

Neuromodulation of Infralimbic-Nucleus Accumbens Shell Circuit to Evaluate Neuropathic Pain-Related Effects on Cognitive Motifs

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Abstract

The corticostriatal circuit plays an important role in several cognitive processes, such as spatial working memory (WM). The medial prefrontal cortex (mPFC) sends dense projections to the *nucleus accumbens* (NAc), a major structure associated with reward-seeking behaviors, goal-directed learning, motivation, and emotional processing. Specifically, the infralimbic cortex-*nucleus accumbens shell* (IL-NAcSh) microcircuit is associated with distinct aspects of reward mnemonic information, relying on spatial data to define optimal behavioral strategies. Pain-related corticostriatal circuit functional connectivity unbalance is considered one source of WM impairments. However, these alterations are not fully understood.

In this thesis project, I intended to investigate the role of the IL-NAcSh circuit in mnemonic information encoding and the impact of this circuit, especially of the NAcSh, in chronic pain-related WM impairments. To address these aims, firstly I evaluated the impact of local NAcSh electrical lesions during the execution of a food-reinforced spatial WM alternation task. In my second task, I examined if chronic pain induced morphological alteration in the NAcSh by performing immunohistochemistry assays. During the full execution of this work, I used the rodent neuropathic pain model spared nerve injury (SNI) to assess the impact in behavioural performance.

The gathered results revealed that the NAcSh lesions and the pain model did not impact the performance of the animals, but caused disturbances in WM information processing and decision-making mechanisms. The animals subjected to the peripheral injury did not display motor impairments, as they exhibited distance, velocity, and patterns of navigation similar to non-injured animals. In addition to these results, SNI-treated animals presented lower mechanical noxious threshold, but did not reveal any differences in inhibitory neuron density.

These results suggest that the IL-NAcSh microcircuit participates in WM and decision-making processes, and may be considered an important therapeutic interface to reverse spatial WM-related deficits in persistent pain cases.

Keywords: working memory, neuropathic chronic pain, infralimbic, *nucleus accumbens* shell, electrical lesions, immunohistochemistry

Resumo

O circuito cortico-estriado tem um importante papel em diversos processos cognitivos, incluindo a memória de trabalho espacial (WM). O córtex pré-frontal medial (mPFC) envia densas projeções para o *núcleo accumbens* (NAc), uma estrutura essencial associada com a execução de comportamentos relacionados com a obtenção de recompensa, a aprendizagem orientada para um objetivo, a motivação e o processamento emocional. Especificamente, o microcircuito córtex infralímbico-*núcleo accumbens shell* (IL-NAcSh) está relacionado com detalhes distintos da informação mnemónica da recompensa, utilizando dados espaciais para definir as melhores estratégias comportamentais. Os desequilíbrios funcionais de conectividade no circuito cortico-estriado provocadas pela dor são considerados uma das fontes de défices de WM. No entanto, estas alterações não estão ainda totalmente compreendidas.

Neste projeto de tese, pretendi investigar o papel do microcircuito IL-NAcSh na codificação de informações mnemónicas e o impacto deste microcircuito, especialmente do NAcSh, nos défices de WM relacionados com a dor crónica. Para tal finalidade, avaliei primeiramente o impacto de lesões elétricas locais no NAcSh na realização de uma tarefa de alternância espacial de WM com reforço de comida. Na minha segunda tarefa, examinei através da realização de ensaios imunohistoquímicos se a dor crónica induziria alterações morfológicas no NAcSh. Durante a realização de todo o trabalho utilizei o modelo de dor neuropática em roedores *spared nerve injury* (SNI) para avaliar o impacto no desempenho comportamental.

Os resultados recolhidos revelaram que as lesões do NAcSh e o modelo de dor não tiveram impacto no desempenho dos animais, mas causaram distúrbios nos mecanismos de processamento de informação de WM e de tomada de decisão. Os animais submetidos à lesão periférica também não apresentaram deficiências motoras, pois exibiram distâncias, velocidades e padrões de navegação semelhantes aos animais não lesionados. Para além destes resultados, os animais SNI apresentaram limiares de dor mecânica inferiores, mas não revelaram quaisquer diferenças na densidade de neurónios inibitórios.

Estes resultados sugerem que o microcircuito IL-NAcSh participa em processos de WM e tomada de decisão, e pode apresentar uma importante interface terapêutica para a melhoria de défices relacionados com a WM espacial em casos de dor persistente.

Palavras-chave: memória de trabalho, dor crónica neuropática, infralímbico, *núcleo accumbens shell*, lesões elétricas, imuno-histoquímica.

Abbreviations

ANOVA	Analysis of variance
AP	Antero-posterior
CCI	Chronic constriction injury
D2R	Dopamine D2 receptor
DA	Dopamine
DAPI	4',6-diamidino-2-phenylindole
DNMS	Delay non-match to sample
DV	Dorso-ventral
F	Friedman one-way repeated measures ANOVA by ranks test
GABA	γ -aminobutyric acid
GAD67	Glutamic acid decarboxylase 67 kDa
IL	Infralimbic cortex
IP	Intraperitoneal
ks	Kolmogorov-Smirnov test
ks2	Two sample Kolmogorov-Smirnov test
KW	Kruskal-Wallis rank test
ML	Medio-lateral
mPFC	Medial prefrontal cortex
NAc	<i>Nucleus accumbens</i>
NAcC	<i>Nucleus accumbens core</i>
NAcSh	<i>Nucleus accumbens shell</i>
NMDA	N-methyl-D-aspartate
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline-Triton
PFA	Paraformaldehyde
PFC	Prefrontal cortex
RAM	Random access memory
SEM	Standard error of mean
SNI	Spared nerve injury

TNF- α Tumor necrosis factor alfa
WM Working memory

I. Introduction

I.1 Working memory

Working memory (WM) is a dynamic process crucial in the short-time maintenance of new information ^[1]. The constant manipulation and storage of active temporary information act as a basis for goal-directed behaviours. WM can be considered a transient updating source to knowledge previously attained, as retrieved memories are revised and transformed to plan forthcoming responses and ensure the best possible outcome ^{[2], [3]}. In a sense, it is similar to a RAM in a computer: once without power, the volatile memory is lost ^[4]. For example, phone numbers are quickly forgotten after written down to prevent the brain from becoming overloaded; however, the crucial information needed was maintained long enough to achieve the desired goal – storing the information acquired in a more reliable format.

Initial studies indicated that the medial prefrontal cortex (mPFC) has a critical role regarding WM maintenance ^{[5], [6]}. When presented with a stimulus, mPFC glutamatergic neurons exhibit increased spiking; moments after the stimulus disappearance, these neurons continue to spike, now at lower rates. This persistent firing activity during the delay period of WM tasks is considered by many to be an indication of the neural mechanism of information retention ^{[7], [8], [9], [10]}. However, persistent spiking is not only metabolically expensive, but also not efficient for optimizing WM information maintenance. The highly organized networks present time and spatial selectivity tuning, controlled by local γ -aminobutyric acid (GABA)-ergic interneurons ^{[11], [12], [13]}. These are in turn regulated by dopamine (DA)-ergic signalling arising from the mesolimbic pathway ^{[14], [15]}. At a cellular and network level, local GABAergic-mediated inhibition results in a mPFC neural activity regulation dependent on cognitive demand, influencing the information acquisition and their transmission to other associated brain areas ^{[11], [14]}. In other words, the input and output of the mPFC data depends on local inhibition levels to ensure accurate and timely responses. For instance, several studies showed that mPFC lesions or local disinhibition through reduced GABAergic and DAergic activity resulted in WM impairments ^{[11], [16], [17], [18], [19]}. By disrupting the mPFC in non-human primates and rats, the animals performed poorly on WM tasks ^{[5], [6]}. The results are

worsened in tasks with longer delay periods: the ability to maintain attentional performance and remember the choice made in the previous trial was reduced in animals with mPFC lesions [20], [21]. Even without mPFC manipulation, external interference (such as distractors) impact greatly the maintenance of information relevant to the on-going task [22]. This indicates an important central regulatory role of local PFC networks in encoding mnemonic information and potentiating its maintenance to execute the task successfully.

Nevertheless, the mPFC is only one portion in a complex of multiple regions that contribute to WM manipulation. Specific thalamic, parietal cortex and striatal neurons also display delay activity and support executive functions of WM [23]. On this subject, inactivation of subset neurons or lesions in these regions result in spatial WM impairments and decision-making deficits [24], [25], [26], [27].

In conclusion, WM is a complex cognitive process that requires the symbiosis of various brain regions' activity. Although a superior role for WM mechanisms has been attributed to the PFC, it cannot subside the role of other areas in WM processes and the crucial intercommunication necessary for transient information updating and strategic planning of impending action. To this day, a complete understanding of the underlying WM impairments observed in neuropsychological, neurodegenerative and chronic pain conditions remains lacking, and further investigation is mandatory.

I.1.1 Spatial working memory tasks in rodents

Rodents have a natural tendency to alternate locations: evolutionary, it is the most efficient strategy to search for food in the wild. The same behavior is found in spatial tasks, as they spontaneously alternate choices on successive trials in order to obtain rewards [28]. The animals use cues outside the maze and maintenance of information regarding the immediate previous trial to correctly plan the next action. There are plenty of tasks designed to evaluate WM mechanisms in rats and mice, but the following four described in detail will focus on spatial learning and memory.

Perhaps the most well-known spatial WM task is the Morris water maze. Navigating in a small pool, the animal must find a platform hidden beneath the water level in order to

rest. In the first trial, the animal greatly explores the maze to find the platform; however, successive trials rely on the temporary knowledge of the platform's location to orient their efforts directly to the platform, thus finding it faster ^[29].

Another task designed to evaluate spatial WM is the radial arm maze. In this case, the animal placed in the centre of the area is presented with a defined number of arms to explore and find food. To optimize the search for all the food contained in the maze, the animal must use previously acquired temporary information to separate explored and unvisited arms to avoid repeating the same locations. Spatial WM grants the animal the possibility to achieve the best possible outcome ^[30].

The development of the T-maze also pried on the natural alternation responses presented by rodents. The animal start in a single corridor and travel to a bifurcation, where they choose one of two arms. After the first trial a delay period is imposed, and the animal need to sustain the spatial WM information of the preceding trial and select the alternative arm. This ability to maintain this temporary data dictates the correct task execution and subsequent reward acquisition ^[31].

There are multiple adaptations to the original T-maze; on example is the eight-figure maze. The T-maze configuration was altered to include routes connecting the feeder locations to the central corridor. Beginning in the central corridor, the animal alternates between two goal locations by navigating in an infinity loop pattern, receiving incentives at goal sites and central corridor. The correct execution of this task relies on the maintenance of the spatial temporal knowledge of the goal location visited in the previous trial ^[32]. This maze has proven to be advantageous in assessing spatial WM in rodents, as it relies on the spontaneous alternation tendency displayed by rodents and requires less external manipulation than other mazes, thus reducing potential stress caused by constant animal repositioning. Simultaneously, it adds the possibility to evaluate delay period activity and behavior, extremely relevant in WM mechanisms.

I.2 The infralimbic-nucleus accumbens shell circuitry

The mPFC is not physiologically homogenous, and can be divided into three distinct subregions underlying different behavioral and cognitive roles: the anterior cingulate, prelimbic, and infralimbic (IL) cortices [33]. Particularly, the IL is considered a key emotional centre involved in cognitive processes such as decision-making and reward-directed learning. The emotional responses are regulated through the extinction of prior learned associations when they detrimentally influence the outcome [34]. It is hypothesised that the acquisition of new temporary information and update of old habits done by the IL supports advantageous behavioral strategies and allows greater exploration of the surrounding environment [34], [35]. In this regard, it has been reported that IL inactivation prevents the acquisition and expression of habitual behavior [36], [37]. Furthermore, IL activity is strictly associated to the facilitation of the establishment of alternative responses and learning mechanisms dependent on WM [38], [39]. In rats, disruption of the IL region resulted in lower performances in spatial tasks, corroborating the hypothesis that IL is involved in the organization of reward-seeking prospective actions [40]. IL selective lesions can also lead to an increased choice impulsivity and loss of flexible behavior, supporting the notion that IL is necessary for attention processes [37].

One important aspect is that long-range excitatory inputs and long-range projection neurons connect the mPFC to a plethora of regions in mostly reciprocal loops, including the thalamus, the hippocampus, the basolateral amygdala, the ventral tegmental area, and the striatum [41]. Regarding the efferents to the striatum specifically (the main output to the basal ganglia), the mPFC sends dense glutamatergic projections to the nucleus accumbens (NAc), a major structure present associated with reward-seeking behaviors, reinforcement learning, motivation, and emotional processing. The interface between limbic, prefrontal and motor regions established by the NAc provides a base for modulating goal-directed actions [42], [43], [44]. Most of the NAc constitution is composed of GABAergic medium spiny neurons (MSNs) – around 90-95% is occupied by these projecting inhibitory neurons [45], [46]. MSNs contain either DA1 or DA2 receptors (D1R and D2R), which have been associated with reward, aversion and locomotion [46]. The classical consensus of DA1-reward/DA2-aversion MSN roles continues to be defined

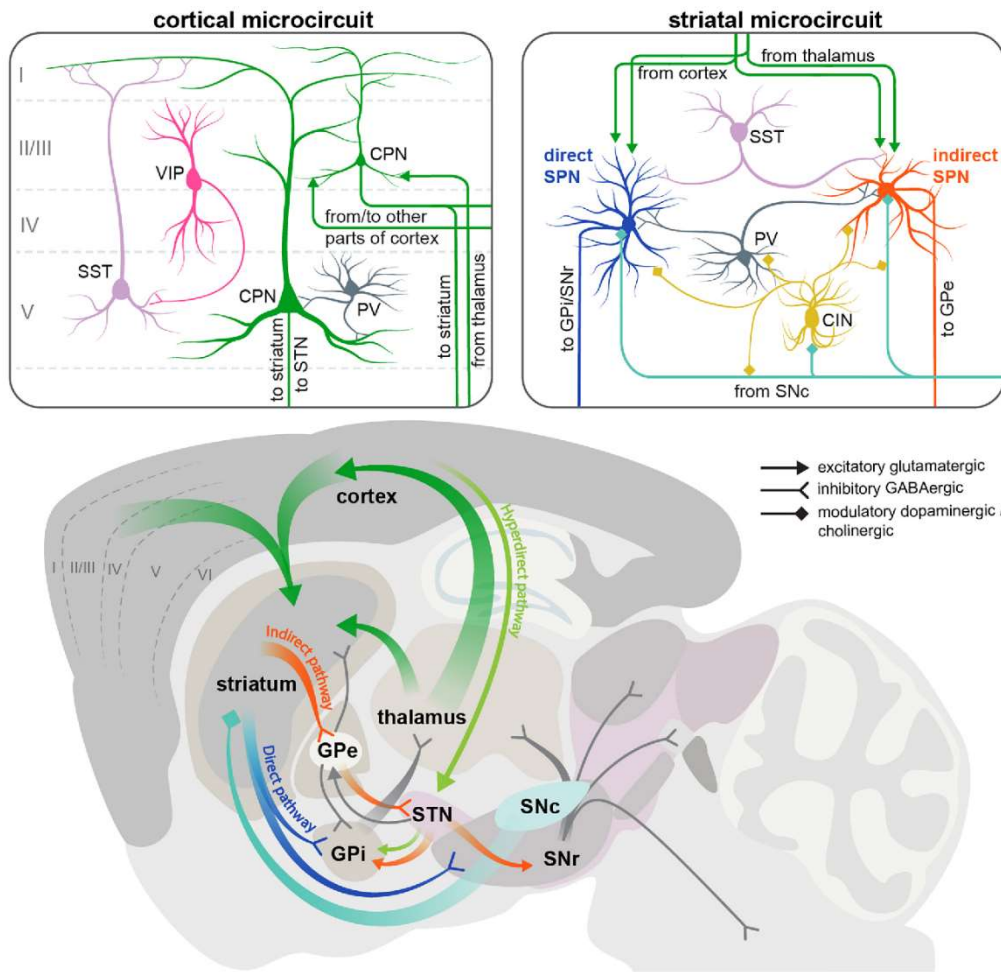


Figure I.2A – Scheme of cortical and striatal microcircuits.

