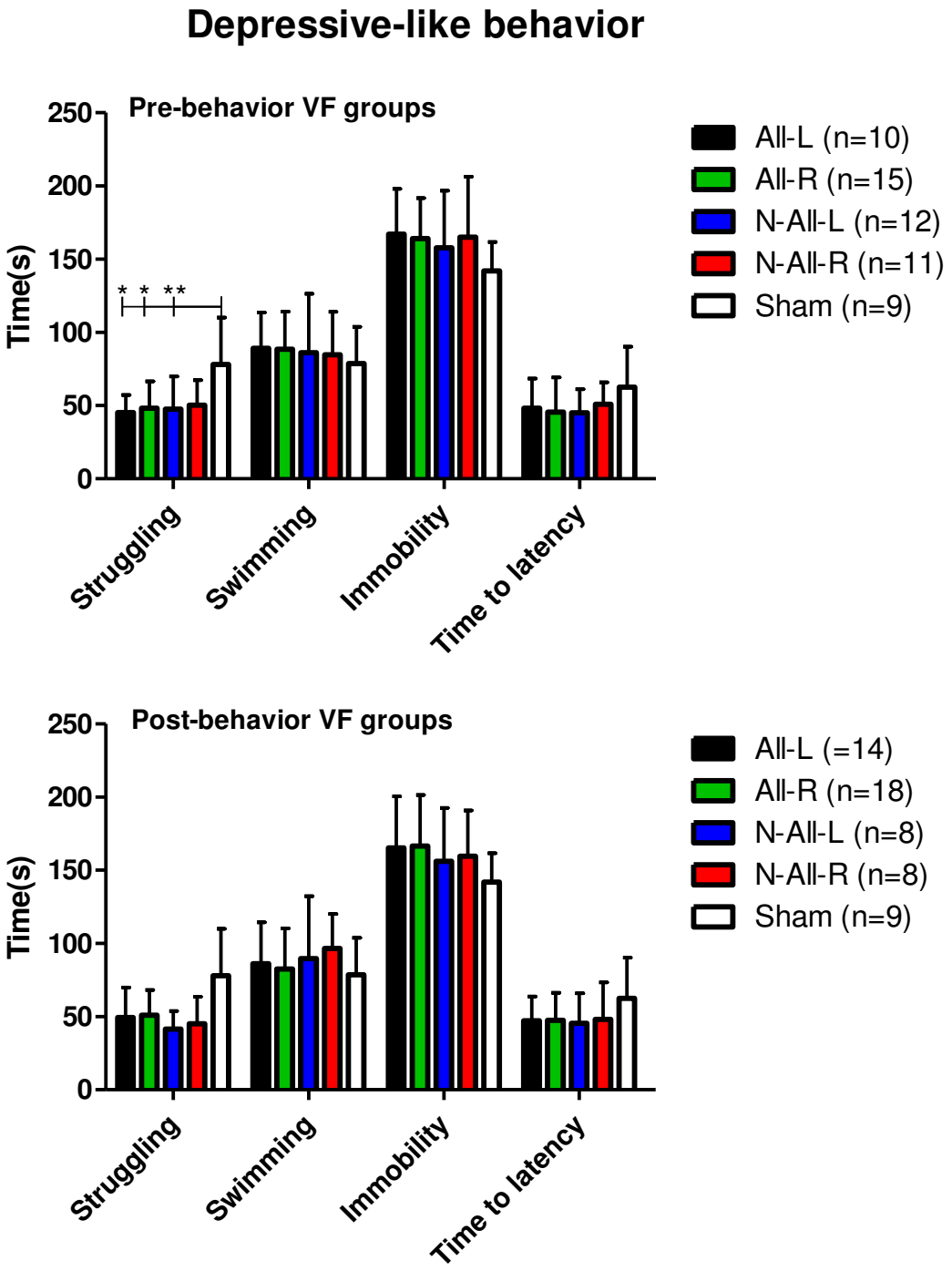

Figure 8- Depressive-like behavior evaluated by swimming, struggling, immobility and latency time in forced swimming test. Spared Nerve Injury animals spend less time struggling than sham animals considering pre-behavior and post-behavior based groups, which reflects a depressive-like behavior. Data presented as mean ± SD, *p<0.05, **p<0.01

No group differences were found between the number of nosepokes during VDS shaping sessions. However, in the first session Sham rats show a tendency to do more correct nosepokes than SNI animals (Fig 11A-B). In VDS test, pre-behavior N-All-R show more impulsive responses than other groups in 12 seconds delay (N-All-R (0.51 ± 0.3) vs Sham (0.12 ± 0.2), p=0.006; N-All-R vs All-L (0.11 ± 0.1), p=0.002) (Fig 11C). Looking at the results in the final 3 seconds delay,

which are the ones that correlate with impulsive behavior (Leite-almeida, et al., 2013), right-injured rats seem more impulsive than left-injured animals, in pre and post-behavior groups (Fig 11C-D).

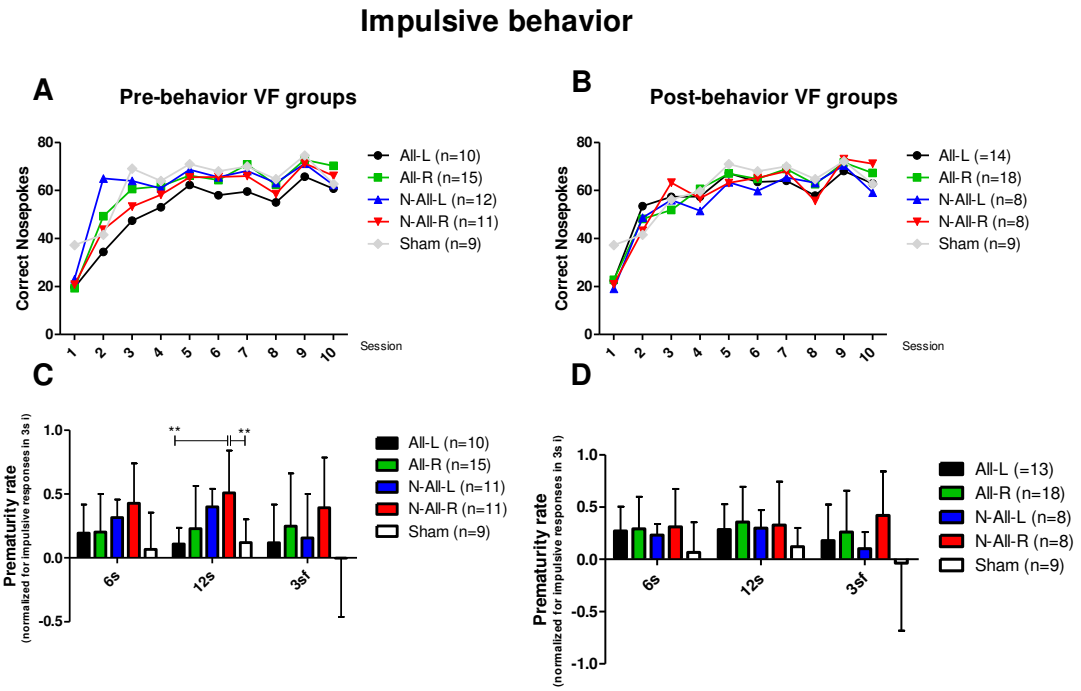

Figure 9- Learning and impulsivity evaluated by the Variable Delay to Signal test. No differences along the number of correct nosepokes along the shaping (training?) sessions were found, reflecting no decrease in learning/motivation capacity in none of the groups neither grouped based in pre (A) nor post behavior (B). At the final 3 seconds delay in VDS test, right lesioned animals show a tendency for increased number of premature responses than sham or left lesioned animals. Concerning 6 and 12 seconds delay... Data presented as mean ± SD, **p<0.01

3.2 Dopaminergic system in chronic pain conditions

To study the status of dopaminergic system in chronic pain conditions a quantification of D_1R and D_2R was done 30 days after neuropathic injury in SNI-L, SNI-R and Sham animals.

No differences were found between SNI-L, SNI-R and Sham body weights throughout the experiment. Regarding allodynia, both SNI-L (0.40 ± 0.2) and SNI-R (0.46 ± 0.6) present lower thresholds compared to Sham (10.09 ± 6.3) animals (SNI-L vs Sham (U=8.50, p=.019) SNI-R vs Sham (U=8.50, p=.001)) (Fig. 12).

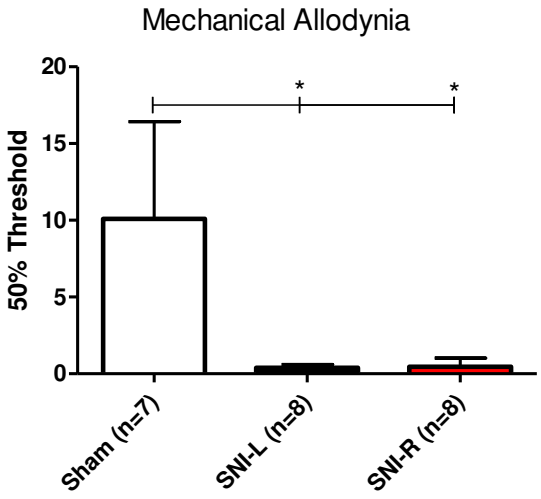

Figure 10. Mechanical Allodynia by the VF test. Sham animals are different from the animals lesioned in the left (SNI-L) and right (SNI-R) paw. Data presented as mean ± SD, *p<0.05

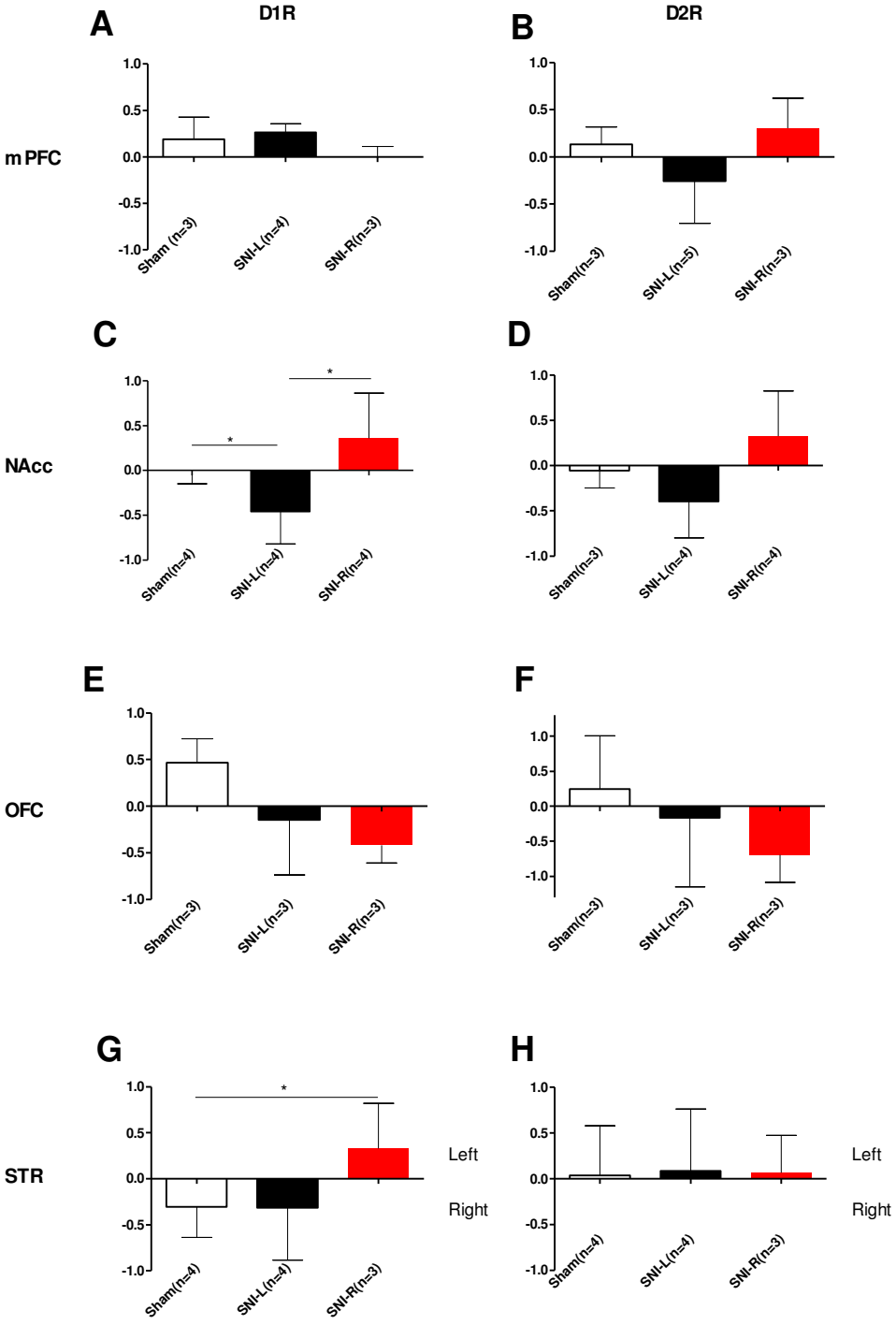

Figure 11- Laterality index of D_1R and D_2R mRNA expression in mPFC, NAcc, OFC and STR. Laterality index of NAcc receptors and D_1R of the mPFC increase to the contralateral side of the lesion. OFC receptors, favors always the right side, independently of lesion side. Data presented as mean ± SD, *p<0.05

mRNA expression of D_1 and D_2 receptors appears to be lateralized in sham animals, although laterality index is not statistically significantly different from 0. This lateralization or lack of it seems to be dependent of both DA receptor and brain nuclei. mPFC (D_1R - 0.19 ± 0.2; D_2R - 0.13 ± 0.2) (Fig 13A-B) and OFC (D_1R - 0.47 ± 0.2; D_2R - 0.24 ± 0.8) (Fig 13E-F) receptors have

a tendency to be more expressed in the left hemisphere than in the right one. In turn, D_2R in STR (0.04 ± 0.5) appears to have increased expression in the right when compared to left hemisphere (Fig 13H) and finally NAcc receptors (D_1R – -0.001 ± 0.1; D_2R - -0.006 ± 0.2) seem to be equally expressed in both sides (Fig 13C-D). With neuropathic lesions the expression of D_1R and D_2R is altered. In NAcc, both D_1R and D_2R, increase in the contralateral side of the lesion (Fig 13C-D). The same seems true for D_2R in mPFC (Fig 13B) and D_1R in STR (Fig 13G). On the other hand, mPFC D_1R increases in the ipsilateral side (Fig 13A). Concerning OFC receptors expression, it increases on the right side (or decreases in the left side), independently of side lesion (Fig 13E-F). D_2R expression in STR appears to remain the same (Fig 13H).

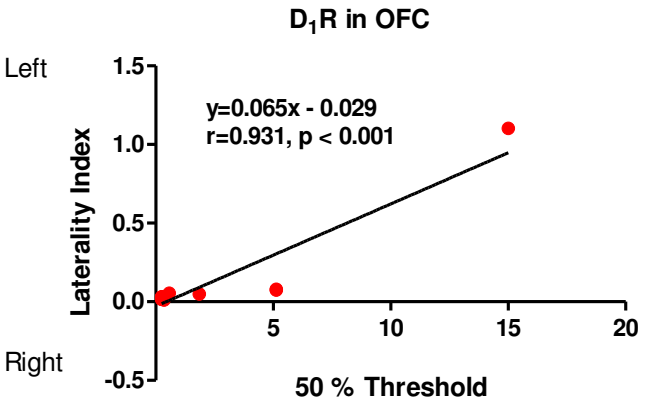

Figure 12- D_1R expression in left OFC and mechanical allodynia. Mechanical allodynia measured by the Von Frey test correlates with the expression of D_1R in the left OFC. This correlation is kept even when the observed outlier is excluded.

Correlation tests between mechanical allodynia and compared expression levels reveal a correlation between 50% threshold values and D_1R expression in the left OFC ($r=0.931$, $p<0.001$) (Fig 14). This correlation is kept even if we exclude the outlier ($r=0.891$, $p<0.001$). No other significant correlations were found.

3.3 Impact of lateralized dopamine depletion in decision-making

A pilot study to evaluate the impact of DA depletion in decision-making was done. Two different doses of 6-OHDA (4 µg and 8 µg) were injected in right or left hemisphere.

No differences were found in the animals' body weight during experimental protocols between lesion side or injected dose. In the days after surgery and during Food deprivation the body weight decreased but this loss was never superior to 15%.

Concerning the number of lever presses per minute along habit induction protocol no differences were found between right or left lesioned animals (Fig 15B) nor between the two injected doses of 6-OHDA (Fig 15A). However, some animals, with different side and dose

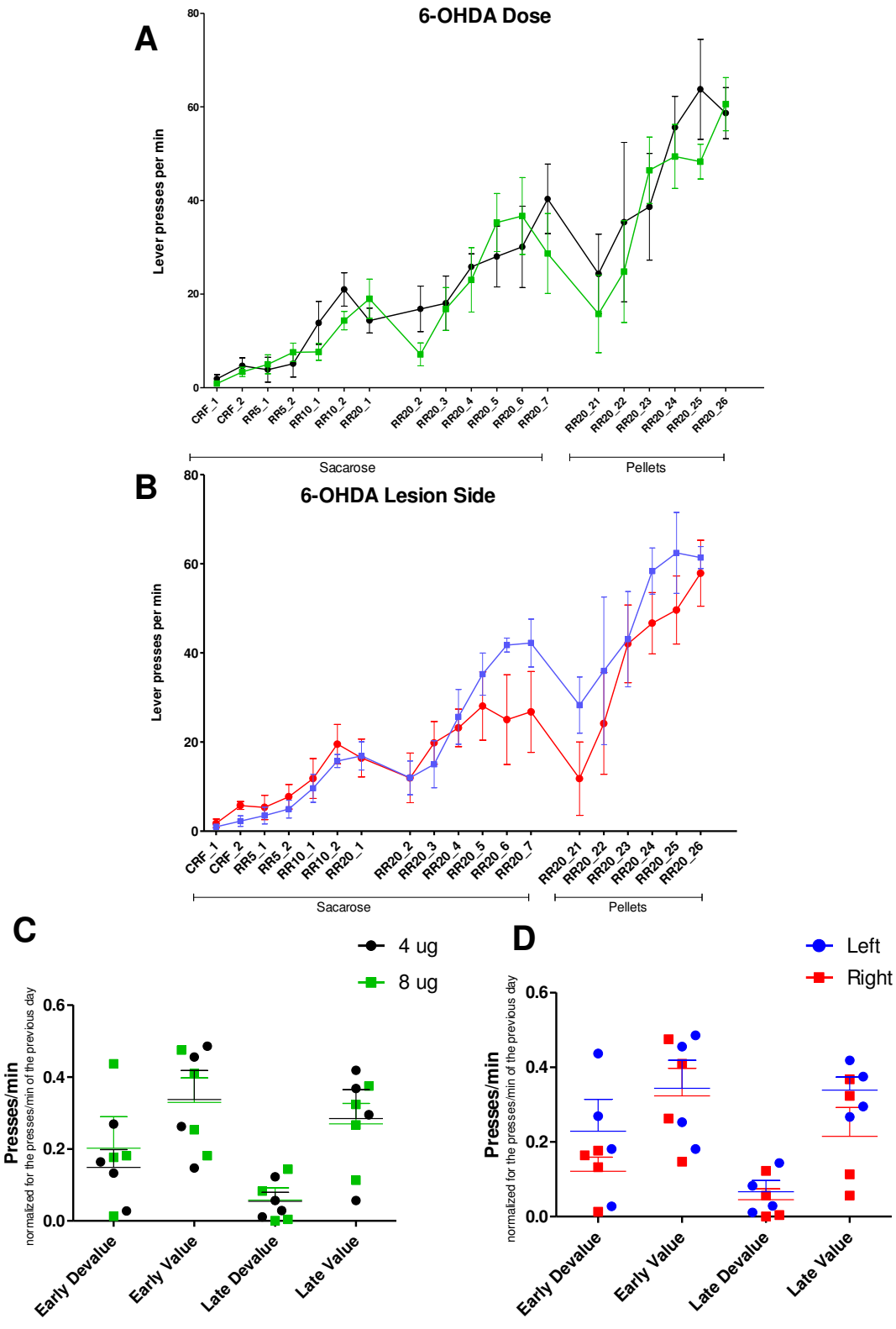

Figure 13. Habit-behavior test. No differences were found between the number of lever presses/min considering 6-OHDA dose (A) and lesion side (B). In the late devaluation day, all the animals decrease the number of lever presses, which indicates that they are not habit-based. Data presented as mean ± SD

lesions, needed more CRF sessions to reach 30 lever presses (Fig 15A-B). At the end of the protocol the number of lever presses/per minute was lower than the one seen in previous experiments and the results were inconclusive, so an extra set of RR20 sessions followed by a devalue and value day was done, with pellets instead of sucrose. Present results reflect the number of lever presses/minutes during extinction test under value or devalue conditions, before (early devalue/value) and after (late devalue/value) habit induction (Fig 15 C-D). Late results reflect the average lever presses/minute after sucrose and pellets habit induction. Each result was normalized to the number of lever presses/minute of previous RR20 session to excluded animals intrinsic variability effects.

No differences were found in learning and devaluation test concerning both lesion side and injected dose (Fig. 14A-D).

Looking to the average number of correct nosepokes along VDS shaping protocol there is no difference between 4 and 8 µg injected rats (Fig 16A) but animals injected in left side have consistently a lower average than animals injected in the right side (Fig 16B). Concerning impulsivity, although not statistically significant, there are differences regarding both dose and side lesion (Fig 16C-D). Rats injected with 4 µg of 6-OHDA in the right hemisphere have more premature responses during 6 seconds, 12 seconds and the final 3 seconds delays than rats injected with 8 µg of 6-OHDA in the left hemisphere. One animal injected in the right side with 4 µg of 6-OHDA was excluded from the results because it presented very high impulsivity values (12.2 at 6 seconds, 9.2 at 12 seconds and 4 at the final 3 seconds). Interestingly, the second animal with high impulsivity values was also injected in the right side with 4 µg of 6-OHDA. Still, there is a tendency to rats injected in the left have a higher impulsivity index than the ones injected in the right side.