Adapted from Blumenstock [47], S. and I. Dudanova, *Cortical and striatal circuits in Huntington's disease*. Frontiers in Neuroscience, 2020. 14: p. 82. [47]

Brain structures - GPe: external segment of the globus pallidus; GPi: internal segment of the globus pallidus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus. Interneurons - CIN: cholinergic interneuron; PV: parvalbumin interneuron; SST: somatostatin interneuron; VIP: vasointestinal peptide interneuron. Projection neurons - CPN: cortical pyramidal neuron; SPN: spiny projection neuron.

by recent studies [46], [48]. Based on functional and cytoarchitectural studies, the NAc can be divided into two different subregions, the core (NAcC) and the shell (NAcSh) [49], [50]. While the NAcC is mostly linked to discrete cue- and context-reward associations to regulate adequate behavior, the NAcSh is responsible for modulating reward-seeking behaviors through spatial information [51], [52], [53]. Studies involving selective lesions and pharmacological blockade of glutamatergic receptors to the NAcSh resulted in spatial information acquisition impairments and reduction in exploratory behaviour [54], [55].

The various connections involving the mPFC and the NAc establish a framework that can be integrated in the corticostriatal circuitry (**Figure 1.2A**). This cortex-striatum close relation serves as a basis for goal-oriented behaviors and decision-making, formation of habits, motivation and task-relevant WM information sorting ^{[56], [57], [58], [59]}. Striatal and cortical neurons are influenced by DA mesolimbic system. For instance, the ventral tegmental area and the substantia nigra send dopaminergic projections to the NAc, controlling information transmission regulation through the NAc and either facilitating or preventing goal-directed learning ^{[60], [61]}. Moreover, NAc modulation depends greatly on time specificity of mPFC-projecting neuron activation ^[60]. For example, the IL directly influences the NAcSh local activity through dense glutamatergic projections, which develop a pivotal role in the neuromodulation of neural ensembles that support reward-seeking behaviors, decision-making, and short-term spatial data for posterior memory integration ^{[37], [39], [52], [62], [63]}. Disrupting IL or NAcSh activity resulted in impaired performances and reduced capacity to retain spatial data ^{[40], [55]}. The infralimbic-nucleus accumbens shell (IL-NAcSh) microcircuit activity is therefore associated with distinct aspects of reward mnemonic information necessary to goal-directed behaviors, relying on spatial information to define optimal behavioral strategies.

1.3 The involvement of infralimbic-nucleus accumbens shell circuitry in pain processing

The official definition of pain describes “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” ^[64]; it cannot be merely associated with signal transmission and processing by neurophysiological processes (nociception) but encompasses psychological and social factors as well. Fundamentally, it is a notion learned through personal experience which ultimately warns the individual about tissue damage ^{[65], [66]}. Pain can be classified into acute (sudden, <3 months) or chronic (lasting longer than 3 months) ^[67]. Chronic pain has no survival-promoting benefits; on the contrary, most times diminishes quality of life due to suffering, catastrophizing and anticipation of future excruciating episodes ^{[65], [68]}. Persistent pain may accompany other symptoms is

various neurological disorders such as multiple sclerosis, Parkinson's disease, and central/peripheral traumatic injury [69]. In normal conditions, specialized neurons in the periphery (nociceptors) transmit nociceptive information through afferent nerve fibers to the dorsal horn of the spinal cord, which in turn relays the information to higher centers in the midbrain and forebrain for integration. However, in neuropathic cases, the organism cannot return to homeostasis: dysfunction or damage to peripheral or central nervous system leads to abnormal integration of nociceptive signals and aberrant activation of multiple brain structures [70]. In the long term, hyperalgesia and allodynia reflect central sensitization and changes in brain connectivity [65], [71]. Studies have identified structures such as thalamus, basal ganglia, mPFC and cerebellum as participants in the so-called "pain-matrix", establishing a connection between sensory and affective pain components and contributing to the conscious perception of pain [70], [72].

The evaluation of pain (or the conscious experience of pain) strongly depends on cognition, as it requires learning and recall of previous experiences. Patients and animal studies often report cognitive impairments derived from persistent pain: attention deficits, changes in decision-making processes, alteration of psychomotor ability, sleep disturbance, and disruption of learning and memory processes, including those associated with working memory [73], [74], [75], [76], [77]. Simple, daily tasks can be compromised due to pain-related WM deficits. Animal models have allowed a better understanding of how both inflammatory and neuropathic chronic pain hijack multiple brain mechanisms and induce WM impairments [78], [79], [80], [81]. For example, high pain-induced tumor necrosis factor alfa (TNF- α) concentrations in the hippocampal formation and reduced DA transmission by blockade of D2 receptors have been associated with WM deficits in chronic pain animals [79], [82]. Moreover, the hypodopaminergic state observed in chronic pain conditions can compromise WM processing in the NAc, since dopamine is essential for spatial WM maintenance in this area [83]. FMRI studies have also uncovered distinct chronic pain-induced connectivity changes between brain regions implicated in spatial WM, such as the mPFC and NAc [84], [85], [86]. The chemical, volumetric and connectivity chronic pain-related alterations observed in multiple brain

regions, including the corticostriatal circuitry, are considered the source of these cognitive impairments [84], [85], [87] (**Figure I.3A**).

The involvement of the NAcSh in persistent pain evaluation and pain encoding has been reported in multiple studies [88], [89], [90]. FMRI studies revealed that the ventral striatum can exhibit different connectivity clusters during nociceptive information processing [50], [91]. At the network level, D2R activation or local lidocaine administration reduced neuropathic behaviors, which indicates that GABAergic MSN neurons in the NAc have an important role in pain modulation and signal transmission through descending pain

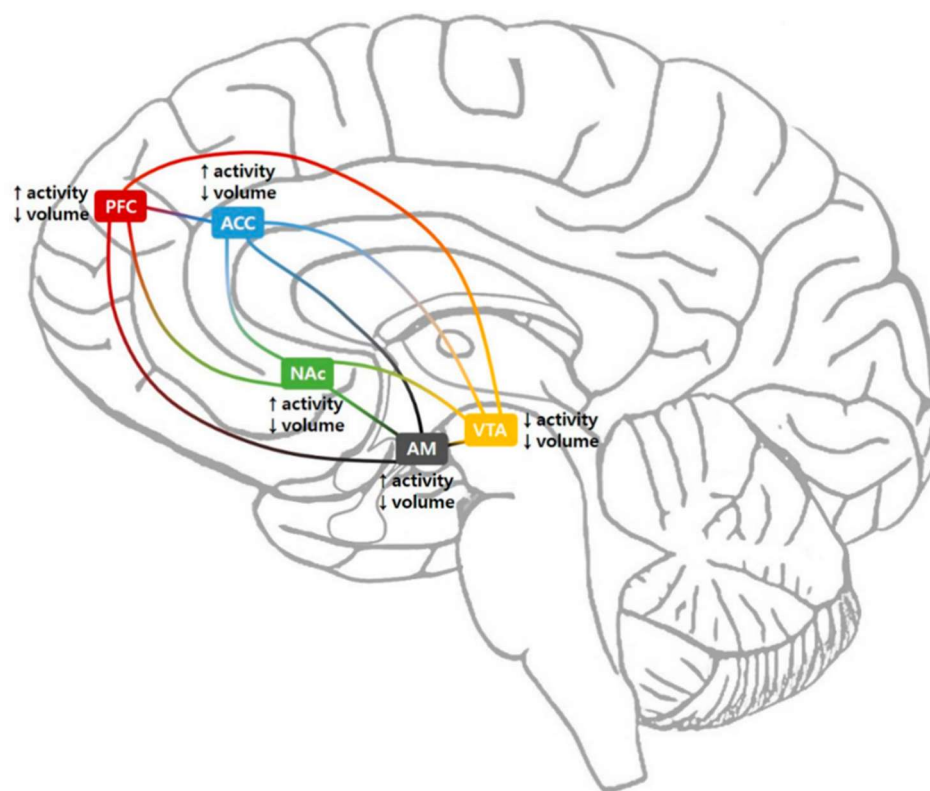


Figure I.3A – Scheme of particular pain-related structural and functional changes in the brain.

Adapted from Yang, S., et al., *Association between chronic pain and alterations in the mesolimbic dopaminergic system*. Brain Sciences, 2020. 10(10): p. 701.^[87]

ACC: anterior cingulate cortex; AM: amygdala; NAc: nucleus accumbens; PFC: prefrontal cortex; VTA: ventral tegmental area.

pathways [86], [92], [93]. On the other hand, the modulation of IL activity through infusion of brain-derived neurotrophic factor or metabotropic glutamate receptor antagonist alleviated pain with long-term effects [94], [95]. This indicates an important role of IL in

facilitating pain-related responses. The IL influence over the NAcSh could therefore enable more pronociceptive or more antinociceptive behaviors.

Increased evidence showed that persistent pain conditions led to an enhanced corticostriatal functional connectivity and impulsivity in humans and rodents [85], [96]. In fact, enhanced corticostriatal connectivity has been considered to be a marker to predict pain chronification [85], [97], [98]. Since these areas are closely related to executive functions such as cognitive flexibility and WM, it is possible the anatomical and connectivity alterations observed in chronic pain conditions are the basis of the pain-related cognitive impairments.

1.3.1 Peripheral neuropathic pain – Spared nerve injury animal model

Neuropathic pain conditions can arise from diverse disease types, which can indicate different physiological mechanisms for neuronal damage [99]. The study of neuropathy in clinical settings is often insufficient or inaccurate as patients often report multiple symptoms/diseases co-existing with persistent pain, introducing high variability to a restricted neuropathy-diagnosed group. Taking this into account, rodent models of neuropathic pain were developed to provide more information about the mechanisms and physiological alterations underlying neuropathic pain conditions. Chronic injury models mimicked human neuropathy of different etiologies and reproduced pain-related symptoms in animals, without any interference from other symptoms or diseases. Several peripheral neuropathy models have been established over the years, such as chronic constriction injury (CCI), spinal nerve ligation, or spared nerve injury (SNI).

In particular, in this thesis I used the spare nerve injury (SNI) model developed by Decosterd and Woolf in 2000 [100]. To perform this model, the common peroneal and tibial branches of the sciatic nerve are ligated and cut, leaving the sural nerve intact. The evaluation of pain hypersensitivity and allodynia is done in the somatotopic zone of the unaffected nerve (lateral portion of the plantar surface of the paw), which is an advantage over most neuropathic models. This model has a rapid pain onset and long-lasting robust effects (6 months to a year), which allow the evaluation of behavioral

responses over a dynamic period of time and better assess treatments for clinical purposes. Apart from this, the animals can still perform basic daily activities: eating, drinking, movement, and normal circadian rhythms are not affected by the SNI. Moreover, autotomy is not present (unlike more severe models), and a low local inflammatory response is observed (for example, lower than noted for CCI). Since it is performed unilaterally, the other (non-injured) paw can be used for comparison of behavioral responses and biochemical alterations with the SNI injured side ^[101].

Overall, the high reproducibility and simple surgical procedure make this an excellent model to study neuropathic-induced physiological and behavioral changes. Although with some limitations, the SNI simulates human neuropathophysiology and allows the evaluation of the plastic alterations correlated with neuropathic cases, including synapse transmission, glial activation, and central sensitization.

I.4 Research aims

The instability of the corticostriatal circuit connectivity present in chronic pain conditions is considered a source of WM deficits. Although the relation between IL-NAcSh is observed, a clearer insight of how neuropathic pain impacts this microcircuit and its associated cognitive function remains absent.

The aim of the current project was to understand the role of the IL-NAcSh circuit in mnemonic information encoding and the impact of this circuit in chronic pain-related WM impairments, particularly focusing on the following questions:

- Aim 1 – How does the malfunctioning of the NAcSh region influence spatial WM performance?
- Aim 2 – What is the impact of NAcSh dysfunction on peripheral neuropathy-related pain responses?
- Aim 3 – Does the neuropathic pain model induce long-term morphological changes in the NAcSh?

II. Research Materials and Methods

II.1. Animal model

Thirty-two adult CD male rats (Charles River Laboratories, France) weighing between 250 and 300 g were used to perform the behavioural procedures throughout my master thesis project (learning phase - $n=32$; probe sessions - Sham: $n= 8$, SNI: $n= 10$). The rats were kept in a 12h light/dark cycle (lights on at 8:00 AM) and were housed in individual ventilated cages (4 rats per cage; Tecniplast IVC – Sealsafe Plus Double Decker, Italy) with controlled temperature ($22\pm 2^{\circ}$ C) and humidity ($55\pm 5\%$). The rats were handled before any training and surgical procedures. The training and behavioural sessions occurred at approximately the same time each day, during the light phase of the circadian cycle. Their body weight was measured 2-3 times per week. The animals were maintained with a 90-95% food deprivation protocol of their *ad libitum* feeding body weights with free access to water throughout the experiments. All experiments were conducted in according to the European Union (2010/63/CE) legislation, and the Research and Ethical Issues of the International Association for the Study of Pain guidelines ^[102]. I made all the efforts to minimize animals suffering and to reduce the number of animals used.

II.2. Surgical procedures

II.2.1. Anaesthesia, stereotaxic placement and brain coordinates

All animals were anaesthetized using a mixture of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg) via intraperitoneal injection (I.P.). The rats were placed in a rodent stereotaxic frame after assessing appropriate anaesthesia levels (complete absence of corneal blink, tail-pinch reflexes and hindpaw withdrawal reflexes). I also used a homoeothermic blanket to maintain the core body temperature during the surgical procedures. The animals' head was fixed using stereotaxic ear bars and incisors bar, and the mandibular angle was adjusted when necessary to ensure the skull was centred and flat to perform the following procedures. To prevent excessive eye dryness

during surgery, eye lubricant was applied (Lubrithal, Dechra). The animal's fur in the top of the head was shaved, the area was cleaned with antiseptic solution (Betadine), and a rostral-caudal incision was made to expose the bone. A 2x2 mm² craniotomy correspondent to the desired site was done using a drill, and the *dura mater* was removed to facilitate the bipolar electrode insertion. The brain coordinates for the NAcSh region were obtained from the Paxinos and Watson (2006) ^[103] stereotaxic brain atlas: Anterior-Posterior (AP): +[1.6-1.8] to bregma, Medial-Lateral (ML): ±[0.4-0.9], and Dorsal-Ventral (DV): -[6.7- 7.0] mm.

II.2.2. NAcSh electrical tissue lesion

The NAcSh electrical lesions were done unilaterally using a bipolar electrode. The electrode was composed by two stainless steel Teflon-coated filaments (probe total diameter = 100-130 µm). The bipolar electrode was placed in a hydraulic micropositioner, and gradually inserted in the brain (~250 µm/min). After reaching the correct location, the bipolar electrode was maintained immobile for 10 minutes before initiating the stimulation protocol. The stimulation protocol consisted of electrical pulses of I=20 µA at 0.5 Hz during 20 sec. After the stimulation, the electrode was maintained immobile for an additional period of 5 minutes before withdrawing.

II.2.3. Rodent model of neuropathic pain

The spared nerve injury model (SNI) was chosen to induce peripheral neuropathic pain, and was performed according to the procedures described by Decosterd and Woolf (2000) ^[100]. This surgery was performed in the same intervention and immediately after the surgical protocol for NAcSh electrical lesion. Briefly, the sciatic nerve plexus of the hindpaw contralateral to the NAcSh lesion was exposed by skin incision and muscle blunt dissection. The tibial and common peroneal nerve branches were ligated with non-absorbent suture silk and a portion of 2-4 mm was axotomized. The sural nerve remained intact. The muscle was re-ligated using absorbent suture coated with polyglycolic acid. The control Sham group was subjected to the same skin incision and muscle dissection, but without nerve lesion. The nerve lesions performed were

counterbalanced between left and right sides. After surgery, the anaesthesia was reverted using atipamezole (5 mg/kg) injected subcutaneously. The analgesic ketoprofen (5-10 mg/kg) and the antibiotic enrofloxacin (5 mg/kg) diluted 1/10 in NaCl 9% mg/ml solution was administered subcutaneously every 24 h for 3-5 days. The rats were monitored daily regarding their overall health status, and were allowed to recover for a minimum of 7-10 days before behavioural testing sessions began.