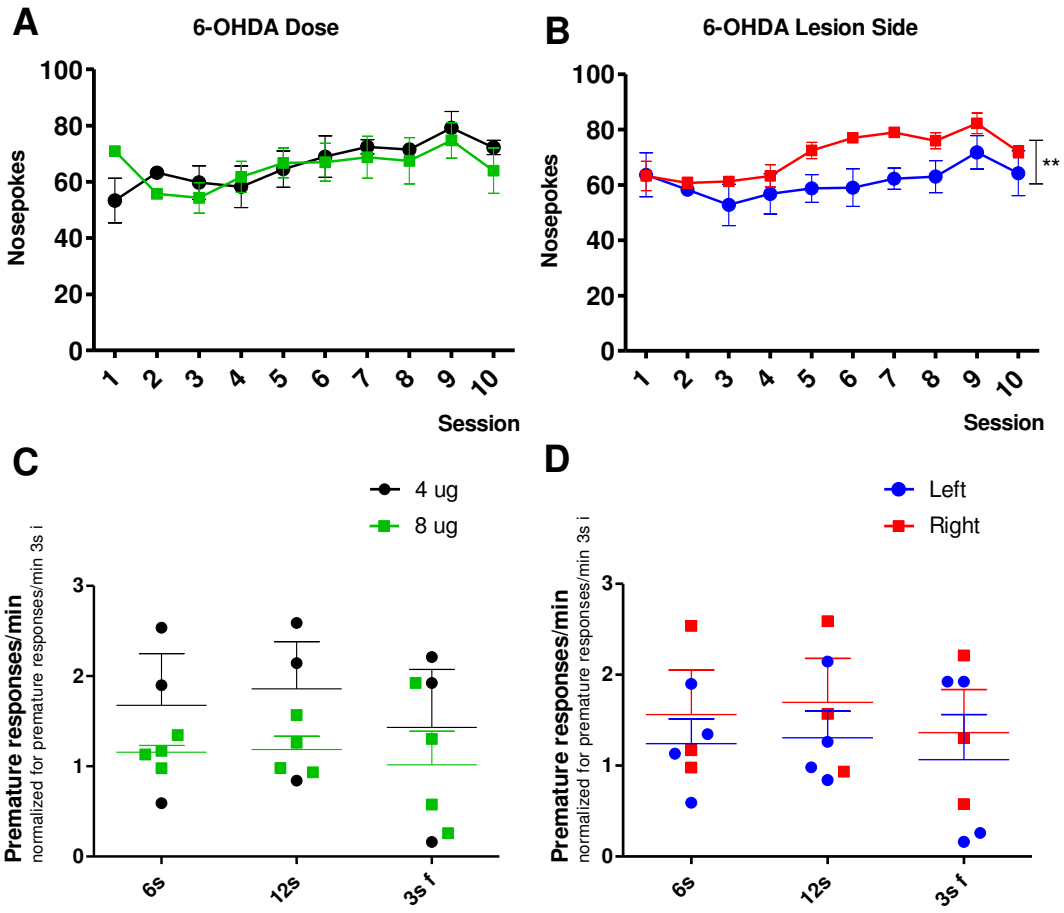

Figure 14- Learning and impulsivity evaluated by the Variable Delay to Signal test. Left lesioned animals present lower number of correct nosepokes along the shaping (training?) sessions than right lesioned animals, which could reflect impairments in learning/motivation capacity (B). No differences were found concerning 6-OHDA dose (A). Regarding prematurity, animals injected with 4 ug of 6-OHDA are apparently more impulsive than the ones injected with 8 ug (C). Right lesioned animals also present higher number of impulsive responses (D). Data presented as mean ± SD, **p<0.01

4. DISCUSSION

4.1 General

Neuropathic pain is frequently comorbid with depression, anxiety and cognitive deficits. It is currently accepted that neuropathic lesions in humans and animals induce pain and that pain maintenance is responsible for the emergence of these emotional and cognitive alterations. However, as dicussed previously (see section 1.3) there is also evidence in the literature that after a peripheral neuropathic lesion, pain and the behavioral alterations can manifest independently. For instance, Dimitrov and colleagues recently reported the presence of emotional deficits in mice in the absence of pain manifestations and Ren and colleagues reported that right-sided SNI animals presented WM deficits whether or not allodynia manifested (Dimitrov, et al., 2014; Ren, et al., 2011). Another set of evidence comes from the temporal mismatch between pain appearance (almost immediately after lesion) and anxiety and depression arise (at least 2 weeks after lesion) (Suzuki, et al., 2007; Yalcin et al., 2011) and the differences in behavioral outcomes in left- and right-sided SNI lesions, although no differences were observed in the pain measurements (Leite-Almeida, et al., 2012, 2014)

To test the hypothesis that pain is not needed to the establishment of emotional and cognitive deficits we decided to study the behavior of allodynic and non-allodynic SNI animals. If the hypothesis proves valid, i.e. that the nerve injury alone can cause emotional and cognitive deficits, then side-specific differences previously observed by the group (Leite-Almeida, et al., 2012) should also be observed in the absence of pain manifestations. Based on this we decided to incorporate the lesion side as a variable in these experiments. The idea was to explore if the lateralization biases previously observed were also present in non-painful neuropathies indicating a critical role of the injury. Therefore the first part of this thesis focused on the behavioral performance of sham, All-L, All-R, N-All-L and N-All-R animals.

The second part of the thesis, in turn, focused on the biochemical alterations behind the initiation and maintenance of chronic pain and its comorbidities. DA appears here as an appealing target as it is individually related with chronic pain, depression, anxiety and cognition. Furthermore, there are evidences of a lateralization in DA levels in the brain and of the influence of this lateralization in anxiety-like behavior (R M Sullivan et al., 2014; Thiel & Schwarting, 2001), which could explain the behavioral differences between left and right-SNI animals. To investigate this possibility we measured the expression of D_1R and D_2R in different areas in both hemispheres in Sham, SNI-L and SNI-R animals 30 days after SNI. At the same time we performed a pilot experiment to study the putative differential effects in decision-making of DA depletion in left or

right hemispheres. Previous unpublished results from our lab showed that after habit induction SNI-R rats, like Sham, are capable of recognizing reward devaluation indicating goal-direct decisions while SNI-L maintain their response level even after devaluation, indicating an impaired transition from habit-based to goal-directed behaviors. Such lateralization differences were also observed in the VDS test. In this case, SNI-R, but not SNI-L animals, present an increased impulsive behavior in comparison to their sham controls (Leite-Almeida, et al., 2012). DA is involved in decision-making, impulsivity and motivation so it is very likely that it plays a role in these behaviors (Arias-Carrión, et al., 2010). Considering that chronic pain induces a decrease in DA release (Jääskeläinen, et al., 2001) we hypothesized that 6-OHDA lesions in the left but not right hemisphere will induce deficits in decision–making similar to the ones observed in SNI-R rats. Therefore, we evaluated the performance of animals lesioned unilaterally with 6-OHDA in habit-induction and VDS test. In this pilot study the effect of two different doses (4 µg and 8 µg) of 6-OHDA were evaluated; this was critical to ensure that 6-OHDA lesioned animals were capable/motivated to learn the shaping protocol of VDS and to acquire habit-based actions.

4.2 Experimental considerations

In all experiments presented in this thesis using the SNI model of neuropathic pain, the analyses started 1 month after the SNI injury installation. As stated before, at this stage behavioral alterations, namely anxiety-like behavior and impaired cognition are already manifesting (Leite-Almeida, et al., 2012; Suzuki, et al., 2007; Yalcin, et al., 2011). Two rat strains (Winstar-Han and Sprague-Dawley) were used in in the experiments. Sprague Dawley were used in the first experimental set following the observation by De Felice and colleagues, who showed that, in this strain, 15% of neuropathic rats do not develop pain (allodynia) (2011). This outcome variation also occurs in other strains, notably in Holtzman rats, which have 50% incidence of pain after peripheral neuropathy; these are not available from European sellers. In our study, the number of SNI individuals with increased threshold to VF probing, was slightly higher than the figures reported by De Felice. However, if only SNI non-responders are considered, then the values are virtually identical between the two studies (see below). The introduction of the Sprague Dawley strain in the study allowed therefore to isolate the effect of the nerve lesion although it reduced our ability to compare results previously obtained with the strain regularly used in our laboratory – Wistar Han.

Other limitation of our studies, and of any study of pain involving animal models, is pain assessment. The major concern is the fact that we can only obtain indirect readouts of pain through either a motor reflex or a conditioned response. Concerning the last, it can be obtained in the Conditioned Place Preference (CPP) paradigm, and is currently the most reliable way to assess ongoing pain (King, et al., 2009). In this test, a conditioned place preference is induced by analgesic drugs specifically in animals with ongoing pain. However, this test implies pharmacological manipulation ideally by intrathecal route, which would introduce another variable to our experiment and would imply another surgical procedure. For that reason SPF were performed in addition to the classical mechanical allodynia measurement with VF. Also, in a tentative to increase our accuracy the SBB was included in the battery of behavioral tests. Although the burrowing behavior it is not a direct measure of pain, it provides an ethologic relevant measure associated with the well-being of rodents. Further, it was described that chronic pain leads to a decreased SBB and that this can be reverted by analgesics (Andrews, et al., 2012).

In our first experience, pain thresholds were unstable across measures of VF and SPF. Although this affected only a minor number of individuals with intermediate thresholds, it introduced some difficulties in the identification of non-allodynic animals. The factors determining these oscillations are not clear. As discussed in the Introduction section, stress and anxiety modulate pain sensation (Bushnell, et al., 2013). However, it seems unlikely that this is the only explanation since variations happen in both directions, i.e. some animals transit to allodynic while others became non-allodynic. Interestingly a clear division between high- and low-responders could only be observed in the post-behavioral test, suggesting a stabilization of the phenotype with time. Nevertheless, considering this variation, results were analyzed taking into account groups divided based on pre- and post-behavior allodynia measurements. Considering the presence of a correlation between VF and SPF results, the classification into allodynic and non-allodynic was based exclusively in VF values being 5 g the cut-off point. Groups based on the pre- and post-behavior divisions show the same tendencies in all the analyzed behavior, which could be one more indication of pain independency in what concerns behavior responses. It is important to stress that groups division into allodynic and non-allodynic is a subjective division. Furthermore, it should be stated that non-allodynic animals have a latent potential to manifest pain as demonstrated after the transient inactivation of the RVM (De Felice, et al., 2011). Curiously, the inverse was also demonstrated i.e. in allodynic animals the same manipulation

transiently treated pain. Such could explain the instability of the phenotype observed in our studies.

Based in the criteria above, from 87 SNI operated animals, 26% and 18% were considered non-allodynic animals in pre and post-behavior measures, respectively. These results, particularly that in the second measurement, were in accordance with the values described by De Felice and colleagues (2011). The lesion side is not a susceptibility factor since number of left- and right-sided non-allodynic animals were very similar (12 and 11 and 8 and 8 in pre- and post-behavior based groups, respectivelly). Allodynic rats with non-allodynic cagemates and sham operated animals were used as controls; all other SNI animals were not included in the behavioral tests for a matter of logistics. Such distribution ensured the best control possible and decreased the impact of external variations on behavioral differences in the groups.

4.3 Behavioral performance in painful and non-painful neuropathic conditions

With this experiment we intend to study if pain was detrimental to the establishment of emotional and cognitive deficits in neuropathic animals. For that we analyzed the behavioral performance of allodynic and non-allodynic rats in a battery of tests.

With this experiment we intended to study if pain was detrimental to the establishment of emotional and cognitive deficits in neuropathic animals. For that we analyzed the behavioral performance of allodynic and non-allodynic rats in a battery of tests.

Results in general supported our hypothesis that the nerve injury alone (in the absence of pain manifestations) is sufficient to induce behavioral disturbances since non-allodynic rats tended to show the same deficits as allodynic animals. Particularly concerning anxiety, in the D/L test we observed a side effect, i.e. left-sided injury was associated with increased anxiety-like behavior in both low- and high-threshold groups. A similar tendency was observed in the SBB. Such is in agreement with a previous observation in Wistar Han rats (Leite-Almeida, et al., 2012) using the SNI model and also with studies in human patients (Gagliese, et al.,1994, 1995; Wasan, et al., 2010). The origin of this asymmetry is not known but a study form Sullivan and colleagues demonstrating that the excitation/inhibition of the right (but not left) infralimbic region of the PFC had anxiogenic/anxiolytic effects (2002) suggests that the SNI-L injury might predominantly affect the function of the contralateral cortex. This hypothesis is supported by a recent observation of the group. In this case, C-fos expression in the rat PFC was analyzed after the completion of a demanding task (attentional set-shifting task). It was observed that the neuronal activity presented a leftward bias in sham controls and in SNI-L. However, this balance

was disrupted in SNI-R animals and this was associated with a poorer performance (Leite-Almeida, et al., 2014). Concerning the VDS, we also observed an interesting tendency for a heightened impulsive behavior in left- and right-sided transcranial stimulation in the PFC differentially affected risk-seeking impulsivity again suggesting an asymmetrical distribution of the PFC functional load (Pripfl, et al., 2013). Analyses of DA receptor expression in ipsi- and contralateral areas to the SNI injury also favor the hypothesis of a selective asymmetric disruption in central function (see discussion below).