II.2.3.1. Evaluation of mechanical responses

The sensory threshold for noxious mechanical stimulation was evaluated 1 hour after the end of every behavioural testing session using von Frey filaments (Somedic, Sweden). The traditional Dixon up-down method with von Frey filaments of logarithmically incremental stiffness (0.064, 0.085, 0.145, 0.32, 0.39, 1.10, 1.70, 3.30, 5.10, 8.30, 17.0, 24.0 g/mm²) was used to measure mechanical hypersensitivity as previously described ^[104]. The rats were individually placed in Plexiglas chambers over a mesh floor and acclimated for at least 10 minutes. Filaments were applied to the somatotopic region of the sural nerve of both paws. Fifty percent withdrawal thresholds were calculated as described previously ^[105].

II.3. Behavior assessment

II.3.1. Behavioral apparatus

Animals were tested in an eight-shaped arena composed by two reward locations (**Fig. II.3.1A**). This spatial working memory task was extracted from a previous study, where different neural activation related to working memory was assessed ^[32]. I used a 90x60 cm arena with 15 cm wide corridors and 30 cm high opaque walls, based on a previously described arena ^[73]. During the habituation period, rats started to explore the behavioral arena in groups of 4 animals, and after in pairs. Food reward pellets were used as motivators to illicit the exploratory activity throughout the whole apparatus. Before the exposition to the arena, the rats were placed in the open field (45x45 cm, 70° wall inclination) for 5-10 minutes before each training session. Pellet delivery automatization

and tracking navigation vectors, zone location and pellet dispenser activation recordings were done using the OpenControl software ^[106]. Behavioral video recordings were acquired using the Bonsai software ^[107].

Three different navigation zones were considered in this arena: the “choice” zone, corresponding the end of the delay zone and the zone preceding the reward locations; the “reward” zone, the zone where the animal receives the reward pellet for a correct alternation; and the “delay” zone, corresponding to navigation trajectory that mediates the navigation between the previous and prospective response. Additionally, for further behavioral analysis it was considered a “decision point” defined as the transition limit between the delay and choice zones.

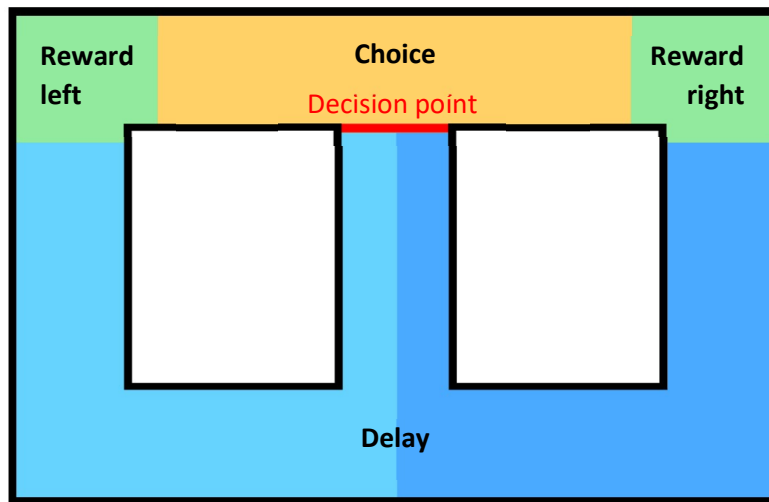


Figure II.3.1A – Scheme of the correct trajectory to travel in the eight-shaped maze.
Yellow area: choice zone; green areas: reward zone; blue areas: delay zone. Red line indicates the decision point.

II.3.2. Spatial alternation task

In the phase one of training, the rats were trained to start in the centre and reach alternatively (left or right) each reward location to receive a chocolate-flavour pellet. Choosing one of the reward locations, the rats must continue the trajectory opposite of the approach and return to the centre to be able to visit the other reward location. The rats were rewarded with a pellet per correct alternation. To teach the correct approaching trajectory rules, I began by placing the rats in the centre facing the choice zone in a 6 maze, blocking the entrance of one of the reward zones. After they made the

choice between left or right arms, the approaching corridor already travelled was blocked. Before the rats reached the centre corridor, I quickly shifted the barrier to block the other path in order to force the rats to navigate to the opposite feeder cup. This forced the rats to complete the maze circuit without repeating the same side multiple times. The 6-maze training stage took approximately 3-4 days to complete, depending on the learning capacity of each set.

After the 6-maze training completion, the rats entered the phase two of training: travelling through the maze without the barriers as guidance, allowing me to construct a learning curve. The rats completed the maze successfully if they travelled the space alternating reward sites and returned to the centre without repeating corridors. If the rat returned to the centre through the same path after eating the first pellet, the pellet was not dispensed (considered an error). The learning phase comprised of 10 days of 12-minute daily sessions. Only the rats with an average of 80% or higher correct trials in the last three sessions (days 8, 9 and 10) were selected to undergo the surgical procedures.

II.4. Histological validation of electrical lesions

To validate the extension of the electric lesions in the NAcSh, in the end of the experiments the rats were anaesthetised using sodium pentobarbital (60 mg/Kg, I.P.) and transcardiacally perfused with 0.1 M phosphate-buffered saline (PBS) for 15-20 minutes followed by paraformaldehyde (PFA) 4% w/v. The brains were collected and post fixated in PFA 4% for 4 hours. Before sectioning, brains were stored in a sucrose 30% w/v solution mixed with azide 1% w/v to prevent fungi growth. The collected brains were kept in the fridge and cut into coronal 30 or 60 μm slices using a freezing microtome (Leica Biosystems). For histological validation, I used 60 μm coronal slices, whereas for immunohistochemistry characterization, I used 30 μm coronal slices (see details below). The slices were stained using thionine to confirm the lesion locations, and then visualized using an optical light microscope. Only the rats with correct NAcSh lesion extension/location were considered for posterior analysis.

II.5. Immunohistochemistry

For immunohistochemistry phenotype characterization, the coronal slices of 30 μm were washed in PBS and incubated for 2 hours in 10% normal goat serum diluted in PBS-Triton (PBS-T). After washing with PBS-T, the slices were incubated for 48 hours in 4°C with an anti-glutamic acid decarboxylase 67 kDa (anti-GAD67) mouse monoclonal antibody (Cat. No. MAB5406, Merck Millipore) diluted 1:1200 in 2% normal goat serum with PBS-T. The sections were then washed in PBS-T and incubated with Alexa 488 goat anti-mouse antibody (Cat. No. A-32723, Thermofisher, Invitrogen) for 1 hour in 4°C, diluted 1:1500 in PBS-T. After the slices were rinsed with PBS, the cell nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI, 2 $\mu\text{g}/\text{mL}$ in glycerol-mounting media). Zeiss Z1 Apotome microscope was used to observe the stained sections. To measure integrated density (fluorescence intensity) values in the NAcSh area, images of the two brain hemispheres with 2.5x magnification were acquired. To visually compare GAD67⁺-expressing neuron density between brain hemispheres, 5 serial z-stacks of the NAcSh region were acquired at x40 magnification (1 μm thickness, 6-11 planes per image).

II.6. Experimental plan

II.6.1. Effect of NAcSh lesion in pain-related spatial working memory deficits

To evaluate the potential effects that arise from the lack of NAcSh proper functioning, I performed unilateral targeted lesions in this brain region. I divided the rats into two experimental groups (SNI and Sham); in each group, I performed electric lesions or control lesions (only electrode insertion in the NAcSh). The handling, maze exploration and 6-maze training described previously took between 8 and 12 days to complete. The rats had daily 12-minute sessions to facilitate the learning process (learning curve). I then performed the central NAcSh or control lesions, and the peripheral SNI or Sham lesions on the same surgery. After this procedure, the rats were allowed to recover for 7-10 days. Before the testing period began, I conducted 2 recall sessions of 5 minutes each to allow the rats to re-habituate to the testing arena. The behavioral probe sessions were conducted 14, 21 and 28 days after the surgical procedures. Each probe session

comprised 12 minutes in the maze. In the end of these sessions, the sensory threshold for noxious mechanical stimulation was evaluated for each rat.

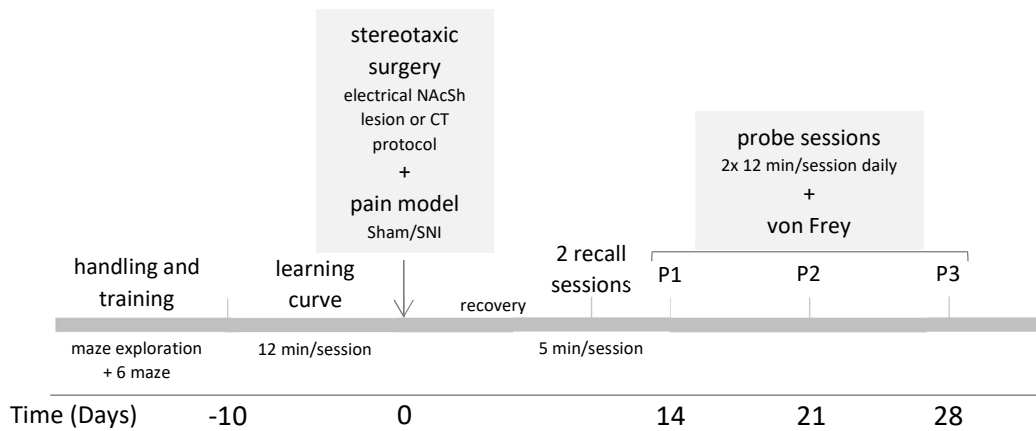


Figure II.6.1A – Experimental Timeline.

After handling, maze exploration and 6-maze training, the animals completed a 10-day learning curve to select the ones to undergo the surgical procedures (day 0). They were allowed to recover for at least 7 days. Two recall sessions were done prior to the first probe session to help the animals remember the task. The animals were tested 14, 21 and 28 days after the surgeries, and the noxious stimuli threshold assessed using the Von Frey filaments test.

II.6.2. Morphological characterization of the NAcSh phenotype of Sham- and SNI-treated rats

Immunohistochemical detection of GABAergic cells was used to assess morphological differences in the NAcSh area in Sham and SNI animals. After finishing the training sessions, the rats were perfused. Brains were collected (see details above) and cut into 30 μm coronal slices to perform the immunohistochemistry. The sections were incubated with primary antibody against GAD67 and secondary antibody Alexa 488 (see details above). Stained slices were observed using a fluorescence microscope. The images were acquired uniformly to prevent additional brightness and contrast manipulation between slices. Five image fields were acquired for each rat hemisphere. Contralateral and ipsilateral hemispheres to the peripheral lesion of each slice were analysed to compare potential differences in fluorescence intensity. Digital images were post-processed using the AxioVision and FIJI/ImageJ software to reduce the acquisition background.

II.7. Data analysis and representation

II.7.1 Behavioral data

Custom MATLAB scripts were used to analyse the following behavioral parameters: total number of trials (including correct left and/or right trials, incorrect trials, and omissions), accuracy rate (% of correct trials), mean response latency of feeder choice, total time spent per navigation zone, total distance travelled, and mean and instantaneous velocity. In the learning phase, the values for each parameter were calculated per animal and per testing session. In the 14-, 21- and 28-days post-surgery probe sessions, the two daily testing session results were determined for each session first and then averaged to obtain the final result to each animal.

Tracking navigation traces for each animal per testing session were obtained using the position coordinates of the animals throughout time acquired via OpenControl I/O software. Correct trials were counted when the animal travelled from the reward location to the path opposite of the approach, returned to the central corridor and chose the opposite reward zone; contrastingly, incorrect trials comprised the same trajectory up until the choice zone, but the animals select the same feeder location. Omissions included navigation routes where 1) the animals move from feeder cup to feeder cup, 2) go around the maze (after visiting the feeder location, the animals continue the path and do not cross the central corridor, but travel through the other corridor), and 3) do the intended path in reverse (from the choice zone, through the central corridor, chose one of the corridors and reach the feeder location).

The total number of trials of each animal in a testing session comprised correct trials, incorrect trials, and omissions performed in that session. To evaluate the impact of the central NAcSh manipulation and peripheral nerve lesion on task execution, the performance or accuracy rate of each session was calculated as the number of correct trials divided by the total number of trials, and expressed as a percentage. To examine period-specific variations in the values obtained for the total trial number and accuracy rate, each session was divided into three periods each with 4 consecutive minutes (hereby designated by early, middle and late periods).

To assess the effects of the central NAcSh manipulation and peripheral nerve lesion on retrospective memory preservation, the time elapsed between choice zone entrance (decision point) and feeder activation was determined – this was defined as the mean response latency per testing session. Feeder activation timestamps were collected via behavioral video recordings and/or accessed by OpenControl I/O software output. The variation of response latency throughout each probe session was evaluated by calculating the cumulative latency. The session was divided into twelve blocks of 1 minute, and the response latency within each block was averaged. The response latency values of each time frame were combined with the previous block (for example, the second cumulative value comprised the summation of the first and second latency values), and the last value measured (end of the session) was further analysed.

To evaluate oscillations in the motor activity of each rat during the maze navigation, the percentage of total time spent per navigation zone was calculated. The maze was divided initially into 7 areas: the 2 feeder locations (reward zone), the 2 lateral corridors and the central corridor until the choice zone (delay zone), the area between feeder locations (choice zone), and an extra area comprising any spatial locations outside those defined above (zones to exclude).

This last area provides a control record of any tracking changes that might have occurred during the task, and assure it is accurately tracking only the rat position in the maze. As such, the allocation to this area was residual (average 2.12% of all probe sessions, which includes the initial 10 seconds (approximately 1.39%) before putting the animal in the maze). The percentage of time per navigation area was calculated using the navigation position coordinates of the animals extracted using the OpenControl I/O software (bin resolution = 50 ms/frame). The number of tracking points in each of the 7 areas were selectively summed to result in the total number of positions per considered maze zone (reward, delay and choice). Each value was then divided by the total number of tracking points excluding those contained in the area outside the defined maze zones, and the final values expressed as a percentage.

The percentage of time spent in each area was calculated per animal and testing session. For the time spent per navigation zone, each video recorded file was divided into a 12x16

grid (grid square resolution = 40x40 dots per inch (dpi)), and each square was coloured between dark red (most time allocated) and dark blue (less time allocated). These heat maps were used to calculate the entropy index. To calculate the entropy index, I used the native Matlab function ^[108]. The average entropy index value was determined for each experimental contingency.

To evaluate potential motor impairments related to the NAcSh lesion and peripheral nerve injury, the total distance of navigation was determined using the Euclidean distance equation ($\text{distance} = \sqrt{((x_1 - x_0)^2 + (y_1 - y_0)^2)}$). The total distance measured per each testing session was then converted to real distance travelled. In order to do that, these values were multiplied by 0.2145 (pixel-real distance conversion constant - 1 pixel = 0.2145 cm).

The instantaneous velocity was calculated applying the same Euclidean distance equation, multiplying the values by 0.2145 (pixel-real distance conversion constant), and dividing each value by the OpenControl binning of 50 ms (0.05 s) to obtain the final values in cm/s. The normalization of instantaneous velocity was done by dividing each value by the maximum instantaneous velocity value obtained. The intra-maze representation of the normalized instantaneous velocity vector was done using the mean of each 5 consecutive values computed with a step of 1 bin. The activity vector of each rat that illustrates the oscillation of the instantaneous velocity during the maze navigation were represented with a colour from dark red (1, maximum velocity) to dark blue (0, minimum velocity). Finally, I calculated the mean navigation velocity of rat per behavior testing session.

II.7.2 Cell quantification data

In order to evaluate possible morphological alterations in tissue phenotype following peripheral nerve lesion, I promoted the quantification of fluorescence intensity in the NAcSh region. To perform this quantification, I executed immunohistochemistry assays. Microphotograph fields centred in the NAcSh were acquired with a magnification of 2.5X. The fluorescence intensity values were obtained per brain hemisphere, and the

difference between ipsi and contralateral hemispheres was measured per slice and later averaged per animal (4 slices/animal). The slice montage was done using the plugin “Grid/Collection Stitching” ^[109], available in the open-source FIJI/ImageJ software (version 1.53c). The measurement of fluorescence intensity was performed also using the same software by applying the native function “ROI manager”. The results were extracted to GraphPad Prism 8.0.1.

II.8. Statistical analysis

The Kolmogorov-Smirnov (*ks*) test with Dallal-Wilkinson-Lilliefors *p*-value correction (GraphPad Prism 8.0.1) was used to evaluate the normality of the data acquired. For data both under and without normality assumptions, for single comparisons I used the non-parametric Wilcoxon matched-pairs signed rank test (two-tailed); for multiple comparisons I used the non-parametric Kruskal-Wallis (*KW*) rank test and the non-parametric Friedman (*F*) one-way repeated measures ANOVA by ranks test, both followed by *post hoc* Dunn's multiple comparisons test. In order to compare the left and right correct trials distributions of the animals in the learning phase, I used the two-sample Kolmogorov-Smirnov test (*ks2*) statistics (MATLAB R13, MathWorks). Correlations between behavioral parameters were made applying the adequate test: under normality assumptions, I used the parametric Pearson coefficient test (two-tailed); without normality assumptions, I used the non-parametric Spearman coefficient test (two-tailed). To compare fluorescence intensity differences between Sham and SNI experimental groups, I used the non-parametric Mann-Whitney U ranks test (two-tailed). Level of significance was set as 5%. Results are presented as the mean $\pm$ standard error of mean (SEM).

III. Results

III.1. Spatial alternation task learning phase

All rats were trained during 10 consecutive days in an 8-figure spatial alternation task. Each daily session comprises 12 min of total duration. Several behavioral parameters were evaluated on each testing session: number of trials performed (including correct trials, incorrect trials, and omissions), percentage of correct trials performed, reward side preference (laterality of rewarded trials), and time spent per behavioral task navigation zone (**Fig. III.1**).

Fig. III.1A illustrates an example of the navigation phenotype of a rat during the beginning and the ending stages of the learning protocol. As shown, the rats performed more errors in early learning sessions, resulting in incorrect direct trajectories between feeders to collect rewards, and delay areas in order to initiate the following trial. As typically observed in goal-directed tasks, there was a phase of rapid improvement followed by a phase of stabilized learning (**Fig. III.1B**, left panel). In terms of the number of trials performed, the present data revealed an increase, but not significant (**Fig. III.1B**, central panel; Wilcoxon test, $p=0.1586$ (n.s.)), resulting also in a decrease of the number of omissions performed from the early (days 2-4; light blue) to the late (days 8-10; light yellow) phase of learning (**Fig. III.1B**, right panel; Wilcoxon test, $p<0.0001$) (For more details, see complementary **Table S.1**).

The rats also exhibited a global increase of the percentage of correct trials performed (**Fig. III.1C**, left panel) from early to late phase in learning (**Fig. III.1C**, right panel; Wilcoxon test, $p<0.0001$).

No significant differences in reward side preference were observed during learning (**Fig. III.1D**). The rats revealed a similar distribution of correct performed trials in either the left or right feeder (**Fig. III.1D**; two-sample Kolmogorov-Smirnov test, $ks2=0.20$, $p=0.9748$).

The percentage of time spent per navigation zone is shown in **Fig. III.1E** (left panel). As expected, globally rats spent more time navigating in the reward locations and in the delay area. Statistical analyses revealed that the rats decreased their percentage of time

spent navigating across the choice area (**Fig. III.1E**, fourth panel; Wilcoxon test, $p < 0,0001$), while increased their time spent in the reward location (**Fig. III.1E**, second panel; Wilcoxon test, $p < 0,0001$) from early to late phase in learning. Finally, the time percentage spent in the delay area did not exhibit a significant discrepancy (**Fig. III.1E**, third panel; Wilcoxon test, $p = 0.1600$ (n.s.)).

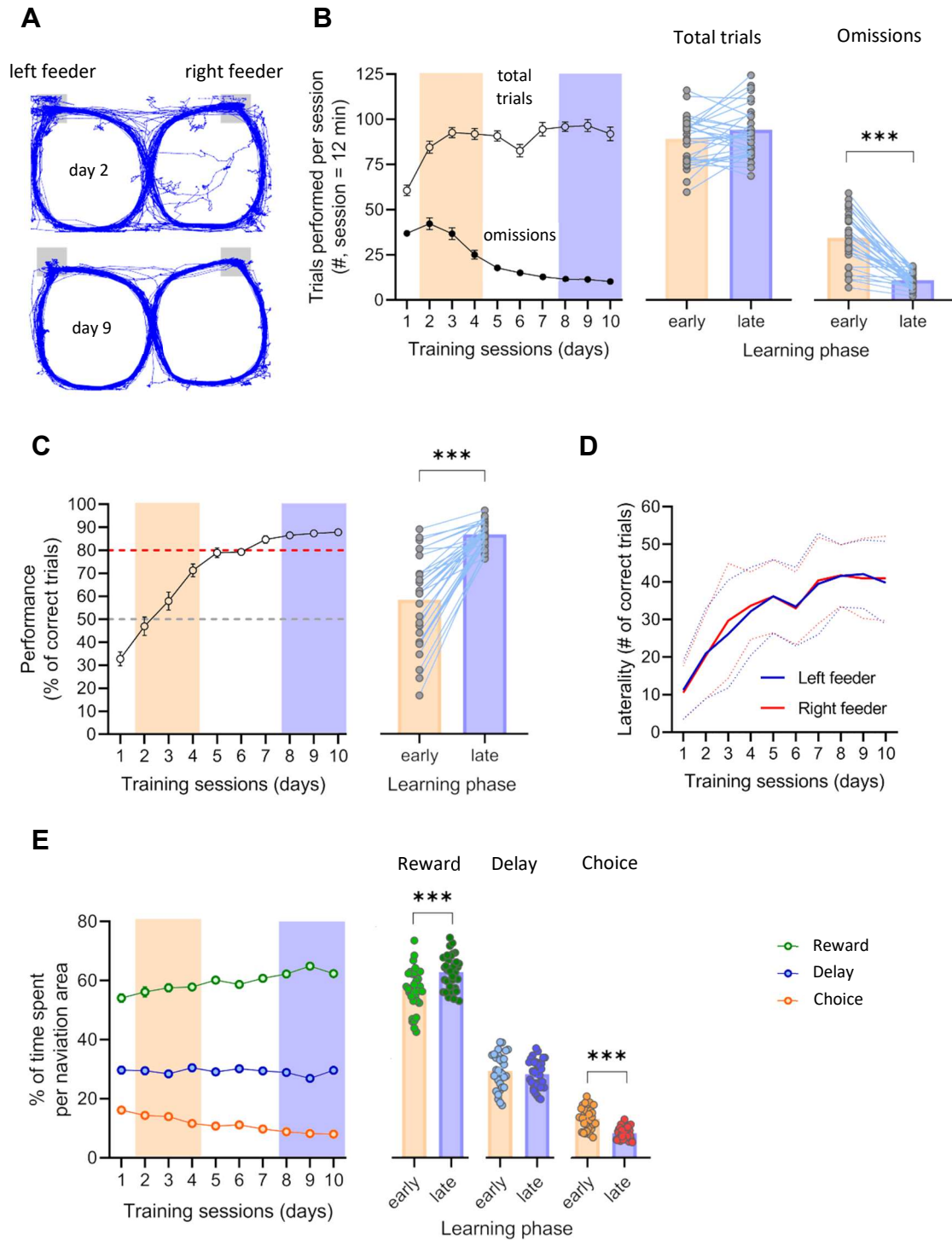


Figure III.1 – Spatial alternation 8-figure working memory task learning curve.

(A) Comparison of the navigation traces of a rat during day 2 and day 9 of the training protocol. The rat navigates more efficiently to obtain reward pellets at the end of the learning phase (note the lack of direct trajectories between feeder locations and delay areas at day 9, present in day 2). Upper left and right gray shaded corners indicate feeder location.

(B) Number of trials and omissions performed per daily testing session (duration = 12 min). Shaded regions denote the range of days from which the early (days 2-4, light blue) and late phase (days 8-10, light yellow) learning curve analyses were performed. The number of omissions performed for all rats decreased significantly from early phase to late phase in learning (right panel), but no significant change was observed in the number of trials performed (central panel).

(C) Mean percentage of correct trials for all rats across 10 daily testing sessions (left panel). Percentage of correct trials for all rats increased significantly from early phase to late phase in learning (right panel).
 (D) Reward side preference of correct performed trials. Rats did not exhibit significant preference for the left or right feeder during learning.
 (E) Percentage of time spent per navigation area (left panel). Rats did not exhibit significant changes between early to late phase during delay area (third panel) navigation, whereas decreased the time spent in the choice area (fourth panel) and increased in the reward location area (second panel).
 Comparisons between learning phases are based on non-parametric Wilcoxon matched-pairs signed rank test (two-tailed). Comparison of reward side preference distributions are based on two-sample Kolmogorov-Smirnov test ($ks2$) statistics was performed. Rats, $n=32$. Values are presented as mean $\pm$ SEM. *** $p<0.001$

III.2 Histological validation of NAcSh lesions

To validate the results obtained during the probe sessions, anatomic location and lesion extension verification was performed (**Fig. III.2**). Histological assessment revealed electrical lesions contained within the selected area, with reduction of number of cells and/or local fibrosis. Only animals with correct NAcSh lesion placement were considered for analysis.

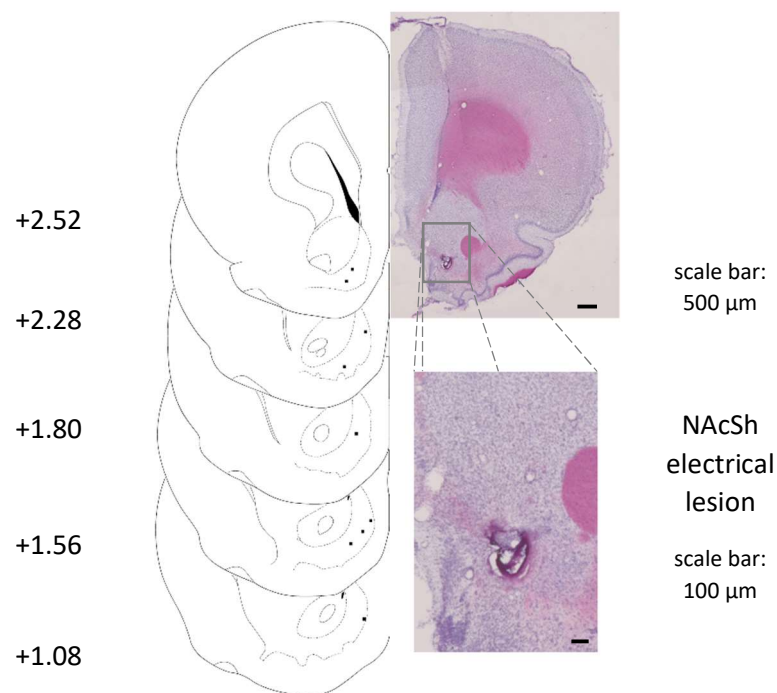


Figure III.2 – Histological NAcSh electrolytic lesion validation

Schematic representations and image example of histological sections for NAcSh electrical lesion assessment. Brain coordinates of coronal sections were assessed using the Paxinos and Watson stereotaxic brain atlas (distances calculated from bregma).

III.3. Spatial alternation task probe sessions

III.3.1. Effects of NAcSh lesions on pain-related working memory performance

After surgical procedures, the animals were placed in one of the four distinct experimental groups: Sham/CT, Sham/NAcSh lesion, SNI/CT and SNI/NAcSh lesion. All the groups were evaluated on working memory performance on the same 8-figure spatial alternation task. The animals were assessed on 2 daily probe sessions on days 14, 21 and 28 post-surgery, and the results averaged to minimize task-unrelated external influence. The results obtained in the probe sessions were compared to a control period (pre-surgery or baseline (CT)) which, in this case, was chosen the late phase of the learning phase. The impact of neuropathic pain and central NAcSh electrical lesions on WM performance were assessed using the following parameters: total number of trials performed (including correct, incorrect trials, and omissions), percentage of correct trials performed, and feeder choice latency (time-window measured between the decision limit and reward cup location) (**Fig. III.3.1**).

For each experimental group, when compared to their respective CT period (baseline), no significant effects were observed across testing sessions (**Fig. III.3.1A**; Friedman test (F): Sham/CT group, $F=3.3$, $p=0.3893$ (n.s.); Sham/NAcSh lesion group, $F=5.7$, $p=0.1411$ (n.s.); SNI/CT group, $F=3.0$, $p=0.4446$ (n.s.); and SNI/NAcSh lesion group: $F=4.2$, $p=0.2603$ (n.s.)). Additionally, no significant changes were observed when compared the behavioral traces of all experimental groups (**Fig. III.3.1A**; Kruskal-Wallis test (KW), $KW=13.19$, $p=0.2808$ (n.s.)). Despite this result, there is an important decrease (but not significant) in the number of trials performed in all experimental groups on day 14 post-surgery compared to the CT period. This can be justified by the absence of exposure to the testing arena during the post-surgery recovery period.

To further evaluate the intragroup variability during the testing days after surgical procedures, I divided each session into 3 different periods: early, middle, and late period. Each period comprised a block of 4 consecutive minutes. Regarding day 14 after surgery (**Fig. III.3.B, top panel**), no statistical differences were found when compared the trials distributions throughout the entire probe session for each experimental group

(Sham/CT group, $F=1.5$, $p= 0.6528$ (n.s.); Sham/NAcSh lesion group, $F=3.5$, $p= 0.2731$ (n.s.); SNI/CT group, $F=4.526$, $p= 0.1111$ (n.s.); SNI/NAcSh lesion group, $F=5.158$, $p= 0.0849$ (n.s.)). Furthermore, no significant changes were observed when evaluated the behavioral median activity between experimental groups ($KW= 10.12$, $p= 0.5198$ (n.s.)). In the case of day 21 (**Fig. III.3.B, centre panel**), statistical analysis revealed significant differences in the total number of trials performed throughout the testing session for SNI/NAcSh-lesioned animals ($F=6.4$, $p=0.0394$); moreover, the *post hoc* test showed a significant increase in the number of trials performed from the early to the late period during the day 21 (Dunn's multiple comparisons test, $p<0.05$). In addition, the comparison of the distribution of trials throughout each experimental group session revealed also no significant effect (Sham/CT group, $F=6.0$, $p=0.0694$ (n.s.); Sham/NAcSh lesion group, $F=0.9333$, $p=0.7778$ (n.s.); SNI/CT group, $F=1.2$, $p=0.6914$ (n.s.)). Furthermore, no significant changes were observed when compared the activity medians between experimental groups ($KW= 11.08$, $p= 0.4363$ (n.s.)). In the case of day 28 post-surgery (**Fig. III.3.B, bottom panel**), no statistical differences were found when compared the trials distributions throughout the entire probe session for each experimental group (Sham/CT group, $F=1.5$, $p= 0.6528$ (n.s.); Sham/NAcSh lesion group, $F=0.5$, $p= 0.9306$ (n.s.); SNI/CT group, $F=6.0$, $p= 0.0540$ (n.s.); SNI/NAcSh lesion group, $F=3.6$, $p= 0.1821$ (n.s.)). Moreover, no significant changes were observed when compared the activity medians between experimental groups ($KW= 7.448$, $p= 0.7617$ (n.s.)). Together, these results suggest that the animals globally maintained the number of trials performed within each probe session.

In terms of the number of omissions performed, each experimental group when compared to their respective CT period revealed no significant changes (**Fig. III.3.1C**; Sham/CT group, $F=2.1$, $p=0.6489$ (n.s.); Sham/NAcSh lesion group, $F=1.0$, $p=0.8585$ (n.s.); SNI/CT group, $F=1.163$, $p=0.7987$ (n.s.); and SNI/NAcSh lesion group, $F=3.98$, $p=0.2755$ (n.s.)). In addition, statistical analysis revealed no significant effects when compared the omissions behavioral traces of all groups ($KW=5.676$, $p=0.8941$ (n.s.)). A more detailed view regarding the number of trials and omissions performed is shown in complementary **Table S.2**. This result indicates that the number of omissions remained unaltered during pre- and post-surgery period.