Although no effect of side or pain was observed, there was a difference concerning Sham and SNI animals in FST and in the distance walked during D/L. Concerning the first there are controversies regarding the temporal emergence of depressive-like behavior with some reporting its manifestation 2 weeks and other only 8 weeks after lesion (Suzuki et al., 2007; Yalcin et al., 2011). Since no differences were found concerning immobility time it is possible that struggling results reflect the motor impairments observed by the distance walked in the D/L test and not a depressive-like phenotype.

Analgesic treatment both in rat and in humans has been thoroughly demonstrated to ameliorate anxiety, depression and cognitive deficits in chronic pain conditions (Amorim, et al. 2014; Borges, Neto, et al., 2014; Hu, et al., 2010; D. A. Seminowicz, et al., 2011). While such observations are apparently in contradiction to our hypothesis, it should be equated that analgesic drugs eliminate pain because they interfere with the same processes e.g. inflammation and neurotransmitters release, that likely underlie the emergence of the comorbidities. Indeed, antidepressants are remarkably effective treatments for neuropathic pain (Ashburn & Staats, 1999).

4.4 Dopaminergic system in chronic pain conditions

With the intention to study the dopaminergic system status in chronic neuropathic pain conditions, a relative quantification, by qPCR, of D_1R and D_2R in left and right mPFC, OFC, NAcc and STR was performed.

Results show a tendency in some brain nuclei (namely D_1R in OFC) to present a basal lateralization which is lost or inverted after the neuropathic lesion. Evidences of lateralized expression of D_1R and D_2R and DA concentration in basal status are reported in rats, marmosets and humans (Molochnikov & Cohen, 2014; Nowak, 1989; Schneider, et al., 1982; Silva, et al., 2007; Vernaleken, et al., 2007). We hypothesized based on the fact that DA is reduced in the CSF of chronic pain patients (Bouckoms, et al., 1992; Legangneux, et al., 2001) and that DA

receptors are increased in the brain of chronic pain patients when compared to healthy controls (Hagelberg, et al., 2003), that neuropathic lesion will lead to a decrease in DA availability, and that such effect is more severe in the hemisphere contralateral to the neuropathic lesion. Expression of D_1R and D_2R in the NAcc and of D_2R in the mPFC, sustain this hypothesis, as the expression of these receptors increases in the contralateral side of the lesion. The same is not true for the other nuclei, implying that DAergic system is not altered in a general way, but specifically depending on the receptor and its localization. Other evidence is that the expression of D_1R and D_2R reflect the same tendency in NAcc and OFC but present different responses to neuropathic lesion in mPFC and STR. These expression differences depend on the brain nuclei, side and receptor and could help to elucidate the reasons behind the different behavioral outcomes observed in Sham, SNI-L and SNI-R rats. In fact, studies with agonists and antagonists specific to each receptor and genetically modified mice have revealed a differential involvement of D_1R and D_2R in some behaviors. For instance, D_1R seem to be more involved in WM, attention and risky decision-making than D_2R (Arnsten, 1998; Granon, et al., 2000; Müller, et al., 1998; Stopper, et al., 2013). On the other hand, D_2R, (but not D_1R) antagonists have an anxiolytic effect as demonstrated in EPM (Rodgers, et al., 1994). The anatomical location of DA receptors has also been demonstrated to be a critical determinant of behavior. For instance, injection of a D2R antagonist in mPFC and OFC increased impulsive choice in a delay reinforced task. D1R antagonism produce the same effect but only at the mPFC (not OFC) (Pardey, Kumar, Goodchild, & Cornish, 2013). In addition, a number of studies specifically addressed the issue of laterality. Thiel and colleagues, for instance reported that rats with increased DA concentration in right over left frontal cortex presented decreased anxiety-like behaviors (2001). However, the opposite was observed by Andersen and Teicher (1999). Also, PD patients with worse disease signs in the left hemisphere show more symptoms of anxiety than the ones with higher damage in the right hemisphere (Fleminger, 1991). The association between DA asymmetries in the PFC and anxiety is still controversial. Our results suggest that SNI-L animals are more anxious-like and that this should reflect reduced levels of DA in right cortex. Other evidence came from the study of Sullivan and colleagues (2014) which report that 6-OHDA right hemisphere lesioned male rats have an anxious-like behavior when compared with left lesioned and control rats.

In pain behavior, a significant number of studies have successfully proved the involvement DAergic activity. Studies in animal models and human brain imaging studies demonstrated a specific involvement of D_2R in chronic pain (Ansah, et al., 2007; Hagelberg, et al., 2003;

Magnusson & Fisher, 2000) specifically, D_2R agonism was shown to diminish pain. Paradoxically, we found a strong correlation between mechanical allodynia and D_1R expression in the left OFC. Surprisingly, this occurred irrespectively of the SNI side. In addition, the expression of DA receptors in the OFC presented an interesting bias towards the right hemisphere, again irrespectively of the SNI side. A similar phenomena has been reported in the basolateral nucleus of the Amygadala (BLA) (Ji & Neugebauer, 2009); In this case, sensitization markers namely neuronal extracellular signal-regulated kinase (ERK) were found to be increased specifically in the right (but not left) BLA. OFC is involved in cognitive evaluation of pain and it presents a decrease of its grey matter volume in chronic pain conditions. Furthermore, DA and 5-HT levels were also shown to be decreased in these conditions resulting in impaired decision-making (Neugebauer, et al., 2009; Seifert, 2012). The OFC and the BLA present extensive bidirectional connectivity which might explain the asymmetry observed in our study. Nevertheless, the relevance of this correlation between pain and D1R in left OFC and its relation with decision making is yet to be explored.

4.5 Impact of lateralized dopamine depletion in decision-making

Previous unpublished studies from the group show that SNI-R rats are more impulsive while SNI-L present an impaired shift from goal-directed to habit-based decisions. DA is involved in impulsivity, habit formation and decision-making. In healthy subjects, de Wit and colleagues showed that depletion of DA (tyrosine depleted diet) leads to a habit instead of goal-direct decision-making (de Wit, et al., 2012). On the other hand, PD patients frequently manifest impulsivity and decision-making disorders when under L-DOPA medication (Osman et al., 2014). Goal-direct control, on the other hand was inversely related with impulsivity, indicating that goal-direct behavior is related with higher values of DA (Hogarth, et al., 2012). It is clear that an imbalance of DA availability, in either direction, is prejudicial to goal-direct behavior and impulsivity. We therefore studied DA's role in this lateralization bias using a unilateral depletion model (6-OHDA). Preliminary results from the habit/goal decision-making test do not show differences in habit establishment concerning lesion side; both groups show goal-directed behavior since number of lever presses decrease in devalue conditions. In the impulsivity paradigm a tendency for higher impulsivity was observed in right-lesioned animals, during the learning (shaping) phase of the paradigm of the test. No differences were however observed in the VDS proper. The two parts of the VDS paradigm reflect different aspects of impulsive behavior, namely response impulsivity and delay tolerance, respectively (Leite-almeida, et al.,

2013). Dopamine differentially modulates these traits, particularly, decreased levels of DA were shown to be associated with a reduction of impulsive responses and with a decreased delay tolerance (Dalley & Roiser, 2012), therefore in contradiction with results from our pilot study. Besides the small sample size which prevents firmer conclusions, these studies have not accounted for possible lateralized effects. Follow up studies will thus be critical to clarify this issue.

4.4 Conclusions

The interaction between neuropathic lesions, chronic pain and emotional/cognitive disturbances is a complex and multifactorial problem still under investigation. Classically, it is assumed that neuropathic lesions, particularly those affecting the somatosensory system, might result in neuropathic pain and the latter is the trigger for the emergence of anxiety, depression and cognitive deficits. In the work presented in this thesis we attempted to demonstrate a new model in which after a peripheral neuropathy, pain and the behavioral dysfunctions emerge in parallel and not as a cause-effect relation. In other words, we hypothesized that the peripheral neuropathic lesion is sufficient to drive central plastic events that underlie the manifestation of anxiety, depression and cognitive deficits. We further assume that these events might occur even in the absence of pain. As discussed before, in "silent" non-painful peripheral neuropathies, pain is latent and can be triggered by manipulations of the RVM, indicating that the injury is active. The differential impacts of left- and right-sided pain on behavior and the delayed emergence of anxiety and depression after pain onset, also support this conceptualization.

The results presented in the first part of this thesis support the suggested model. Impaired anxiety-like behavior in left but not right-sided lesioned animals, appeared independent of high or low allodynia thresholds following the same side bias previously observed by our group. A similar observation was made for impulsive behavior. Such observations implied that the central impact of the lesion had to obey to a lateralized logic. Dopamine was in this context an interesting target. The obtained results varied according to the analyzed area. As a general trend, an upward variation was observed, particularly in the contralateral sites to the peripheral nerve injury. Such is suggestive of a compensatory mechanism to decreased DA availability as described in several other basic and clinical studies. Nevertheless, such requires future studies specifically addressing this issue. Also, pharmacological or optogenetic unilateral manipulations of DAergic activity should also be performed in order to extend/complement our observations. The causality proof is critical to validate our results.

It is estimated that less than 5% of the individuals experience pain after traumatic nerve injury. Nothing is however known about the behavioral impact on the other 95% (Sunderland, 1993.). The magnitude of the problem increases if other more common neuropathies like viral infections, neurotoxic agents and metabolic disease are also equated. The work presented here is the first attempt to study the impact of "silent neuropathies" in brain function. Many questions are left unanswered particularly relating with the involvement of other neurotransmitter systems (e.g. serotonine) and the kinetics of the phenomena. Other aspect of relevance, particularly from a translational perspective deals with the susceptibility factors determining the emergence of pain in some individuals. These could furnish valuable information for potential therapeutical targets.

6. REFERENCES

Ahlbeck, K. (2011). Opioids: a two-faced Janus. *Current Medical Research and Opinion, 27*(2), 439-448.

Almeida, A., Leite-Almeida, H., & Tavares, I. (2006). Medullary control of nociceptive transmission: Reciprocal dual communication with the spinal cord. *Drug Discovery Today: Disease Mechanisms*, *3*(3), 305–312.

Almeida, T. F., Roizenblatt, S., & Tufik, S. (2004). Afferent pain pathways: a neuroanatomical review. *Brain Research*, *1000*(1-2), 40–56.

Amorim, D., David-Pereira, A., Pertovaara, A., Almeida, A., & Pinto-Ribeiro, F. (2014). Amitriptyline reverses hyperalgesia and improves associated mood-like disorders in a model of experimental monoarthritis. *Behavioural Brain Research*, *265C*, 12–21.

Andersen, S. L., & Teicher, M. H. (1999). Serotonin laterality in amygdala predicts performance in the elevated plus maze in rats. *Neuroreport*, *10*(17), 3497–500.

Andrews, N., Legg, E., Lisak, D., Issop, Y., Richardson, D., Harper, S., Pheby, T., Huang, W., Burgess, G., Machin, I., Rice, A. S. (2012). Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. *European Journal of Pain (London, England)*, *16*(4), 485–495.