To evaluate the impact of the central NAcSh manipulation and peripheral nerve lesion on the behavioral accuracy to perform the task, I calculated the percentage correct trials performed per session. These results are shown in **Fig. III.3.1D**. Globally, rats performed the behavioral task with performances above 80% during all probe sessions. For each experimental when compared the probe sessions performed after surgery with the CT period (before surgery), no significant differences were observed (Sham/CT group, $F=2.7$, $p=0.5076$; Sham/NAcSh lesion group, $F=1.5$, $p=0.7539$; SNI/CT group, $F=2.28$, $p=0.5612$; SNI/NAcSh lesion group, $F=2.52$, $p=0.5206$). In addition, statistical analysis revealed no significant effects when compared the omissions behavioral traces of all groups ($KW=11.23$, $p=0.4246$ (n.s.)).

To evaluate if the accuracy rate oscillates throughout the probe session, I again computed their activity by time-windows (early, middle and late blocks of 4 consecutive minutes; **Fig. III.3.1E**). In the case of day 14 (**Fig. III.3.1E, top panel**), no significant effect was observed (Sham/CT group, $F=2.7$, $p=0.5076$ (n.s.)); Sham/NAcSh lesion group, $F=1.5$, $p=0.7539$ (n.s.); SNI/CT group, $F=2.28$, $p=0.5612$ (n.s.); SNI/NAcSh lesion group, $F=2.52$, $p=0.5206$ (n.s.)). In respect to the behavioral activity medians of the experimental groups, statistical analyses also revealed no significant effect between them ($KW= 10.12$, $p=0.5198$ (n.s.)). For day 21 (**Fig. III.3.1E, central panel**), no significant differences were obtained for all groups throughout the time-window periods (Sham/CT group, $F=3.5$, $p=0.2731$ (n.s.); Sham/NAcSh lesion group, $F=2.533$, $p=0.3611$ (n.s.); SNI/CT group, $F=3.6$, $p=0.1821$ (n.s.); SNI/NAcSh lesion group, $F=2.8$, $p=0.3673$ (n.s.)). In respect to the behavioral activity medians of the experimental groups, statistical analyses also revealed no significant effect between them ($KW= 8.304$, $p=0.6859$ (n.s.)). Finally for day 28 (**Fig. III.3.1E, bottom panel**), no significant differences were obtained for all groups throughout the time-window periods (Sham/CT group, $F=0.0$, $p>0.9999$ (n.s.); Sham/NAcSh lesion group, $F=4.5$, $p=0.1250$ (n.s.); SNI/CT group, $F=0.4$, $p=0.9537$ (n.s.); SNI/NAcSh lesion group, $F=1.2$, $p=0.6914$ (n.s.)). In respect to the behavioral activity medians of the experimental groups, statistical analyses also revealed no significant effect between them ($KW= 8.708$, $p=0.6488$ (n.s.)).

Nevertheless, it is important to observe that intragroup variability diminished as probe sessions passed. On day 14, especially at the early period of the testing session, SNI/CT

group presents greater variability than the other groups. This could explain the lack of differences observed on day 14. Even though the animals presented identical performance values, this could be a consequence of ponderation at decision point. These animals could have changes in the time spent to choose and approach the correct cup, although they present a similar percentage of correct trials.

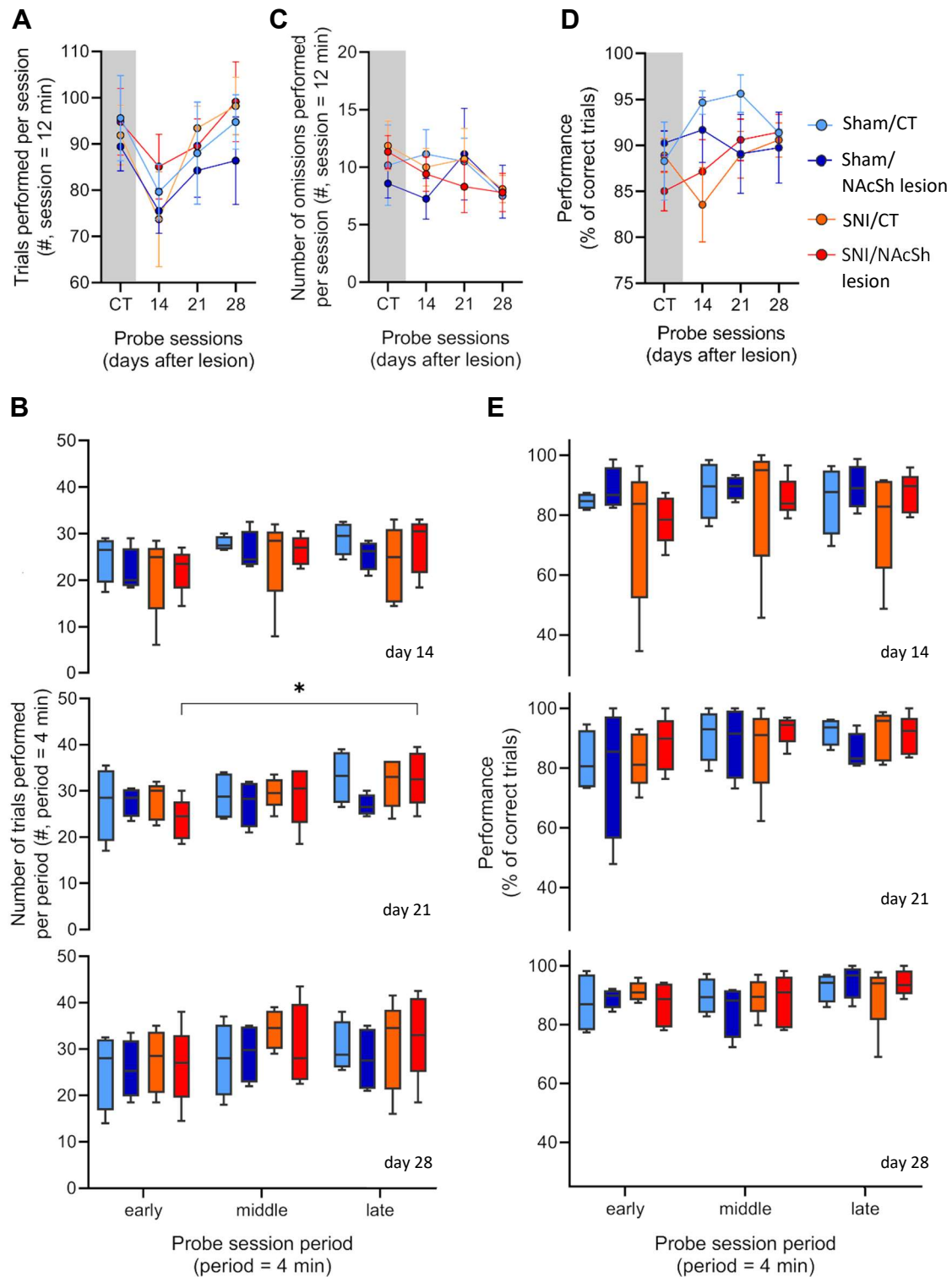


Figure III.3.1 – Trial number and performance did not differ between experimental groups.

- (A) Averaged number of trials performed per two daily testing sessions (duration = 12 min).
 (B) Averaged number of trials performed on days 14 (top panel), 21 (centre panel), 28 (bottom panel) throughout each probe session period (blocks of 4-minute: early, middle and late).
 (C) Averaged number of omissions performed per two daily testing sessions.
 (D) Percentage of correct trials performed.

(E) Percentage of correct trials performed on days 14 (top panel), 21 (centre panel) and 28 (bottom panel) throughout each probe session period.

Comparisons between experimental groups are based on non-parametric Kruskal-Wallis rank test. Intra-group comparisons are based on non-parametric Friedman one-way repeated measures ANOVA by ranks test. Sham/CT, $n=4$; Sham/NAcSh lesion, $n=4$; SNI/CT, $n=5$; SNI/NAcSh lesion, $n=5$. Values are presented as mean $\pm$ SEM. * $p<0.05$.

III.3.2. Effects of NAcSh lesions on pain-related retrospective memory preservation

To evaluate the impact of the central NAcSh manipulation and peripheral nerve lesion on retrospective memory preservation, I calculated the latency response which comprises the time spent between decision point and feeder location (**Materials and Methodology, Fig. II.3.1A**). This parameter provides insight regarding the working memory maintenance of the previous navigated path, as the animals need to remember it in order to choose the correct alternative feeder. My data revealed significant differences when compared the Sham/CT and Sham/NAcSh lesion groups to their respective CT period (**Fig. III.3.2A**; Sham/CT group, $F=9.3$, $p=0.0115$; Sham/NAcSh lesion group, $F=8.1$, $p=0.0329$), but the latency responses remained unaffected in the SNI/CT and SNI/NAcSh lesion groups (SNI/CT group, $F=4.92$, $p=0.2096$ (n.s.); SNI/NAcSh lesion group, $F=7.32$, $p=0.0548$ (n.s.)). In terms of the *post hoc* test analysis, the data revealed an increase of the latency response of the Sham/CT rats when compared days 14 and 21 in respect to CT baseline (Dunn's multiple comparisons test, both $p<0.05$). In the case of Sham/NAcSh lesion group, *post hoc* analysis revealed a higher latency response when compared days 14 and 28 in respect to CT period (both $p<0.05$). Finally, when compared the traces of response latency of all experimental groups statistical analyses revealed a significant effect ($KW=21.71$, $p=0.0268$); moreover, *post hoc* analysis revealed that SNI/CT group has significantly worse latency responses than Sham/CT animals on day 28 post-surgery ($p<0.05$). These results indicate that these experimental groups are differently affected by the peripheral lesion.

In order to further assess the variation of response latency throughout the 12 minutes comprising each probe session, the cumulative latency was calculated for each group. This was done by dividing the session into twelve 1-minute time blocks, calculating the latency responses within each time frame, and averaging the latency values in each block. The latency responses of each block were added with the previous obtained values (for example, the second cumulative value comprised the summation of the first

and second latency values), and the last values (end of each session) were analysed. The corresponding graphs are presented in **Fig. III.3.2B**. For day 14, statistical analysis revealed significant cumulative latency differences between experimental groups at the end of the session ($KW=9.346$, $p=0.0117$). However, *post hoc* analysis did not reveal significant changes when compared all experimental groups. Regarding day 21, no significant differences were found between groups at the end of the testing session ($KW=5.912$, $p=0.1096$). Finally, on day 28 the cumulative response latency was significantly different between experimental groups ($KW=8.216$, $p=0.0274$); moreover, *post hoc* analysis revealed that SNI/CT rats exhibited a higher response latency when compared to Sham/CT rats (Dunn's multiple comparisons test, $p<0.05$). These results suggest that rats with peripheral nerve lesion exhibit distinct feeder-choice time spans.

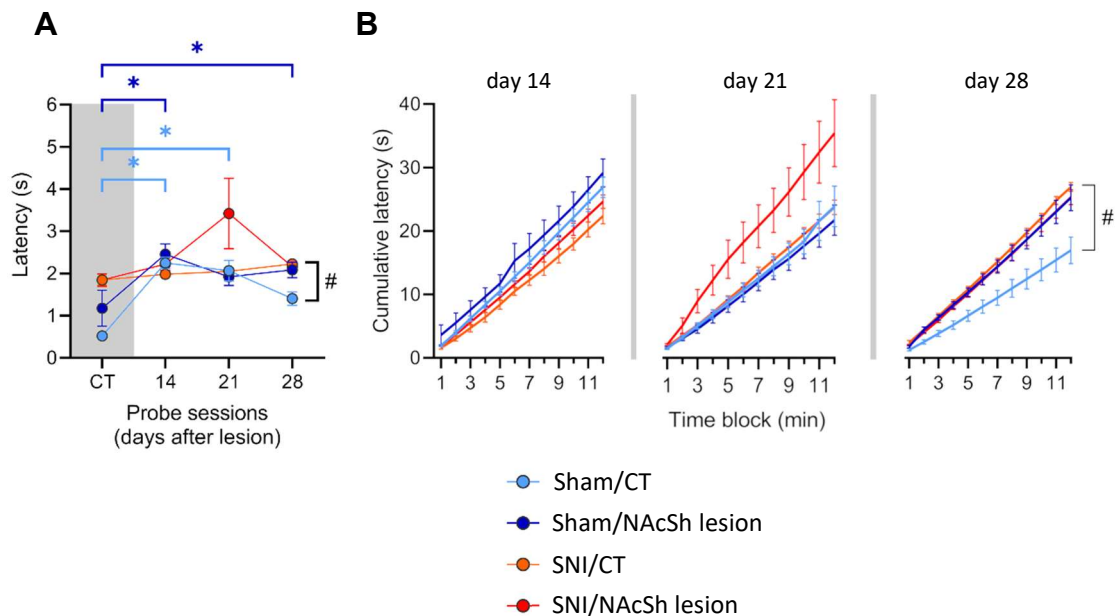


Figure III.3.2 – Response latency improved in Sham/CT animals 28 days after surgery, but not for the other experimental groups.

(A) Averaged time spent between the decision point and the feeder location (response latency). Sham/CT group displayed a significant worse response latency on days 14 and 21. Sham/NAcSh-lesioned animals also presented a significant increase of their response latency on days 14 and 28 in respect to baseline (CT). Moreover, SNI/CT group has a significantly higher response latency when compared to Sham/CT animals on day 28 post-surgery.

(B) Cumulative response latency throughout the 12-minute testing sessions on days 14 (left panel), 21 (centre panel) and 28 (right panel) (time block = 1 minute). SNI/CT animals spend more time choosing the correct feeder to approach than Sham/CT animals on day 28 testing session.

Comparisons between experimental groups are based on non-parametric Kruskal-Wallis rank test. Intra-group comparisons are based on non-parametric Friedman one-way repeated measures ANOVA by ranks test. Sham/CT, $n=4$; Sham/NAcSh lesion, $n=4$; SNI/CT, $n=5$; SNI/NAcSh lesion, $n=5$. Values are presented as mean $\pm$ SEM. Friedman test, $*p<0.05$; Kruskal-Wallis test, $\#p<0.05$.

III.3.3 Correlation between performance percentage and response latency

The time taken to choose the correct feeder to approach can greatly impact the performance values, since this deliberation could dictate the success or failure of the trial. Therefore, the variability in performance values observed in day 14, especially at the early period of the testing session, could be affected by the latency responses each animal presents.

To determine if the correct trial percentage values is directly associated to the time necessary to choose the correct feeder, the correlation between performance values and latency responses in the early period of the day 14 probe session was executed (**Fig. III.3.3A**). No significant correlations were observed between latency responses and performance values (Spearman coefficient test, Sham/CT group, $R=-0.4$, $p=0.75$ (n.s.); Sham/NAcSh lesion group, $R=0$, $p>0.99$ (n.s.); SNI/CT group, $R=0.7$, $p=0.23$ (n.s.); SNI/NAcSh lesion group, $R=0.7$, $p=0.23$ (n.s.)). Despite the fact of both Sham groups displayed negative slopes and both SNI groups exhibited a positive tendency, these statistical results indicate that there is not direct relationship between the accuracy rate and response latency.

On the other hand, the animals have similar performance values in day 28, yet differences in latency responses between Sham/CT and SNI/CT were found. To explore this potential association, the correlation between the latency responses and respective performance on day 28 post-surgery was made (**Fig. III.3.3B**). Statistical analysis revealed no significant correlations for all experimental groups (Sham/CT group, $R=0.8$, $p=0.33$; Sham/NAcSh lesion group, $R=-0.8$, $p=0.33$; SNI/CT group, $R=0.3$, $p=0.68$; SNI/NAcSh lesion group, $R=0.1$, $p=0.95$). These statistical results indicate that there is not direct relationship between the accuracy rate and response latency during day 28 post lesion.

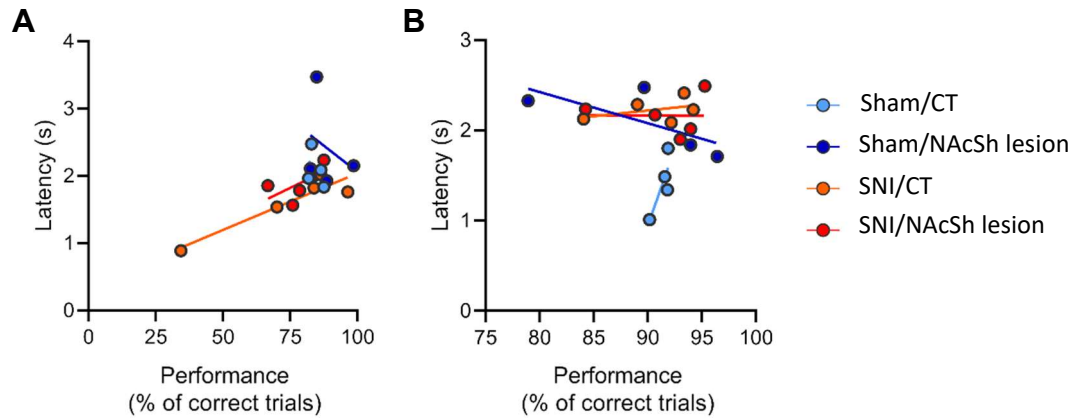


Figure III.3.3 – Performance accuracy rate is not directly correlated to the mean response latency.

Relationship between accuracy rate (% of correct trials) and response latency for the days 14 (**A**) and 28 (**B**) after lesion. No significant correlation was observed during both probe testing sessions.

Despite these results, on day 14 SNI-treated rats tend to take more errors when they cross the decision point and reach the feeder area with lower latency values. In the case of day 28, the lower response latency observed in the Sham/NAcSh lesion and SNI/NAcSh lesion groups may lead to higher performance and vice-versa. For the Sham/CT and SNI/CT groups, the lower response latency tended to be correlated with a poor performance.

Correlation analysis was based on the non-parametric Spearman coefficient test. Sham/CT, $n=4$; Sham/NAcSh lesion, $n=4$; SNI/CT, $n=5$; SNI/NAcSh lesion, $n=5$.

III.3.4. Effects of NAcSh lesions on pain-related motor performance

The inherent motor component associated with the 8-figure spatial alternation task allows for the evaluation of motor performance of the intervened animals. This was done by analysing the percentage of time spent per behavioral task navigation zone, total distance travelled per probe session, and mean and instantaneous velocity vectors. An example of the divisions of the maze and the navigation tracking trace is shown in **Fig. III.3.4**.

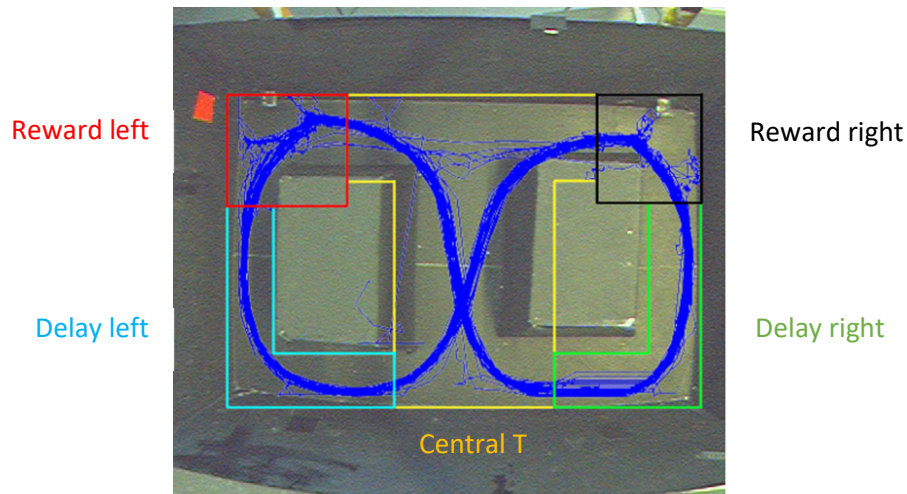


Figure III.3.4 – Maze divisions and navigation tracking trace extracted through video-based spatial vectors.

The central T area used in the tracking automated pellet distribution was later divided into 2 separate areas for analysis, comprising the central corridor and the choice zone between feeders.

III.3.4.1. Effects on maze area distribution

The time distribution per maze zone is presented in **Fig. III.3.4.1A**. Regarding the reward zone (**Fig. III.3.4.1A, left panel**), the SNI/NAcSh lesion group exhibited a significant oscillation over the testing sessions ($F=8.28$, $p=0.0313$); moreover, on day 21 *post hoc* analysis revealed that SNI/NAcSh lesion group rats increased the time spent in the reward zone when compared to CT period (Dunn's multiple comparisons test, $p<0.05$). Apart from this result, no other differences were observed for the other experimental groups (Sham/CT group, $F=3.0$, $p=0.4321$ (n.s.); Sham/NAcSh group, $F=6.9$, $p=0.0678$ (n.s.); SNI/CT group, $F=3.48$, $p=0.3720$ (n.s.)). The comparison between all experimental groups over the testing sessions revealed no differences ($KW=8.315$, $p=0.6848$ (n.s.)). For the delay zone (**Fig. III.3.4.1A, central panel**), statistical analysis indicated no significant differences when compared the CT period with probe sessions for each experimental group (Sham/CT group, $F=2.1$, $p=0.6489$ (n.s.); Sham/NAcSh group, $F=5.7$, $p=0.1411$ (n.s.); SNI/CT group, $F=3.96$, $p=0.2982$ (n.s.); SNI/NAcSh group, $F=4.92$, $p=0.2096$ (n.s.)). Moreover, no changes were found between delay time traces of all experimental groups ($KW=9.963$, $p=0.5337$ (n.s.)). The choice zone (**Fig. III.3.4.1A, right panel**) did not present significant alterations when compared CT period with probe sessions for each experimental group (Sham/CT group, $F=2.1$, $p=0.6489$ (n.s.);

Sham/NAcSh group, $F=3.9$, $p=0.3241$ (n.s.); SNI/CT group, $F=3.48$, $p=0.3720$ (n.s.); SNI/NAcSh group, $F=4.44$, $p=0.2261$ (n.s.)). Moreover, the comparison of the delay zone traces of all experimental groups did not present significant differences ($KW=6.343$, $p=0.8495$ (n.s.)). These results suggest that the animals did not change significantly their time allocated per each considered maze area during behavior probe sessions.

In order to illustrate the alterations in the time spent across the maze, I divided the behavioral arena into a grid of activity. The coloured grid images in **Fig. III.3.4.1B** represent a quantitative approach to time-spent distribution per navigation zone. Each square was coloured between dark red (most time allocated) and dark blue (less time allocated). The heat maps of all experimental groups exhibit similar time allocations throughout the maze, with most time spent in the reward zone (dark red) and reduced allocation to delay and choice areas (light blue, green and yellow).

Then, I used these heat maps to calculate the entropy index (**Fig. III.3.4.1C**). The statistical analysis revealed that the Sham/NAcSh lesion group exhibited significant differences comparing probe sessions entropy indexes ($F=6.5$, $p=0.0417$); moreover, *post hoc* analysis indicated a significant decrease of entropy index comparing day 14 and day 28 probe sessions ($p<0.05$). No further significant differences were found for the other experimental groups (Sham/CT group, $F=2.0$, $p=0.4306$ (n.s.); SNI/CT group, $F=0.4$, $p=0.9537$ (n.s.); SNI/NAcSh group, $F=2.8$, $p=0.3673$ (n.s.)). Finally, no changes were revealed when comparing the entropy index traces of all experimental groups ($KW=13.73$, $p=0.2483$ (n.s.)). These results indicate that the Sham/NAcSh-lesioned animals allocated the time in the maze more uniformly 4 weeks after surgery.

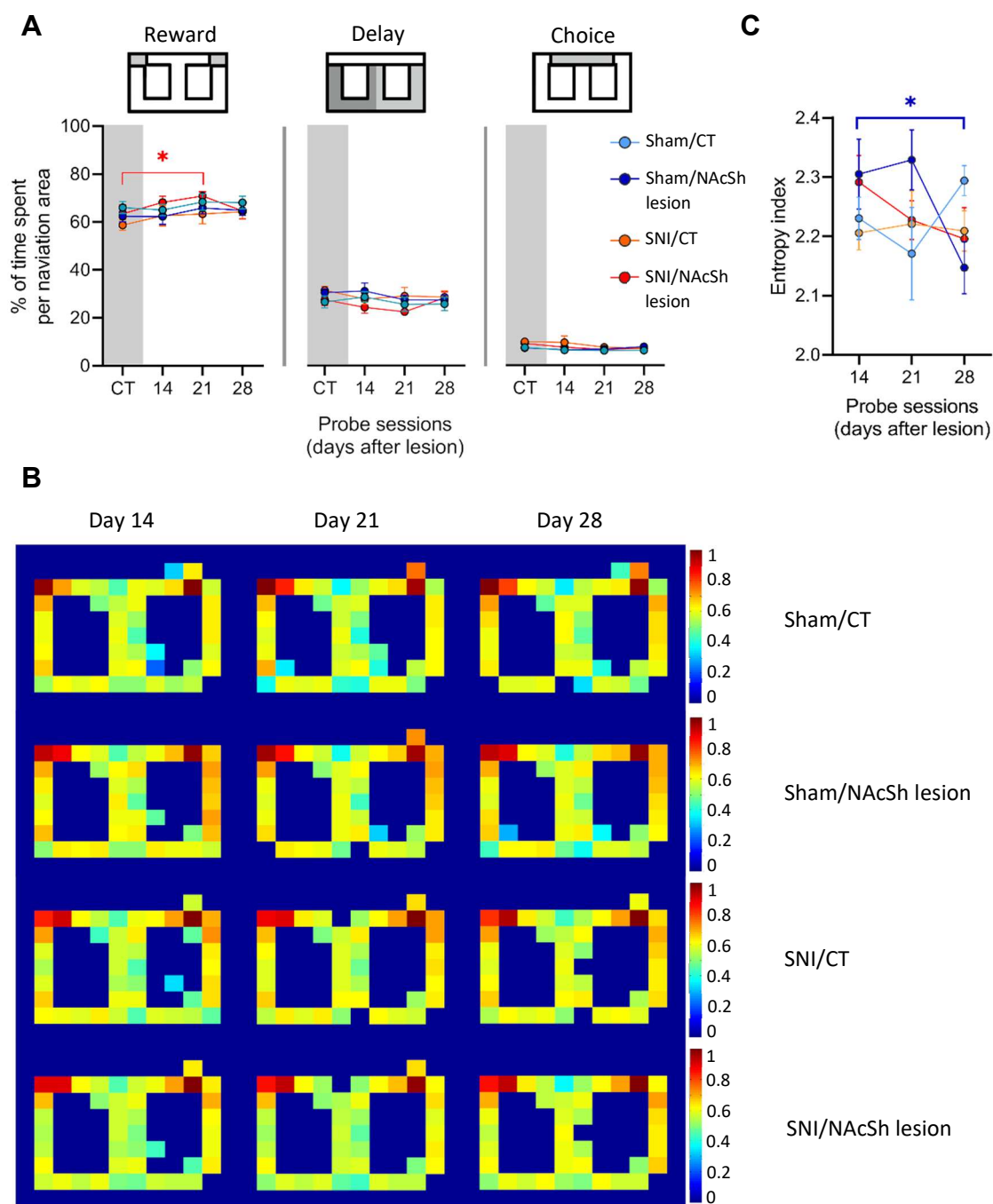


Figure III.3.4.1 – Peripheral nerve lesion did not alter navigation patterns in the spatial alternation task.

(A) Percentage of time spent by all experimental groups per navigation zone. Data presented per navigation zone: reward (left panel), delay (central panel), and choice (right panel). SNI/NACSh-lesioned animals increased their time allocation in the reward zone in day 21 compared to the CT period.

(B) Heat map of normalized time spent per square (bin=40 pixels/square).

(C) Probe session navigation entropy index. Sham/NACSh lesion groups present a significant decrease in entropy index from day 14 to day 28 post-surgery, indicating a uniform navigation pattern.

Comparisons between experimental groups are based on non-parametric Kruskal-Wallis rank test. Intragroup comparisons between probe session days are based on non-parametric Friedman one-way repeated measure ANOVA by ranks test. Rats, $n=18$. Values are presented as mean $\pm$ SEM. * $p<0.05$.

III.3.4.2. Effects on locomotion and velocity

One important aspect to consider is that zone percentage distribution does not account for total distance travelled: this is associated partially to the number of trials the animal performs. The results show that no significant differences in total distance travelled were found when comparing the probe sessions to the CT period of each experimental group (**Fig. III.3.4E**; Sham/CT group, $F=2.1$, $p=0.6489$ (n.s.); Sham/NAcSh lesion group, $F=1.2$, $p=0.7997$ (n.s.); SNI/CT group, $F=6.36$, $p=0.0933$ (n.s.); SNI/NAcSh lesion group, $F=6.36$, $p=0.0933$ (n.s.)). Moreover, no significant changes were found between the total distance traces of all experimental groups ($KW=10.55$, $p=0.4818$ (n.s.)). This indicates that the SNI groups do not present motor impairments to perform the task, as the total distance covered does not differ significantly from the Sham groups.

To evaluate the potential alterations in pacing throughout the maze due to pain-triggering movements, the mean velocity was determined (**Fig.III.3.4.2A**). When compared the probe sessions of each experimental group to the CT period, statistical analysis did not display significant differences (Sham/CT group, $F=2.1$, $p=0.6489$ (n.s.); Sham/NAcSh lesion group, $F=1.2$, $p=0.7997$ (n.s.); SNI/CT group, $F=6.36$, $p=0.0933$ (n.s.); SNI/NAcSh lesion group, $F=6.36$, $p=0.0933$ (n.s.)). In addition, no significant alterations were observed for the mean velocity traces of all experimental groups ($KW=10.12$, $p=0.5195$ (n.s.)). This lack of statistical differences shows that the neuropathic pain model did not influence the animals' capacity to execute motor actions.

Although the overall mean velocity was similar, SNI/NAcSh-lesioned animals had a significant increase in reward zone time on day 21. This is indicative of a reduction in the overall time spent in the remaining considered arena zones, which suggests also that probably these rats tend to use a higher velocity to navigate in these zones.

A critical point in the maze that could suggest alterations in the navigation accuracy of the animals is the central corridor, just before the decision point. To further analyse this possible difference, the mean velocity in the central corridor of the maze was calculated (**Fig.III.3.4.2B**). Statistical analysis did not reveal significant differences between probe sessions for each experimental group (Sham/CT group, $F=1.5$, $p=0.6528$ (n.s.); Sham/NAcSh lesion group, $F=0.5$, $p=0.9306$ (n.s.); SNI/CT group, $F=1.6$, $p=0.5216$ (n.s.);

SNI/NAcSh lesion group, $F=1.6$, $p=0.5216$ (n.s.)). In addition, no significant changes were found between experimental groups ($KW=19.23$, $p=0.0571$ (n.s.)). This result suggests that mean velocity in the central corridor remained unaltered when comparing the traces of all experimental groups. Although not significant, the Sham/NAcSh lesion group presents lower mean velocities in the central corridor compared to the other experimental groups.

To explore possible oscillations of the navigation velocity vector throughout each session, I measured the instantaneous velocity. These values were presented as a normalization between 0 and 1. The images were acquired for one session of one animal and represent an example of each group (**Fig.III.3.4.2C**). It is important to note that each group does not exhibit differences as the probe sessions progress. Moreover, the experimental groups present similar navigation patterns in each probe session. The animals increase their velocity immediately after leaving the feeder area (dark red) and begin to decrease upon arrival at the decision point (orange-yellow).