Ansah, O. B., Leite-Almeida, H., Wei, H., & Pertovaara, A. (2007). Striatal dopamine D2 receptors attenuate neuropathic hypersensitivity in the rat. *Experimental Neurology*, *205*(2), 536–546.

Apkarian, A. V., Sosa, Y., Krauss, B. R., Thomas, P. S., Fredrickson, B. E., Levy, R. E., Harden, R. N., Chialvo, D. R. (2004). Chronic pain patients are impaired on an emotional decision-making task. *Pain*, *108*(1-2), 129–136.

Apkarian, A. V., Sosa, Y., Sonty, S., Levy, R. M., Harden, R. N., Parrish, T. B., & Gitelman, D. R. (2004). Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(46), 10410–10415.

Apkarian, A. V., Bushnell, M. C., Treede, R.-D., & Zubieta, J.-K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain (London, England)*, *9*(4), 463–484.

Arias-Carrión, O., Stamelou, M., Murillo-Rodríguez, E., Menéndez-González, M., & Pöppel, E. (2010). Dopaminergic reward system: a short integrative review. *International Archives of Medicine*, *3*, 24.

Arnsten, A. (1998). Catecholamine modulation of prefrontal cortical cognitive function. *Trends in Cognitive Sciences*, *2*(11), 436-447.

Ashburn, M. A., & Staats, P. S. (1999). Management of chronic pain. *Lancet*, *353*(9167), 1865–1869.

Azevedo, L. F., Costa-Pereira, A., Mendonça, L., Dias, C. C., & Castro-Lopes, J. M. (2012). Epidemiology of chronic pain: a population-based nationwide study on its prevalence, characteristics and associated disability in Portugal. *The Journal of Pain : Official Journal of the American Pain Society*, *13*(8), 773–783.

Bair, M., & Robinson, R. (2003). Depression and pain comorbidity: a literature review. *Archives of Internal Medicine, 163*(20), 2433–2445.

Basbaum, A. I., Bautista, D. M., Scherrer, G., & Julius, D. (2009). Cellular and molecular mechanisms of pain. *Cell*, *139*(2), 267–284.

Basile, A., & Janowsky, A. (2007). Characterization of the antinociceptive actions of bicifadine in models of acute, persistent, and chronic pain. *Journal of Pharmacology and Experimental Therapeutics*, *321*(3), 1208–1225.

Beecher, H. K. (1946). Pain in Men Wounded in Battle. *Annals of Surgery*, *123*(1), 96–105.

Beiske, A. G., Loge, J. H., Rønningen, A., & Svensson, E. (2009). Pain in Parkinson's disease: Prevalence and characteristics. *Pain*, *141*(1-2), 173–117.

Berryman, C., Stanton, T. R., Jane Bowering, K., Tabor, A., McFarlane, A., & Lorimer Moseley, G. (2013). Evidence for working memory deficits in chronic pain: a systematic review and meta-analysis. *Pain*, *154*(8), 1181–1196.

Bingel, U., & Tracey, I. (2008). Imaging CNS modulation of pain in humans. *Physiology (Bethesda, Md.)*, *23*, 371–380.

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M. C., Ploner, M., & Tracey, I. (2011). The effect of treatment expectation on drug efficacy: imaging the analgesic benefit of the opioid remifentanil. *Science Translational Medicine*, *3*(70), 70ra14.

Borges, G., Neto, F., Mico, J. A., & Berrocoso, E. (2014). Reversal of monoarthritis-induced affective disorders by diclofenac in rats. *Anesthesiology*, *120*(6), 1476–90.

Bouckoms, A. J., Sweet, W. H., Poletti, C., Lavori, P., Carr, D., Matson, W., Gamache, P., Aronin, N. (1992). Monoamines in the brain cerebrospinal fluid of facial pain patients. *Anesthesia Progress*, *39*(6), 201–208.

Braz, J., Solorzano, C., Wang, X., & Basbaum, A. I. (2014). Transmitting Pain and Itch Messages: A Contemporary View of the Spinal Cord Circuits that Generate Gate Control. *Neuron*, *82*(3), 522–536.

Brefel-Courbon, C., Payoux, P., Thalamas, C., Ory, F., Quelven, I., Chollet, F., Montastruc, J. L., Rascol, O. (2005). Effect of levodopa on pain threshold in Parkinson's disease: a clinical and positron emission tomography study. *Movement Disorders: Official Journal of the Movement Disorder Society*, *20*(12), 1557–1563.

Brown, A. S., & Gershon, S. (1993). Dopamine and depression. *Journal of Neural Transmission. General Section*, *91*(2-3), 75–109.

Brown, J. L., Liu, H., Maggio, J. E., Vigna, S. R., Mantyh, P. W., & Basbaum, A. I. (1995). Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis. *The Journal of Comparative Neurology*, *356*(3), 327–344.

Burkey, A. R., Carstens, E., & Jasmin, L. (1999). Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *19*(10), 4169–4179.

Bushnell, M. C., Ceko, M., & Low, L. a. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nature Reviews. Neuroscience*, *14*(7), 502–511.

Calejesan, a a, Kim, S. J., & Zhuo, M. (2000). Descending facilitatory modulation of a behavioral nociceptive response by stimulation in the adult rat anterior cingulate cortex. *European Journal of Pain (London, England)*, *4*(1), 83–96.

Casey, K. L. and Melzack, R. (1968). *The Skin Senses*. Chapter 20: Sensory, Motivational, and Central Control Determinants of Pain, pp. 423-439

Chaplan, S. R., Bach, F. W., Pogrel, J. W., Chung, J. M., & Yaksh, T. L. (1994). Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods*, *53*(1), 55–63.

Chaplan, S. R., Guo, H.-Q., Lee, D. H., Luo, L., Liu, C., Kuei, C., Velumian, A. A., Butler, M. P., Brown, S. M., Dubin, A. E. (2003). Neuronal Hyperpolarization-Activated Pacemaker Channels Drive Neuropathic Pain. *Journal of Neurosciences.*, *23*(4), 1169–1178.

Chinta, S. J., & Andersen, J. K. (2005). Dopaminergic neurons. *The International Journal of Biochemistry & Cell Biology*, *37*(5), 942–946.

Chudler, E. H., & Lu, Y. (2008). Nociceptive behavioral responses to chemical, thermal and mechanical stimulation after unilateral, intrastriatal administration of 6-hydroxydopamine. *Brain Research*, *1213*, 41–47.

Coffeen, U., López-Avila, A., Ortega-Legaspi, J. M., del Angel, R., López-Muñoz, F. J., & Pellicer, F. (2008). Dopamine receptors in the anterior insular cortex modulate long-term nociception in the rat. *European Journal of Pain (London, England)*, *12*(5), 535–543.

Cools, R., & D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biological Psychiatry*, *69*(12), 113–125.

Crawley, J., & Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacology, Biochemistry, and Behavior*, *13*(2), 167–170.

Cui, M., Feng, Y., Adoo, D. J. M. C., & Willis, W. D. (1999). Periaqueductal Gray Stimulation-Induced Inhibition of Nociceptive Dorsal Horn Neurons in Rats Is Associated with the Release of Norepinephrine, Serotonin, and Amino Acids 1, *289*(2), 868–876.

Dahlstroem, A., & Fuxe, K. (1964). Evidence for the Existence of Monoamine-Containing Neurons in the Central Nervous System. I. Demonstration of Monoamines in the Cell Bodies of Brain Stem Neurons. *Acta Physiologica Scandinavica Supplementum*, SUPPL 232:1–55.

Dalley, J. W., & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience*, *215*, 42–58.

De Felice, M., Sanoja, R., Wang, R., Vera-Portocarrero, L., Oyarzo, J., King, T., Ossipov, M. H., Vanderah, T. W., Lai, J., Dussor, G. O., Fields, H. L., Price, T. J., Porreca, F. (2011). Engagement of descending inhibition from the rostral ventromedial medulla protects against chronic neuropathic pain. *Pain*, *152*(12), 2701–2709.

De Wit, S., Standing, H. R., Devito, E. E., Robinson, O. J., Ridderinkhof, K. R., Robbins, T. W., & Sahakian, B. J. (2012). Reliance on habits at the expense of goal-directed control following dopamine precursor depletion. *Psychopharmacology*, *219*(2), 621–631.

Deacon, R. M. J. (2006). Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. *Nature Protocols*, *1*(1), 118–121.

Decosterd, I., & Woolf, C. J. (2000). Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain*, *87*(2), 149–158.

DeVeaugh-Geiss, A. M., West, S. L., Miller, W. C., Sleath, B., Gaynes, B. N., & Kroenke, K. (2010). The adverse effects of comorbid pain on depression outcomes in primary care patients: results from the ARTIST trial. *Pain Medicine*, *11*(5), 732–741.

Diatchenko, L., Slade, G. D., Nackley, A. G., Bhalang, K., Sigurdsson, A., Belfer, I., Goldman, D., Xu, K., Shabalina, S. A., Shagin, D., Max, M. B., Makarov, S. S., Maixner, W. (2005). Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Human Molecular Genetics*, *14*(1), 135–143.

Dick, B. D., & Rashiq, S. (2007). Disruption of attention and working memory traces in individuals with chronic pain. *Anesthesia and Analgesia*, *104*(5), 1223–1229.

Dieb, W., Ouachikh, O., Durif, F., & Hafidi, A. (2014). Lesion of the dopaminergic nigrostriatal pathway induces trigeminal dynamic mechanical allodynia. *Brain and Behavior*, *4*(3), 368–380.

Dimitrov, E. L., Tsuda, M. C., Cameron, H. A., & Usdin, T. B. (2014). Anxiety- and Depression-Like Behavior and Impaired Neurogenesis Evoked by Peripheral Neuropathy Persist following Resolution of Prolonged Tactile Hypersensitivity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(37), 12304–12312.

Djouhri, L., & Lawson, S. N. (2004). Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Research. Brain Research Reviews*, *46*(2), 131–145.

Dogrul, A., Ossipov, M. H., & Porreca, F. (2009). Differential mediation of descending pain facilitation and inhibition by spinal 5HT-3 and 5HT-7 receptors. *Brain Research*, *1280*, 52–59.

Dworkin, R. H. (1994). Pain insensitivity in schizophrenia: a neglected phenomenon and some implications. *Schizophrenia Bulletin*, *20*(2), 235–48.

Eisenhofer, G., Kopin, I. J., & Goldstein, D. S. (2004). Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacological Reviews*, *56*(3), 331–349.

Elbert, T., Flor, H., Birbaumer, N., Knecht, S., Hampson, S., Larbig, W., & Taub, E. (1994). Extensive reorganization of the somatosensory cortex in adult humans after nervous system injury. *Neuroreport*, *5*(18), 2593–2597.

Emerson, N. M., Zeidan, F., Lobanov, O. V, Hadsel, M. S., Martucci, K. T., Quevedo, A. S., Starr, C. J., Nahman-Averbuch, H., Weissman-Fogel, I., Granovsky, Y.,Yarnitsky, D., Coghill, R. C. (2014). Pain sensitivity is inversely related to regional grey matter density in the brain. *Pain*, *155*(3), 566–573.

Fang, F. G., Haws, C. M., Drasner, K., Williamson, A., & Fields, H. L. (1989). Opioid peptides (DAGO-enkephalin, dynorphin A(1-13), BAM 22P) microinjected into the rat brainstem: comparison of their antinociceptive effect and their effect on neuronal firing in the rostral ventromedial medulla. *Brain Research*, *501*(1), 116–128.

Felten, D. L., & Sladek, J. R. (1983). Monoamine distribution in primate brain V. Monoaminergic nuclei: anatomy, pathways and local organization. *Brain Research Bulletin*, *10*(2), 171–284.

Fields, H. L., Bry, J., Hentall, I., & Zorman, G. (1983). The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *3*(12), 2545–2552.

Finnerup, N. B., Otto, M., McQuay, H. J., Jensen, T. S., & Sindrup, S. H. (2005). Algorithm for neuropathic pain treatment: an evidence based proposal. *Pain*, *118*(3), 289–305.

Fleminger, S. (1991). Left-sided Parkinson's disease is associated with greater anxiety and depression. *Psychological Medicine*, *21*(3), 629–38.

Flor, H., Braun, C., Elbert, T., & Birbaumer, N. (1997). Extensive reorganization of primary somatosensory cortex in chronic back pain patients. *Neuroscience Letters*, *224*(1), 5–8.

Gagliese, L., Schiff, B. B., & Taylor, A. (1995). Differential consequences of left- and right-sided chronic pain. *The Clinical Journal of Pain*, *11*(3), 201–207.

Gebhart, G. F. (2004). Descending modulation of pain. *Neuroscience and Biobehavioral Reviews*, *27*(8), 729–737.

Geerse, G.-J., van Gurp, L. C. A., Wiegant, V. M., & Stam, R. (2006). Individual reactivity to the open-field predicts the expression of stress-induced behavioural and somatic pain sensitisation. *Behavioural Brain Research*, *174*(1), 112–118.

Geha, P. Y., Baliki, M. N., Harden, R. N., Bauer, W. R., Parrish, T. B., & Apkarian, A. V. (2008). The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. *Neuron*, *60*(4), 570–581.

Genro, J. P., Kieling, C., Rohde, L. A., & Hutz, M. H. (2010). Attention-deficit/hyperactivity disorder and the dopaminergic hypotheses. *Expert Review of Neurotherapeutics*, *10*(4), 587–601.

Gonçalves, L., Silva, R., Pinto-Ribeiro, F., Pêgo, J. M., Bessa, J. M., Pertovaara, A., Sousa, N., Almeida, A. (2008). Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat. *Experimental Neurology*, *213*(1), 48–56.

Granon, S., Passetti, F., Thomas, K. L., Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *20*(3), 1208–1215.

Grégoire, S., Michaud, V., Chapuy, E., Eschalier, A., & Ardid, D. (2012). Study of emotional and cognitive impairments in mononeuropathic rats: effect of duloxetine and gabapentin. *Pain*, *153*(8), 1657–1663.

Grisart, J. M., & Plaghki, L. H. (1999). Impaired selective attention in chronic pain patients. *European Journal of Pain*, *3*(4), 325–333.

Gureje, O., & Korff, M. Von. (1998). Persistent pain and well-being. *JAMA: The Journal of the American Medical Association, 280*(2), 147–152.

Hagelberg, N., Forssell, H., Aalto, S., Rinne, J. O., Scheinin, H., Taiminen, T., Någren, K., Eskola, O., Jääskeläinen, S. K. (2003). Altered dopamine D2 receptor binding in atypical facial pain. *Pain*, *106*(1-2), 43–48.

Hagelberg, N., Forssell, H., Rinne, J. O., Scheinin, H., Taiminen, T., Aalto, S., Luutonen, S., Någren, K., Jääskeläinen, S. (2003). Striatal dopamine D1 and D2 receptors in burning mouth syndrome. *Pain*, *101*(1-2), 149–154.

Hagelberg, N., Martikainen, I. K., Mansikka, H., Hinkka, S., Någren, K., Hietala, J., Scheinin, H., Pertovaara, A. (2002). Dopamine D2 receptor binding in the human brain is associated with the response to painful stimulation and pain modulatory capacity. *PAIN*, *99*(1-2), 273–279.

Hains, B. C., & Waxman, S. G. (2006). Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(16), 4308–4317.

Hammond, D. L., & Yaksh, T. L. (1984). Antagonism of stimulation-produced antinociception by intrathecal administration of methysergide or phentolamine. *Brain Research*, *298*(2), 329–337.

Han, H. C., Lee, D. H., & Chung, J. M. (2000). Characteristics of ectopic discharges in a rat neuropathic pain model. *Pain*, *84*(2-3), 253–261.

Hardy, S. G., & Haigler, H. J. (1985). Prefrontal influences upon the midbrain: a possible route for pain modulation. *Brain Research*, *339*(2), 285–293.

Harriott, A. M., & Gold, M. S. (2009). Contribution of primary afferent channels to neuropathic pain. *Current Pain and Headache Reports*, *13*(3), 197–207.

Hart, R. P., Martelli, M. F., & Zasler, N. D. (2000). Chronic pain and neuropsychological functioning. *Neuropsychology Review*, *10*(3), 131–149.

Hasnie, F. S., Wallace, V. C. J., Hefner, K., Holmes, A., & Rice, A. S. C. (2007). Mechanical and cold hypersensitivity in nerve-injured C57BL/6J mice is not associated with fear-avoidance- and depression-related behaviour. *British Journal of Anaesthesia*, *98*(6), 816–822.

Heinricher, M. M., Morgan, M. M., Tortorici, V., & Fields, H. L. (1994). Disinhibition of off-cells and antinociception produced by an opioid action within the rostral ventromedial medulla. *Neuroscience*, *63*(1), 279–288.

Heinricher, M. M., Tavares, I., Leith, J. L., & Lumb, B. M. (2009). Descending control of nociception: Specificity, recruitment and plasticity. *Brain Research Reviews*, *60*(1), 214–225.

Helmstetter, F. J., Tershner, S. A., Poore, L. H., & Bellgowan, P. S. (1998). Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. *Brain Research*, *779*(1-2), 104–118.

Hentall, I. D., Pinzon, A., & Noga, B. R. (2006). Spatial and temporal patterns of serotonin release in the rat's lumbar spinal cord following electrical stimulation of the nucleus raphe magnus. *Neuroscience*, *142*(3), 893–903.

Hogarth, L., Chase, H. W., & Baess, K. (2012). Impaired goal-directed behavioural control in human impulsivity. *Quarterly Journal of Experimental Psychology (2006)*, *65*(2), 305–316.

Hooley, J. M., & Delgado, M. L. (2001). Pain insensitivity in the relatives of schizophrenia patients. *Schizophrenia Research*, *47*(2-3), 265–273.

Hu, Y., Yang, J., Hu, Y., Wang, Y., & Li, W. (2010). Amitriptyline rather than lornoxicam ameliorates neuropathic pain-induced deficits in abilities of spatial learning and memory. *European Journal of Anaesthesiology*, *27*(2), 162–168.

Iversen, S. D., & Iversen, L. L. (2007). Dopamine: 50 years in perspective. *Trends in Neurosciences*, *30*(5), 188–193.

Jääskeläinen, S. K., Rinne, J. O., Forssell, H., Tenovuo, O., Kaasinen, V., Sonninen, P., & Bergman, J. (2001). Role of the dopaminergic system in chronic pain – a fluorodopa-PET study. *Pain*, *90*(3), 257–260.

Jay, G. W., & Barkin, R. L. (2014). Neuropathic pain: etiology, pathophysiology, mechanisms, and evaluations. *Disease-a-Month : DM*, *60*(1), 6–47.

Jensen, T. S., & Yaksh, T. L. (1984). Effects of an intrathecal dopamine agonist, apomorphine, on thermal and chemical evoked noxious responses in rats. *Brain Research*, *296*(2), 285–293.

Ji, G., & Neugebauer, V. (2009). Hemispheric lateralization of pain processing by amygdala neurons. *Journal of Neurophysiology*, *102*(4), 2253–2264.

Jordan, K. D., & Okifuji, A. (2011). Anxiety disorders: differential diagnosis and their relationship to chronic pain. *Journal of Pain & Palliative Care Pharmacotherapy*, *25*(3), 231–245.

Kesner, R. P., & Churchwell, J. C. (2011). An analysis of rat prefrontal cortex in mediating executive function. *Neurobiology of Learning and Memory*, *96*(3), 417–431.

King, T., Vera-Portocarrero, L., Gutierrez, T., Vanderah, T. W., Dussor, G., Lai, J., Fields, H. L., Porreca, F. (2009). Unmasking the tonic-aversive state in neuropathic pain. *Nature Neuroscience*, *12*(11), 1364–1366.

Kontinen, V. K., Kauppila, T., Paananen, S., Pertovaara, A., & Kalso, E. (1999). Behavioural measures of depression and anxiety in rats with spinal nerve ligation-induced neuropathy. *Pain*, *80*(1-2), 341–346.

Kouneiher, F., Charron, S., & Koechlin, E. (2009). Motivation and cognitive control in the human prefrontal cortex. *Nature Neuroscience*, *12*(7), 939–945.

Kuchinad, A., Schweinhardt, P., Seminowicz, D. A., Wood, P. B., Chizh, B. A., & Bushnell, M. C. (2007). Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(15), 4004–4007.

Landau, S. M., Lal, R., O'Neil, J. P., Baker, S., & Jagust, W. J. (2009). Striatal dopamine and working memory. *Cerebral Cortex (New York, N.Y. : 1991)*, *19*(2), 445–454.

Larisch, R., Meyer, W., Klimke, A., Kehren, F., Vosberg, H., & Müller-Gärtner, H. W. (1998). Left-right asymmetry of striatal dopamine D2 receptors. *Nuclear Medicine Communications*, *19*(8), 781–787.

Legangneux, E., Mora, J. J., Spreux-Varoquaux, O., Thorin, I., Herrou, M., Alvado, G., & Gomeni, C. (2001). Cerebrospinal fluid biogenic amine metabolites, plasma-rich platelet serotonin and [3H]imipramine reuptake in the primary fibromyalgia syndrome. *Rheumatology (Oxford, England)*, *40*(3), 290–296.

Leite-Almeida, H., Almeida-Torres, L., Mesquita, A. R., Pertovaara, A., Sousa, N., Cerqueira, J. J., & Almeida, A. (2009). The impact of age on emotional and cognitive behaviours triggered by experimental neuropathy in rats. *Pain*, *144*(1-2), 57–65.

Leite-Almeida, H., Cerqueira, J. J., Wei, H., Ribeiro-Costa, N., Anjos-Martins, H., Sousa, N., Pertovaara, A., Almeida, A. (2012). Differential effects of left/right neuropathy on rats' anxiety and cognitive behavior. *Pain*, *153*(11), 2218–2225.

Leite-Almeida, H., Guimarães, M. R., Cerqueira, J. J., Ribeiro-Costa, N., Anjos-Martins, H., Sousa, N., & Almeida, A. (2014). Asymmetric c-fos expression in the ventral orbital cortex is associated with impaired reversal learning in a right-sided neuropathy. *Molecular Pain*, *10*, 41, 1-6.

Leite-almeida, H., Melo, A., Pêgo, J. M., Bernardo, S., Milhazes, N., Borges, F., & Sousa, N. (2013). Variable delay-to-signal : a fast paradigm for assessment of decision and response impulsivity in rodents Variable delay-to-signal : a fast paradigm for assessment of decision and response impulsivity in rodents. *Frontiers in Behavioral Neuroscience*, *7*(154), 1-9.

Lima, D. (2002). The medullary dorsal reticular nucleus as a pronociceptive centre of the pain control system. *Progress in Neurobiology*, *66*(2), 81–108.

Ling, J., Campbell, C., Heffernan, T. M., & Greenough, C. G. (2007). Short-term prospective memory deficits in chronic back pain patients. *Psychosomatic Medicine*, *69*(2), 144–148.

López-Avila, A., Coffeen, U., Ortega-Legaspi, J. M., del Angel, R., & Pellicer, F. (2004). Dopamine and NMDA systems modulate long-term nociception in the rat anterior cingulate cortex. *Pain*, *111*(1-2), 136–143.

Lorenz, J., Minoshima, S., & Casey, K. L. (2003). Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. *Brain*, *126*(5), 1079–1091.