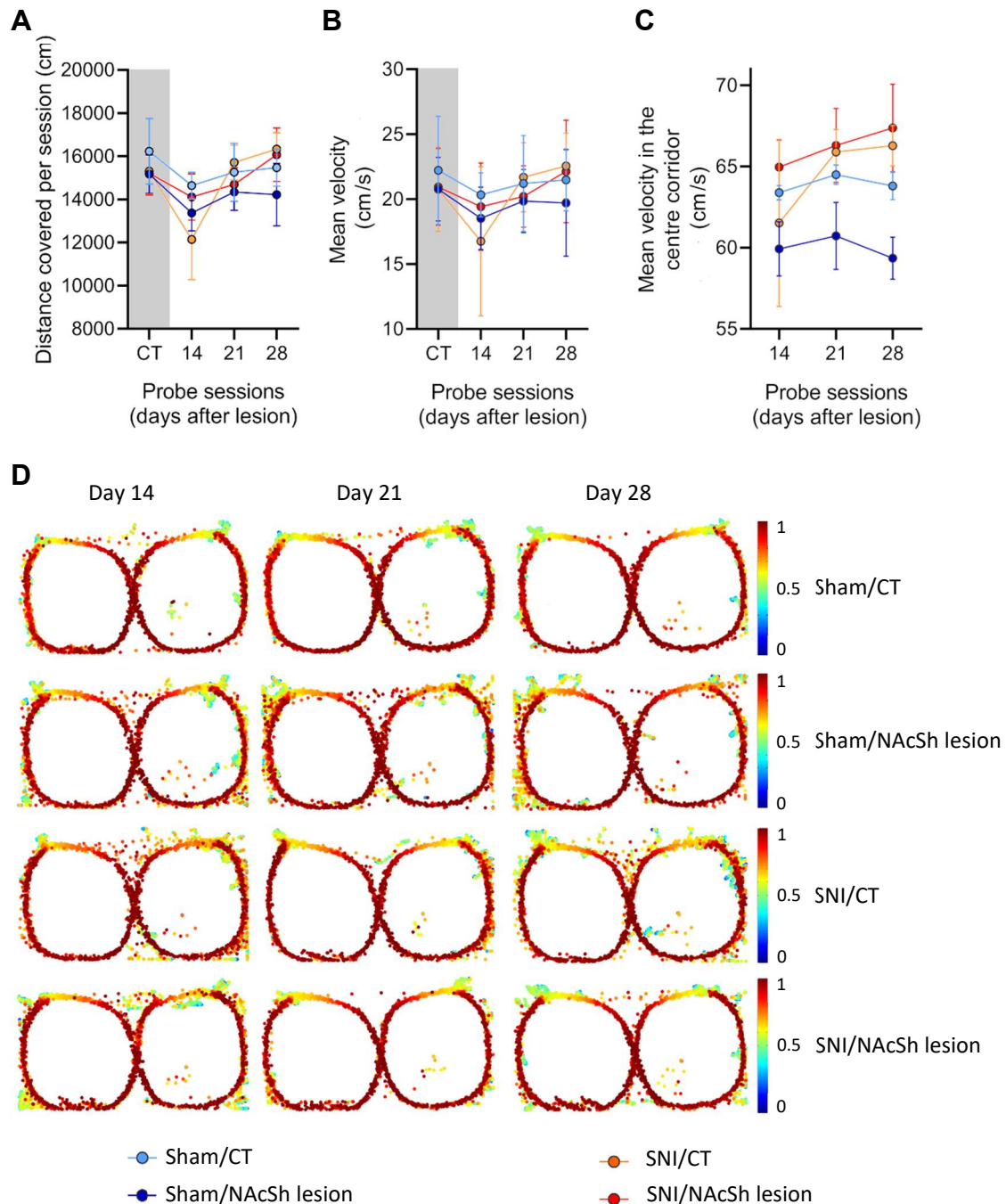


Figure III.3.4.2 – Peripheral nerve lesion did not induce motor impairments during the performance of the spatial alternation task.

(A) Total distance travelled per probe session. No significant alterations were found for all experimental groups, indicating that SNI animals did not present motor incapacities to execute the task.

(B) Mean velocity per probe session. The animals did not exhibit differences in mean velocity, suggesting that SNI groups had no motor impairments to perform the task.

(C) Mean velocity in the central corridor. The velocity values remained unaltered for all experimental groups throughout the probe sessions.

(D) Examples of scatter of normalized instantaneous velocity calculated per session.

Comparisons between experimental groups are based on non-parametric Kruskal-Wallis rank test. Intragroup comparisons between probe session days are based on non-parametric Friedman one-way repeated measure ANOVA by ranks test. Rats, $n=18$. Values are presented as mean $\pm$ SEM.

III.3.5. Relationship between the mean navigation velocity in the central corridor and the percentage of time spent in the reward location

The increase in the time spent in the reward zone by the SNI/NAcSh lesion animals comparing the CT period and the day 21 probe session could indicate a reduced capacity to quickly shift the attention to the next trial to execute. Furthermore, the velocity achieved in the centre corridor could suggest a necessity to navigate the maze faster in order to still remember the correct feeder to choose at the decision point stage. This could imply impaired capacity to retain temporary information and move on to the next alternation. To assess if the mean centre velocity is directly associated to the reward zone time percentage, the correlation between the two variables was performed.

On day 14, statistical analysis revealed a positive correlation between reward time percentage and mean navigation velocity in the central corridor for SNI/CT group (**Fig. III.3.5A**; $R=1.0$, $p=0.0167$); however, no further correlations were found (Sham/CT group: $R=-0.4$, $p=0.75$; Sham/NAcSh lesion group: $R=-0.2$, $p=0.9167$; SNI/NAcSh lesion group: $R=0.7$, $p=0.2333$). Regarding day 21, no significant correlations were observed (**Fig. III.3.5B**; Sham/CT group: $R=-0.2$, $p=0.9167$; Sham/NAcSh lesion group: $R=0.4$, $p=0.75$; SNI/CT group: $R=0.9$, $p=0.0833$; SNI/NAcSh lesion group: $R=0.7$, $p=0.2333$). Finally, on day 28 statistical analysis revealed a positive correlation for the SNI/NAcSh group (**Fig. III.3.5C**; $R=1.0$, $p=0.0167$), but not for the other experimental groups (Sham/CT group: $R=-0.6$, $p=0.4167$; Sham/NAcSh lesion group: $R=0.2$, $p=0.9167$; SNI/CT group: $R=0.6$, $p=0.35$). These results indicate a direct correlation between the mean navigation velocity in the central corridor and the time spent in the reward zone for the SNI-treated rats. Although some correlations were non-significant, the SNI-treated rats displayed a similar tendency for all probe sessions.

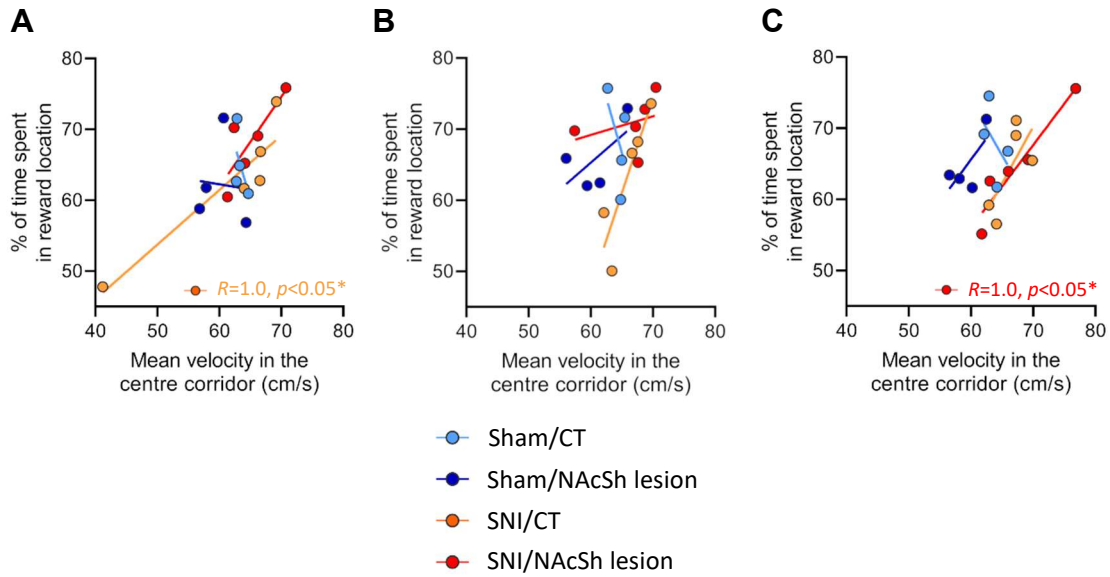


Figure III.3.5 – The time spent in the reward zone is directly correlated with the mean navigation velocity across the central corridor in SNI-treated rats.

Relationship between mean centre velocity and reward zone time allocation in days 14 (A), 21 (B) and 28 (C) post-surgery. Significant correlations were observed for the SNI/CT group in day 14, and for SNI/NAcSh lesion group in day 28. SNI-treated animals tend to spend more time in the reward zone if they cross the central corridor faster. Moreover, the Sham/NAcSh lesion group tended to spend more time in the reward zone if they crossed the central corridor more slowly in day 14; however, this tendency was altered, and Sham/NAcSh-lesioned animals tended to behave similarly to SNI groups in days 21 and 28 post-surgery (faster in the central corridor, more time in reward zone).

Correlation analysis was based on the non-parametric Spearman coefficient test. Sham/CT, $n=4$; Sham/NAcSh lesion, $n=4$; SNI/CT, $n=5$; SNI/NAcSh lesion, $n=5$. $*p<0.05$

III.3.6 Effects of NAcSh lesions on nociception

The sensory threshold for noxious mechanical stimulation was evaluated using von Frey filaments, and the measurements occurred 1 hour after the second daily probe session. Statistical analysis revealed significant differences when comparing both SNI groups to their respective CT period (SNI/CT group: $F=13.91, p=0.0039$; SNI/NAcSh lesion group: $F=13.50, p=0.0003$), but not for the Sham groups (Sham/CT group: $F=6.0, p=0.1667$; SNI/NAcSh lesion group: $F=13.50, p=0.0003$). *Post hoc* analysis indicated a significant decrease in mechanical noxious threshold for SNI/CT animals when comparing the CT period to the day 28 post surgery ($p<0.05$). *Post hoc* analysis also revealed a significant mechanical noxious threshold reduction for the SNI/NAcSh lesion group comparing the CT period to days 14 and 21 ($p<0.01$ and $p<0.05$, respectively). Moreover, significant changes were found between experimental groups ($KW=35.54, p=0.0002$), and *post hoc* analysis showed a significant difference in mechanical noxious threshold when

comparing Sham/NAcSh lesion and SNI/NAcSh lesion groups in day 14 post-surgery ($p < 0.05$). These data indicate that the neuropathic pain model was well established, and SNI/NAcSh-lesioned animals need less pressure than the Sham/NAcSh lesion group to evoke pain responses. Furthermore, it is important to note that the SNI/NAcSh lesion group display a slightly higher threshold value than the SNI/CT group, although this difference is not significant.

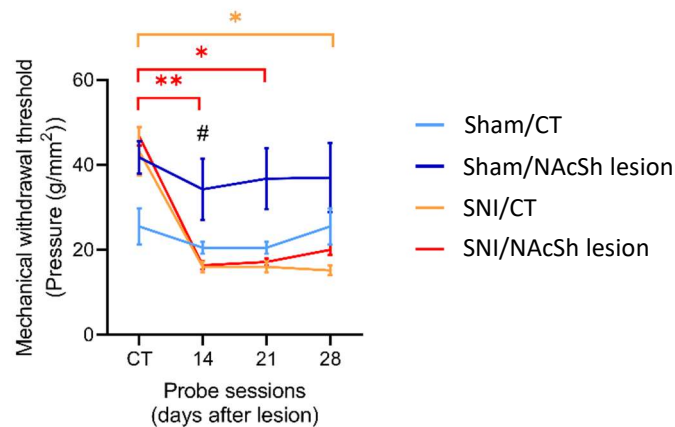


Figure III.3.6 – SNI groups display significantly lower post-surgery mechanical noxious threshold.

Mechanical noxious threshold was evaluated using the von Frey filaments test. When individually compared to the CT baseline period (*), both SNI groups displayed a significant decreased of their mechanical threshold to elicit a hindpaw response. When compared together (#), SNI/NAcSh-lesioned animals exhibited a significant lower mechanical threshold when compared to Sham/NAcSh-lesioned animals during day 14 post-surgery.

Comparisons between groups are based on non-parametric Kruskal-Wallis rank test. Intragroup comparisons are based on non-parametric Friedman one-way repeated measure ANOVA by rank. Rats, $n=18$. Values are presented as mean $\pm$ SEM. Friedman test, * $p < 0.05$, ** $p < 0.01$. Kruskal-Wallis test, # $p < 0.05$.

III.3.7 Effects of the neuropathic pain model on NAcSh morphology phenotype

In order to evaluate pain-related changes in NAcSh inhibitory GAD67-expressing neurons, I used an immunohistochemical protocol and measured the local tissue fluorescence intensity of these neurons. These quantifications were done using images with a 2.5X magnification (**Fig. III.3.7A**). Further observation of GAD67⁺-neurons was done with 40x magnification (**Fig. III.3.7B**). Then, the local NAcSh tissue fluorescence intensity was calculated using the difference between ipsi- and contralateral NAcSh region of each coronal slice. Finally, the experimental groups were analysed according to their peripheral injury (Sham or SNI lesion, including both CT and NAcSh lesions).

Statistical analysis did not reveal any significant changes in the NAcSh GAD65-expressing neurons between Sham- and SNI-treated animals (**Fig. III.3.7C**; Mann-Whitney U test (U), $U=14$, $p=0.9307$). This result suggests that peripheral nerve lesion did not produce significant changes in local NAcSh inhibitory neurons.

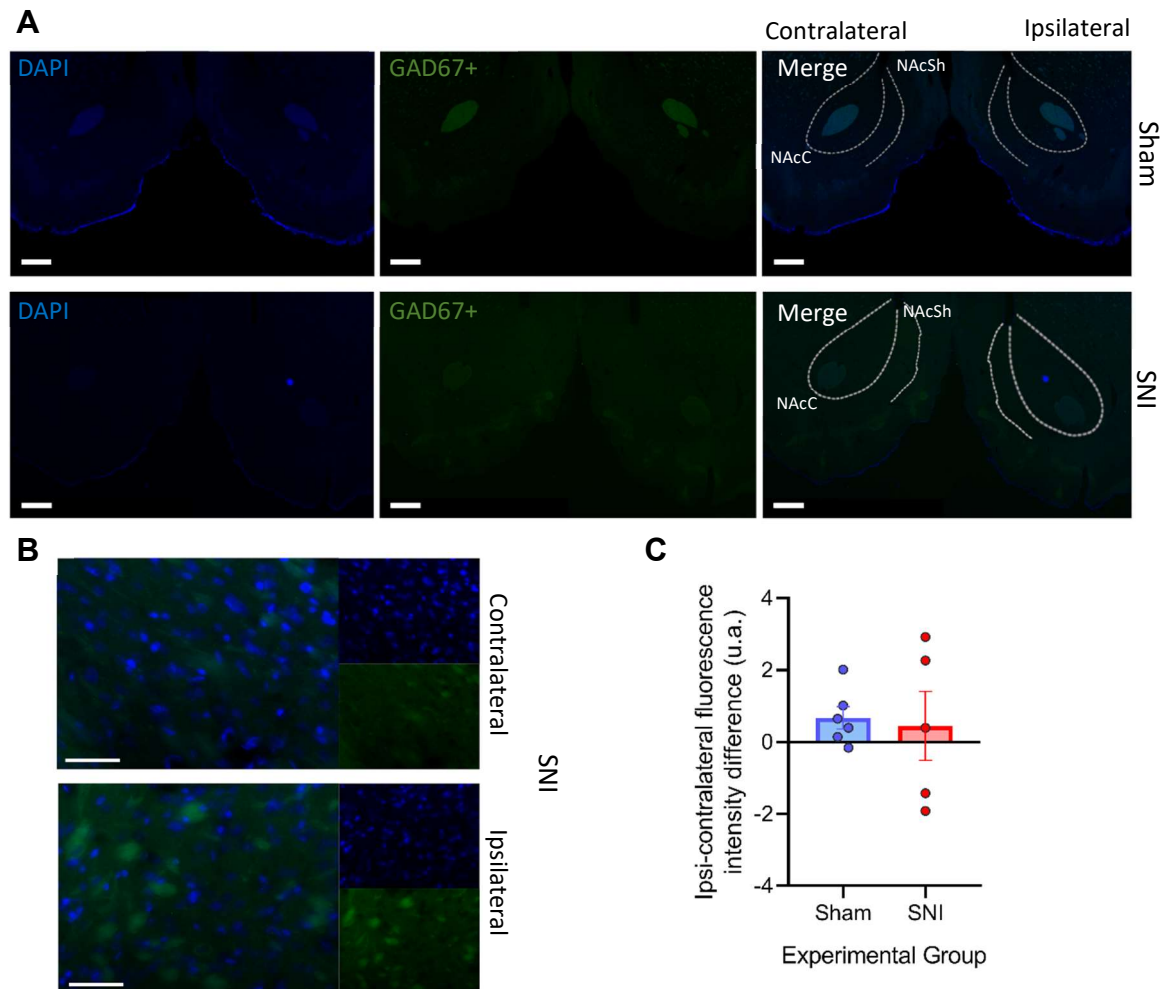


Figure III.3.7 – SNI group do not present changes in NAcSh inhibitory GAD67⁺-neurons density compared to Sham group.