Louilot, A., Le Moal, M., & Simon, H. (1986). Differential reactivity of dopaminergic neurons in the nucleus accumbens in response to different behavioral situations. An in vivo voltammetric study in free moving rats. *Brain Research*, *397*(2), 395–400.

Luerding, R., Weigand, T., Bogdahn, U., & Schmidt-Wilcke, T. (2008). Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. *Brain : A Journal of Neurology*, *131*(Pt 12), 3222–3231.

Lutz, J., Jäger, L., de Quervain, D., Krauseneck, T., Padberg, F., Wichnalek, M., Beyer, A., Stahl, R., Zirngibl, B., Morhard, D., Reiser,M., Schelling, G. (2008). White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. *Arthritis and Rheumatism*, *58*(12), 3960–3960.

Magnusson, J. E., & Fisher, K. (2000). The involvement of dopamine in nociception: the role of D(1) and D(2) receptors in the dorsolateral striatum. *Brain Research*, *855*(2), 260–266.

Maihöfner, C., Handwerker, H. O., Neundörfer, B., & Birklein, F. (2003). Patterns of cortical reorganization in complex regional pain syndrome. *Neurology*, *61*(12), 1707–1715.

Mansikka, H., Erbs, E., Borrelli, E., & Pertovaara, A. (2005). Influence of the dopamine D2 receptor knockout on pain-related behavior in the mouse. *Brain Research*, *1052*(1), 82–87.

Marchand, S. (2008). The physiology of pain mechanisms: from the periphery to the brain. *Rheumatic Diseases Clinics of North America*, *34*(2), 285–309.

Martenson, M. E., Cetas, J. S., & Heinricher, M. M. (2009). A possible neural basis for stress-induced hyperalgesia. *Pain*, *142*(3), 236–244.

Matsuzawa-Yanagida, K., Narita, M., Nakajima, M., Kuzumaki, N., Niikura, K., Nozaki, H., ... Suzuki, T. (2008). Usefulness of antidepressants for improving the neuropathic pain-like state and pain-induced anxiety through actions at different brain sites. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *33*(8), 1952–1965.

May, A. (2008). Chronic pain may change the structure of the brain. *Pain*, *137*(1), 7–15.

Mazzoni, P., Shabbott, B., & Cortés, J. C. (2012). Motor control abnormalities in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(6), a009282.

Menefee, L. A., Cohen, M. J., Anderson, W. R., Doghramji. K., Frank, E. D., Lee, H. (2000). Sleep Disturbance and Nonmalignant Chronic Pain: A Comprehensive Review of the Literature. *Pain Medicine*, *1*(2), 156–1572.

Merskey, H. & Bogduk, N. (1994). Part III: Pain Terms, A Current List with Definitions and Notes on Usage*. Classification of Chronic Pain: descriptions of chronic pain syndromes and definitions of pain terms*, pp. 209-214.

Merzenich, M. M., Nelson, R. J., Stryker, M. P., Cynader, M. S., Schoppmann, A., & Zook, J. M. (1984). Somatosensory cortical map changes following digit amputation in adult monkeys. *The Journal of Comparative Neurology*, *224*(4), 591–605.

Millan, M. J. (1999). The induction of pain: an integrative review. *Progress in Neurobiology*, *57*(1), 1–164.

Millan, M. J. (2002). Descending control of pain. *Progress in Neurobiology*, *66*(6), 355–474.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., & Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiological Reviews*, *78*(1), 189–225.

Molochnikov, I., & Cohen, D. (2014). Hemispheric differences in the mesostriatal dopaminergic system. *Frontiers in Systems Neuroscience*, *8*, 110.

Moreira, R. (2011). The efficacy and tolerability of bupropion in the treatment of major depressive disorder. *Clinical Drug Investigation*, *31 Suppl 1*, 5–17.

Moriarty, O., McGuire, B. E., & Finn, D. P. (2011). The effect of pain on cognitive function: a review of clinical and preclinical research. *Progress in Neurobiology*, *93*(3), 385–404.

Morin, C. M., Gibson, D., Wade, J., (1998). Self-reported sleep and mood disturbance in chronic pain patients. *Clinical Journal of Pain, 14*(4), 311-314.

Müller, U., von Cramon, D. Y., & Pollmann, S. (1998). D1- versus D2-receptor modulation of visuospatial working memory in humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(7), 2720–2728.

Nagasako, E. M., Oaklander, A. L., & Dworkin, R. H. (2003). Congenital insensitivity to pain: an update. *Pain*, *101*(3), 213–219.

Neugebauer, V., Galhardo, V., Maione, S., & Mackey, S. C. (2009). Forebrain pain mechanisms. *Brain Research Reviews*, *60*(1), 226–242.

Nowak, G. (1989). Lateralization of neocortical dopamine receptors and dopamine level in normal Wistar rats. *Polish Journal of Pharmacology and Pharmacy*, *41*(2), 133–137.

Obermann, M., Rodriguez-Raecke, R., Naegel, S., Holle, D., Mueller, D., Yoon, M. S., Theysohn, N., Blex, S., Diener, H. C., Katsarava, Z. (2013). Gray matter volume reduction reflects chronic pain in trigeminal neuralgia. *NeuroImage*, *74*, 352–358.

Oliveras, J. L., Hosobuchi, Y., Redjemi, F., Guilbaud, G., & Besson, J. M. (1977). Opiate antagonist, naloxone, strongly reduces analgesia induced by stimulation of a raphe nucleus (centralis inferior). *Brain Research*, *120*(2), 221–229.

Osman, M., Ryterska, A., Karimi, K., Tu, L., Obeso, I., Speekenbrink, M., & Jahanshahi, M. (2014). The effects of dopaminergic medication on dynamic decision making in Parkinson's disease. *Neuropsychologia*, *53*, 157–164.

Ossipov, M., Dussor, G., & Porreca, F. (2010). Central modulation of pain. *The Journal of Clinical Investigation, 120*(11), 3779–3787.

Ossipov, M. H. (2012). The perception and endogenous modulation of pain. *Scientifica*, *2012*, 1–25.

Ottoni, E. B. (2000). EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions. *Behavior Research Methods, Instruments, & Computers : A Journal of the Psychonomic Society, Inc*, *32*(3), 446–449.

Pardey, M. C., Kumar, N. N., Goodchild, A. K., & Cornish, J. L. (2013). Catecholamine receptors differentially mediate impulsive choice in the medial prefrontal and orbitofrontal cortex. *Journal of Psychopharmacology (Oxford, England)*, *27*(2), 203–212.

Pavese, N. (2012). PET studies in Parkinson's disease motor and cognitive dysfunction. *Parkinsonism & Related Disorders*, *18 Suppl 1*, 96–99.

Pertovaara, A. (2006). Noradrenergic pain modulation. *Progress in Neurobiology*, *80*(2), 53–83.

Pertovaara, A., Martikainen, I. K., Hagelberg, N., Mansikka, H., Någren, K., Hietala, J., & Scheinin, H. (2004). Striatal dopamine D2/D3 receptor availability correlates with individual response characteristics to pain. *The European Journal of Neuroscience*, *20*(6), 1587–1592.

Ploner, M., Freund, H.-J., & Schnitzler, A. (1999). Pain affect without pain sensation in a patient with a postcentral lesion. *Pain*, *81*(1-2), 211–214.

Pons, T. P., Garraghty, P. E., Ommaya, A. K., Kaas, J. H., Taub, E., & Mishkin, M. (1991). Massive cortical reorganization after sensory deafferentation in adult macaques. *Science*, *252*(5014), 1857–1860.

Porreca, F. (2002). Chronic pain and medullary descending facilitation. *Trends in Neurosciences*, *25*(6), 319–325.

Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730–32.

Potvin, S., Larouche, A., Normand, E., de Souza, J. B., Gaumond, I., Grignon, S., & Marchand, S. (2009). DRD3 Ser9Gly polymorphism is related to thermal pain perception and modulation in chronic widespread pain patients and healthy controls. *The Journal of Pain : Official Journal of the American Pain Society*, *10*(9), 969–975.

Potvin, S., & Marchand, S. (2008). Hypoalgesia in schizophrenia is independent of antipsychotic drugs: a systematic quantitative review of experimental studies. *Pain*, *138*(1), 70–78.

Pripfl, J., Neumann, R., Köhler, U., & Lamm, C. (2013). Effects of transcranial direct current stimulation on risky decision making are mediated by "hot" and "cold" decisions, personality, and hemisphere. *The European Journal of Neuroscience*, *38*(12), 3778–3785.

Quintão, N. L. M., Balz, D., Santos, A. R. S., Campos, M. M., & Calixto, J. B. (2006). Long-lasting neuropathic pain induced by brachial plexus injury in mice: Role triggered by the pro-inflammatory cytokine, tumour necrosis factor alpha. *Neuropharmacology*, *50*(5), 614–620.

Raghavendra, V., Tanga, F., & DeLeo, J. A. (2003). Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *The Journal of Pharmacology and Experimental Therapeutics*, *306*(2), 624–6230.

Rainville, P., Duncan, G. H., Price, D. D., Carrier, B., & Bushnell, M. C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science*, *277*(5328), 968–971.

Rasu, R. S., Vouthy, K., Crowl, A. N., Stegeman, A. E., Fikru, B., Bawa, W. A., & Knell, M. E. (2014). Cost of pain medication to treat adult patients with nonmalignant chronic pain in the United States. *Journal of Managed Care Pharmacy : JMCP*, *20*(9), 921–928.

Ren, W.-J., Liu, Y., Zhou, L. J., Li, W., Zhong, Y., Pang, R. P., Xin, W. J., Wei, X. H., Wang, J., Zhu, H. Q., Wu, C. Y., Qin, Z. H., Liu, G., Liu, X. G. (2011). Peripheral nerve injury leads to working memory deficits and dysfunction of the hippocampus by upregulation of TNF-α in rodents. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *36*(5), 979–992.

Reyes Del Paso, G. A., Pulgar, A., Duschek, S., & Garrido, S. (2012). Cognitive impairment in fibromyalgia syndrome: the impact of cardiovascular regulation, pain, emotional disorders and medication. *European Journal of Pain (London, England)*, *16*(3), 421–429.

Reynolds, D. V. (1969). Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science*, *164*(3878), 444–445.

Richardson, D. E., & Akil, H. (1977). Pain reduction by electrical brain stimulation in man. Part 1: Acute administration in periaqueductal and periventricular sites. *Journal of Neurosurgery*, *47*(2), 178–183.

Riva, R., Mork, P. J., Westgaard, R. H., Okkenhaug Johansen, T., & Lundberg, U. (2012). Catecholamines and heart rate in female fibromyalgia patients. *Journal of Psychosomatic Research*, *72*(1), 51–57.

Robbins, T. W., & Roberts, a C. (2007). Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cerebral Cortex (New York, N.Y. : 1991)*, *17 Suppl 1*, i151–60.

Rodgers, R. J., Nikulina, E. M., & Cole, J. C. (1994). Dopamine D1 and D2 receptor ligands modulate the behaviour of mice in the elevated plus-maze. *Pharmacology, Biochemistry, and Behavior*, *49*(4), 985–995.

Rogers, R. D. (2011). The roles of dopamine and serotonin in decision making: evidence from pharmacological experiments in humans. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *36*(1), 114–32. doi:10.1038/npp.2010.165

Saadé, N. E., Atweh, S. F., Bahuth, N. B., & Jabbur, S. J. (1997). Augmentation of nociceptive reflexes and chronic deafferentation pain by chemical lesions of either dopaminergic terminals or midbrain dopaminergic neurons. *Brain Research*, *751*(1), 1–12.

Savitz, J., Solms, M., & Ramesar, R. (2006). The molecular genetics of cognition: dopamine, COMT and BDNF. *Genes, Brain, and Behavior*, *5*(4), 311–328.

Schestatsky, P., Kumru, H., Valls-Solé, J., Valldeoriola, F., Marti, M. J., Tolosa, E., & Chaves, M. L. (2007). Neurophysiologic study of central pain in patients with Parkinson disease. *Neurology*, *69*(23), 2162–2169.

Schiff, B. B., & Gagliese, L. (1994). The consequences of experimentally induced and chronic unilateral pain: reflections of hemispheric lateralization of emotion. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *30*(2), 255–267.

Schmidt-Wilcke, T., Leinisch, E., Gänssbauer, S., Draganski, B., Bogdahn, U., Altmeppen, J., & May, A. (2006). Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. Pain, 125(1-2), 89–97.

Schneider, L. H., Murphy, R. B., & Coons, E. E. (1982). Lateralization of striatal dopamine (D2) receptors in normal rats. *Neuroscience Letters*, *33*(3), 281–284.

Scott, D. J., Stohler, C. S., Koeppe, R. A., & Zubieta, J.-K. (2007). Time-course of change in [11C]carfentanil and [11C]raclopride binding potential after a nonpharmacological challenge. *Synapse*, *61*(9), 707–714.

Seeman, P. (1987). Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse*, *1*(2), 133–152.

Seifert, F. (2012). Decision making in the chronic pain patient (and rodent): contribution of the orbitofrontal cortex. Pain, 153(8), 1553–1554.

Seminowicz, D. a, Laferriere, A. L., Millecamps, M., Yu, J. S. C., Coderre, T. J., & Bushnell, M. C. (2009). MRI structural brain changes associated with sensory and emotional function in a rat model of long-term neuropathic pain. *NeuroImage*, *47*(3), 1007–1014.

Seminowicz, D. A., Wideman, T. H., Naso, L., Hatami-Khoroushahi, Z., Fallatah, S., Ware, M. A., Jarzem, P., Bushnell, M. C., Shir, Y., Ouellet, J. A., Stone, L. S. (2011). Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(20), 7540–7550.

Shah, T. H., & Moradimehr, A. (2010). Bupropion for the treatment of neuropathic pain. *The American Journal of Hospice & Palliative Care*, *27*(5), 333–336.

Sheng, H. Y., Qu, C. L., Huo, F.-Q., Du, J. Q., & Tang, J. S. (2009). D2-like but not D1-like dopamine receptors are involved in the ventrolateral orbital cortex-induced antinociception: a GABAergic modulation mechanism. *Experimental Neurology*, *215*(1), 128–134.

Silva, M. A. de S., Topic, B., Lamounier-Zepter, V., Huston, J. P., Tomaz, C., & Barros, M. (2007). Evidence for hemispheric specialization in the marmoset (Callithrix penicillata) based on lateralization of behavioral/neurochemical correlations. *Brain Research Bulletin*, *74*(6), 416–428.

Simons, L. E., Elman, I., & Borsook, D. (2014). Psychological processing in chronic pain: a neural systems approach. *Neuroscience and Biobehavioral Reviews*, *39*, 61–78.

Sindrup, S. H., & Jensen, T. S. (1999). Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain*, *83*(3), 389–400.

Sohn, J. H., Lee, B. H., Park, S. H., Ryu, J. W., Kim, B. O., & Park, Y. G. (2000). Microinjection of opiates into the periaqueductal gray matter attenuates neuropathic pain symptoms in rats. *Neuroreport*, *11*(7), 1413–1416.

Sommer, C. (2004). Serotonin in pain and analgesia: actions in the periphery. *Molecular Neurobiology*, *30*(2), 117–1125.

Sommer, C., Petrausch, S., Lindenlaub, T., & Toyka, K. V. (1999). Neutralizing antibodies to interleukin 1-receptor reduce pain associated behavior in mice with experimental neuropathy. *Neuroscience Letters*, *270*(1), 25–28.

Sorkin, L. S., & Doom, C. M. (2000). Epineurial application of TNF elicits an acute mechanical hyperalgesia in the awake rat. *Journal of the Peripheral Nervous System : JPNS*, *5*(2), 96–100.

Stopper, C. M., Khayambashi, S., & Floresco, S. B. (2013). Receptor-specific modulation of risk-based decision making by nucleus accumbens dopamine. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *38*(5), 715–728.

Strobel, C., Hunt, S., Sullivan, R., Sun, J., & Sah, P. (2014). Emotional regulation of pain: the role of noradrenaline in the amygdala. *Science China. Life Sciences*, *57*(4), 384–390.

Study, R. E., & Kral, M. G. (1996). Spontaneous action potential activity in isolated dorsal root ganglion neurons from rats with a painful neuropathy. *Pain*, *65*(2-3), 235–242.

Sullivan, R. M., Dufresne, M. M., Siontas, D., Chehab, S., Townsend, J., & Laplante, F. (2014). Mesocortical dopamine depletion and anxiety-related behavior in the rat: Sex and hemisphere differences. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *54*, 59–66.

Sullivan, R. M., & Gratton, A. (2002). Prefrontal cortical regulation of hypothalamic–pituitary–adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology*, *27*(1-2), 99–114.

Sun, Q., Tu, H., Xing, G.-G., Han, J.-S., & Wan, Y. (2005). Ectopic discharges from injured nerve fibers are highly correlated with tactile allodynia only in early, but not late, stage in rats with spinal nerve ligation. *Experimental Neurology*, *191*(1), 128–136.

Sunderland, S. (1993). Nerves and nerve injuries. London: Churchill Livingstone.

Suzuki, T., Amata, M., Sakaue, G., Nishimura, S., Inoue, T., Shibata, M., & Mashimo, T. (2007). Experimental neuropathy in mice is associated with delayed behavioral changes related to anxiety and depression. *Anesthesia and Analgesia*, *104*(6), 1570–1577.

Tang, N. K. Y., & Crane, C. (2006). Suicidality in chronic pain: a review of the prevalence, risk factors and psychological links. *Psychological Medicine*, *36*(5), 575–586.

Taylor, R. S. (2006). Epidemiology of refractory neuropathic pain. *Pain Practice : The Official Journal of World Institute of Pain*, *6*(1), 22–26.

Thiel, C. M., & Schwarting, R. K. (2001). Dopaminergic lateralisation in the forebrain: relations to behavioural asymmetries and anxiety in male Wistar rats. *Neuropsychobiology*, *43*(3), 192–199.

Tomer, R., Slagter, H. A., Christian, B. T., Fox, A. S., King, C. R., Murali, D., ... Davidson, R. J. (2014). Love to win or hate to Lose? Asymmetry of dopamine D2 receptor binding predicts sensitivity to reward versus punishment. *Journal of Cognitive Neuroscience*, *26*(5), 1039–1048.

Treede, R.-D., Jensen, T. S., Campbell, J. N., Cruccu, G., Dostrovsky, J. O., Griffin, J. W., ... Serra, J. (2008). Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology*, *70*(18), 1630–1635.

Treister, R., Pud, D., Ebstein, R. P., Laiba, E., Gershon, E., Haddad, M., & Eisenberg, E. (2009). Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans. *Pain*, *147*(1-3), 187–193.

Treister, R., Pud, D., & Eisenberg, E. (2013). The dopamine agonist apomorphine enhances conditioned pain modulation in healthy humans. *Neuroscience Letters*, (548), 115–119).

Vernaleken, I., Weibrich, C., Siessmeier, T., Buchholz, H.-G., Rösch, F., Heinz, A., Cumming, P., Stoeter, P., Bartenstein, P., Gründer, G. (2007). Asymmetry in dopamine D(2/3) receptors of caudate nucleus is lost with age. *NeuroImage*, *34*(3), 870–8.

Viguier, F., Michot, B., Hamon, M., & Bourgoin, S. (2013). Multiple roles of serotonin in pain control mechanisms–implications of 5-HT_7 and other 5-HT receptor types. *European Journal of Pharmacology*, *716*(1-3), 8–16.

Villemure, C., & Bushnell, M. C. (2002). Cognitive modulation of pain: how do attention and emotion influence pain processing? *Pain*, *95*(3), 195–199.

Walteros, C., Sánchez-Navarro, J. P., Muñoz, M. A., Martínez-Selva, J. M., Chialvo, D., & Montoya, P. (2011). Altered associative learning and emotional decision making in fibromyalgia. *Journal of Psychosomatic Research*, *70*(3), 294–301.

Wang, J., Goffer, Y., Xu, D., Tukey, D. S., Shamir, D. B., Eberle, S. E., Zou, A. H., Blanck, T. J., Ziff, E. B. (2011). A single subanesthetic dose of ketamine relieves depression-like behaviors induced by neuropathic pain in rats. *Anesthesiology*, *115*(4), 812–821.

Wasan, A. D., Anderson, N. K., & Giddon, D. B. (2010). Differences in pain, psychological symptoms, and gender distribution among patients with left- vs right-sided chronic spinal pain. *Pain Medicine*, *11*(9), 1373–1380.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews. Neuroscience*, *5*(6), 483–494.

Wood, P. B., Patterson, J. C., Sunderland, J. J., Tainter, K. H., Glabus, M. F., & Lilien, D. L. (2007). Reduced presynaptic dopamine activity in fibromyalgia syndrome demonstrated with positron emission tomography: a pilot study. *The Journal of Pain : Official Journal of the American Pain Society*, *8*(1), 51–58.

Woolf, C. J. (2010). What is this thing called pain? *The Journal of Clinical Investigation*, *120*(11), 3742–3744.

Woolf, C. J., & Mannion, R. J. (1999). Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet*, *353*(9168), 1959–1964.

Wrigley, P. J., Press, S. R., Gustin, S. M., Macefield, V. G., Gandevia, S. C., Cousins, M. J., Middleton, J.W., Henderson, L. A., Siddall, P. J. (2009). Neuropathic pain and primary somatosensory cortex reorganization following spinal cord injury. *Pain*, *141*(1-2), 52–59.

Wu, J., Xiao, H., Sun, H., Zou, L., & Zhu, L. Q. (2012). Role of dopamine receptors in ADHD: a systematic meta-analysis. *Molecular Neurobiology*, *45*(3), 605–620.

Xie, W., Strong, J. A., Meij, J. T. A., Zhang, J. M., & Yu, L. (2005). Neuropathic pain: early spontaneous afferent activity is the trigger. *Pain*, *116*(3), 243–256.

Xu, J. T., Xin, W. J., Zang, Y., Wu, C. Y., & Liu, X. G. (2006). The role of tumor necrosis factor-alpha in the neuropathic pain induced by Lumbar 5 ventral root transection in rat. *Pain*, *123*(3), 306–321.

Xu, Q., & Yaksh, T. L. (2011). A brief comparison of the pathophysiology of inflammatory versus neuropathic pain. *Current Opinion in Anaesthesiology*, *24*(4), 400–407.

Yalcin, I., Bohren, Y., Waltisperger, E., Sage-Ciocca, D., Yin, J. C., Freund-Mercier, M. J., & Barrot, M. (2011). A time-dependent history of mood disorders in a murine model of neuropathic pain. *Biological Psychiatry*, *70*(10), 946–953.

Zengin-Toktas, Y., Ferrier, J., Durif, F., Llorca, P. M., & Authier, N. (2013). Bilateral lesions of the nigrostriatal pathways are associated with chronic mechanical pain hypersensitivity in rats. *Neuroscience Research*, *76*(4), 261–264.

Zhuo, M., & Gebhart, G. F. (1997). Biphasic Modulation of Spinal Nociceptive Transmission From the Medullary Raphe Nuclei in the Rat. *J Neurophysiol*, *78*(2), 746–758.

Zhuo, M., & Gebhart, G. F. (2002). Modulation of noxious and non-noxious spinal mechanical transmission from the rostral medial medulla in the rat. *Journal of Neurophysiology*, *88*(6), 2928–2941.