(A) Comparative images of NAcSh inhibitory GAD67⁺-expressing neurons in the nucleus accumbens (white dashed lines) of Sham- and SNI-treated animals. Scale bar: 500 μ m

(B) Illustrative images of contralateral and ipsilateral hemispheres of SNI-treated animals. Scale bar: 50 μ m.

(C) Average difference between ipsi and contralateral hemispheres of Sham and SNI animals. No changes were found between groups, indicating a lack of NAcSh phenotype morphological alteration in SNI animals.

Comparisons between groups are based on non-parametric Mann-Whitney U rank test. Rats, $n=11$. Values are presented as mean $\pm$ SEM.

IV. Discussion

The present study aimed to clarify the impact of chronic pain in the IL-NAcSh microcircuit, and how this affects spatial WM performance. The results gathered indicate that 1) the performance levels of the animals did not present differences between groups, however only Sham/CT animals improved latency responses; 2) SNI-treated animals did not exhibit motor impairments in the spatial WM task; 3) SNI-treated animals displayed significantly lower post-surgery mechanical noxious threshold, while not revealing alterations in NAcSh inhibitory neuron density compared to Sham-treated animals.

IV.1 IL-NAcSh microcircuit influence on spatial WM performance

Chronic pain alters a plethora of neurophysiological mechanisms, contributing to WM impairments [78], [79], [80], [81], [82]. Prolonged peripheral neuropathy always evolves to central sensitization. The long-term up-regulation of central pain pathways produces alterations in brain volume and connectivity [68], [71]. The mPFC has been considered a critical structure in WM processes, and intra-mPFC connectivity alterations were observed in the 8-figure maze task [32]. Previous works from our laboratory have also reported connectivity changes (namely in the cortico-hippocampal and cortico-thalamic pathways) and how it led to the compromise of learning and memory mechanisms, especially WM-dependent processes [73], [110], [111]. Nonetheless, other areas that receive dense mPFC projections have been explored as potential players in pain-related cognitive deficits, such as the NAc brain area. The NAc participates in learning and memory processes, and regulates reward-seeking behaviors [58], [59]. The GABAergic MSN neurons activity is altered during nociceptive information processing [86], [92], [93]; this pain-related disruption of the NAc activity can therefore induce impairments in spatial WM tasks, as observed in studies where the NAcSh is not functioning properly [54], [55], [112].

In the current study, the SNI injury did not diminish the number of trials done by the animals and did not impair performance values in the 8-figure maze spatial alternation

task, indicating that the rules acquired during the learning phase were not lost and allowed the SNI-injured animals to overcome pain-related WM impairments. Furthermore, SNI-injured animals had a similar number of omissions to Sham animals. Multiple studies have reported improvement of performance values of SNI-injured animals after dynamic training periods. For instance, previous work using the same task did not observe differences between Sham and SNI groups from day 5 post-surgery until the end of the testing period (21 days post-surgery) ^[73]. Moreover, SNI groups also improved the performance values within the training period in various WM tasks, such as the 8-figure maze, T-maze, and delay non-match to sample (DNMS) ^{[78], [80], [82], [113]}. This suggests that probably the initial impairment observed in the early phase after the lesion may be connected with an inflammatory process related to the surgical intervention, and not with a direct effect of peripheral nerve lesion.

As the mediator between cortical and limbic structures, the NAc provides a base for goal-directed behaviors and short-term spatial data retention for posterior integration ^{[42], [63]}. In this work, NAcSh-lesioned animals did not present performance differences from non-lesioned animals, suggesting an intact capacity to retain temporary information. This contrasts to previous work, where selective N-methyl-D-aspartate (NMDA) excitotoxic lesions or non-NMDA infusion in the NAcSh impaired spatial WM ^{[55], [114]}. Furthermore, electrolytic lesion in this area also led to mnemonic impairments in a DNMS T-maze study ^[112]. Since these studies performed bilateral NAcSh lesions, the lack of WM impairments observed in this work could be explained by the unilateral lesion strategy used. The WM deficits derived from the contralateral NAcSh malfunctioning may therefore be compensated by the intact ipsilateral NAcSh structure.

Although the performance values were similar between experimental groups, the latency responses were altered differently according to the peripheral and/or central treatment. Sham-treated animals increased latency response 14 days after surgical procedures, but Sham/CT animals improved over time while Sham/NAcSh-lesioned animals latency values remained high 4 weeks post-surgery. Previous work on spatial learning and spontaneous alternation using a T-maze had already described similar results: slower choice responses in animals with electrolytical lesions in the NAcSh ^[115]. This result indicates that NAcSh lesions in Sham animals exerted influence on goal-

directed actions, but it did not interfere with temporary information retention as the performance values were not distinct between Sham groups.

Moreover, SNI/CT animals took significantly more time choosing the correct feeder compared to Sham/CT animals 4 weeks after surgery, suggesting that the peripheral injury in animals without central lesions affected choice time efficacy 28 days post-peripheral injury but it did not impact WM performance. This increase in latency response time is also observed in a prior study in which the SNI group significantly increases the latency response of correct trials for 3- and 6-second delay intertrial intervals in the DNMS task ^[74]. WM tasks require the temporary information acquired in previous trials to be clearly and rapidly available for adequate responses when necessary. In this work, chronic pain interfered with temporary information processing velocity 4 weeks after surgery as it led to slower response times, but it did not alter WM maintenance. In other words, chronic pain animals without central lesions took longer to choose, but choose correctly nonetheless. It is also important to note that SNI-injured animals latency responses remain constant comparing the probe sessions to the CT period, indicating incapacity to improve choice velocity over time.

WM performance and latency response has been observed to be negatively correlated in WM tasks ^[74]. In this work, no correlation was observed for the early period of the day 14 nor for the day 28 probe sessions. This suggests that task accuracy cannot be used as predictor of choice timing and vice-versa.

The combination of lack of performance differences and distinct latency responses could be explained by the learning strategy adopted: the 6-maze training before exposing the animals to the maze rules and the recall sessions before the probe testing may have led to a better understanding and retention of these rules, even after the surgical procedures. Repeated learning sessions make it less likely to forget acquired rules, which reflect on the % of correct trials (performance) on testing sessions. In this regard, SNI did not display differences in performance values after a number of testing sessions possibly due to overtraining in the task.

On the other hand, the latency values were different between experimental groups, which indicates the WM-associated choice mechanisms are altered significantly in

Sham/NAcSh-lesioned animals and prevented choice efficacy improvement over time for all experimental groups except for the animals without peripheral and central injury. This could suggest that the task was not difficult enough to evaluate the full extent of WM differences between the groups, which could be overcome by extending the delay areas (increasing the time between the beginning of the trial and the moment to choose the correct feeder to approach). The delay increase strategy could separate potential performance differences currently absent in this work, since it heavily influences the success of the future trials.

Overall, the central and/or peripheral treatments had no significant effects in the performance values obtained in the 8-figure spatial alternation task, which revealed no WM impairments. Despite this result, the time taken to choose the correct feeder between groups revealed disparities related to the central and/or peripheral treatment. SNI-injured animals, as well as Sham/NAcSh-lesioned animals, did not diminish latency responses over time as observed for Sham/CT animals, indicating altered WM processing mechanisms important for later goal-directed actions (such as choosing the correct feeder).

IV.2 IL-NAcSh microcircuit influence on motor capacity

The brain connectivity and synchronicity adaptations observed in persistent pain not only disrupt WM information processing for obtaining rewards but may also impact spatial components of reward-seeking actions. Previous work observed reduced connectivity between the hippocampus and the mPFC, which contributed to diminish spatial information integration^[73]. Moving through certain locations activate particular place cells in the hippocampus, which exhibit specific firing sequences for each navigation memory^{[116], [117]}. The order of activation is correlated to the previous field trajectory, and similar but faster population bursts were observed during pauses in maze exploration^{[117], [118]}. The hippocampus is also greatly connected to the NAc, and this relationship supports integration and retrieval of spatial data^{[51], [119]}. Since chronic pain disturbs NAc-hippocampus connectivity, this indicates that the navigation patterns of pain animals may be altered after injury.

In this work, the SNI/NAcSh-lesioned animals increased the time spent in the reward zone 21 days after surgery when compared to CT period. Apart from this result, no changes were observed in allocation time per each considered maze zone during the probe sessions. This is in accordance with previous work by our laboratory, where the time spent in the delay and choice zone was similar between Sham and SNI animals [73], [113]. Contrasting results were observed in another study with inflammatory pain [111], which indicates that the lack of differences between groups derived exclusively from the neuropathic pain model.

Moreover, there was lack of differences between NAcSh-lesioned and non-lesioned animals as well. The NAc receives important projections from both the mPFC and the hippocampus, contributing to the processing of spatial temporary information [39], [62], [63]. However, the NAcC and the NAcSh encode different WM components: local infusions of NMDA receptor antagonist in the NAcC heavily disrupted the specific spatial sequence to find the reward learned by the animals, but the same was not observed for the NAcSh [120]. Regarding the same study, NAcSh lesions produced impairments in WM in the 8-arm radial maze, but not in the Morris water maze task. This indicates that NAcSh lesions impair selective spatial WM processes; in this work, no spatial impairments were observed.

The evaluation of navigation pattern using the entropy index did not reveal any alterations during the testing sessions. The exception was observed for Sham/NAcSh-lesioned animals, as they navigated more uniformly in day 28 post surgery compared to day 14. This could be due to increased exploratory activity 2-weeks post-surgery, something that has been reported in NAcSh lesion studies [54]. Since I performed unilateral lesions, this was reduced overtime by the compensation possibly of non-lesioned NAcSh animals, and produced more uniform patterns of navigation 4-weeks post-lesion. SNI/NAcSh-lesioned animals follow a similar decrease in entropy index over time, though this is not significant. Navigation patterns are influenced by both the NAc and the hippocampus. Although previous work has demonstrated that place cells functioning is altered in peripheral neuropathy cases [105], and the hippocampal-cortical connectivity is weaker [73], in this case the spatial memory prior to the surgery (learning

phase) and the rules of the task were kept intact (similar performance), which resulted in unchanged navigation patterns between experimental groups.

Depending on etiology, chronic pain patients may or may not exhibit impairments on walking coordination and kinematics ^{[121], [122]}. Limitation of movement to reduce painful occurrences is a normal protective response after injury, leading to a decrease in muscle strength and gait control ^{[123], [124]}. In animal studies, the same is observed using different pain models ^{[77], [80], [125]}.

In the current work, there were no differences observed in total distance travelled and mean velocity between experimental groups, suggesting that the peripheral injury did not induce motor impairments. This is in agreement with previous studies that have reported similar distances travelled by Sham and SNI animals ^{[80], [101]}. When observing the instantaneous velocity scatter graphs, all experimental groups display similar plots, with red and dark red for most of the course shifting to yellow-green from the choice area until the feeder cups.

Furthermore, although not significant, it is interesting to note that SNI-injured animals exhibit the higher distance and velocity values 4-weeks after surgery. In contrast to the performance values observed previously, this result may indicate that SNI animals may display enhanced reward salience but not valence in the task. Borsook and colleagues ^[126] have also described that changes induced by persistent pain in reward processing led to an increase in motivational salience to pain-related cues. The hypodopaminergic state observed in the NAc due to chronic pain has initially an anti-reward effect; however, the between-system anti-reward sensitization in conjunction with the downregulation of the reward-system transform normal motivational mechanisms. This combination is considered an important hallmark between a controlled pain state and an escalating, emotional-driven and opioid addiction-prone pain state ^[126].

The delay portion before the decision point puts into evidence any alterations in the navigation accuracy. Although the mean velocity in the central corridor remained unaltered, the Sham/NAcSh-lesioned animals present the lowest centre velocity values, which explains the slightly lower number of trials observed. More importantly, SNI-injured animals display faster central corridor velocities than the Sham animals. Since

the delay zone time did not alter between experimental groups, this suggests that the SNI groups may cross the central corridor faster to maintain the temporary information long enough to reach the decision point and still remember the correct feeder to approach. Combining this result with the latency responses, the SNI-injured animals are the fastest in the central corridor, yet also take more time choosing the feeder.

Previous studies have reported a tendency in chronic pain patients to exhibit impairments in temporary information processing ^{[127], [128]}. Animals that finish one trial need to quickly adapt to start the next trial, retaining the information regarding the previous path in order to choose correctly in the future. In this case, the relationship between mean velocity in the central corridor and the time spent at the reward location aimed to assess potential patterns displayed by SNI-injured animals. The SNI experimental groups exhibited positive correlations between these two variables for day 14 (SNI/CT group) and day 28 (SNI/NAcSh lesion group) post-surgery, and displayed a similar tendency for all probe sessions. This suggests that animals with neuropathic pain have a combination of reduced capacity to quickly shift the attention to the next trial and impaired capacity to retain temporary information long enough to perform the next alternation.

Moreover, the Sham/NAcSh lesion group also has the same tendency from 3-weeks after surgery and present mean central velocity-reward zone time correlations akin to SNI-injured animals, indicating that the NAcSh lesion in animals without chronic pain also impairs attention and WM processes similarly to the SNI procedure. Indeed, lesions to the NAcSh led to changes in information processing speed and switch of goal-related strategy, and influenced goal-directed behaviors in the DNMS T-maze task ^[112]. The NAcSh therefore plays a role not only in WM mechanisms, but also in attentional shifting within WM tasks, as the Sham/NAcSh-lesioned animals change from a Sham/CT negative tendency profile to one similar to SNI-injured animals.

In summary, the SNI-injured animals do not display any motor impairments related to the neuropathic pain model, as the total distance travelled and mean velocity are unchanged from the Sham animals. Moreover, all experimental groups except Sham/CT display positive correlations/tendencies between velocity in the central corridor and the

time spent in the reward zone. This implies that the peripheral injury and/or the NAcSh lesion induced alterations in cognitive flexibility mechanisms related to WM.

IV.3 IL-NAcSh microcircuit influence in nociception

Central sensitization and connectivity alterations caused by chronic pain are reflected through behavioral modifications [68], [71]. In fact, cognitive impairments including spatial WM deficits are considered a manifestation of central pain-induced changes in brain structures such as the mPFC and the NAc [84], [85], [86], [87].

In the present work, the SNI model was correctly established, and SNI-injured animals presented significantly reduced mechanical noxious thresholds after surgery. Moreover, SNI/NAcSh-lesioned animals exhibited lower mechanical thresholds when compared to Sham/NAcSh-lesioned animals for day 14 post-surgery, but not the following 21 and 28 days. Although not significant, the SNI/NAcSh lesion group slightly increased the threshold comparing to the SNI/CT group, which helps explain the lack of differences between the two NAcSh-lesioned groups in the 3- and 4-weeks testing.

The involvement of the NAc in pain processing has long been demonstrated through various studies [88], [89], [90], [97], [129], [130]. In an evolutionary standpoint, the NAc plays an equally critical part in reward and guiding the individual to avoid tissue damage. The act of increasing the chance of survival and learning from the experience is in its essence rewarding, so the contribution of the NAc becomes apparent in relation to chronic pain contexts [131]. Furthermore, the NAc has extensive cortical and limbic connections, and projects to motor structures to modulate goal-directed behaviors [42], [43], [44]. The influence of the IL over the NAc in chronic pain conditions can be noted through the increase in corticostriatal connectivity, and the NAcSh indirect pathway is heavily modulated by the IL glutamatergic projections to this region [85], [96], [97], [98]. In neuropathic pain cases, the MSNs of the indirect pathway of the NAcSh becomes hyperexcited, amplifying neuropathic pain [97]. The contrary effect is observed with lidocaine infusions in the NAcSh [86], [132], and this is akin to the slight increase in mechanical noxious threshold observed for SNI/NAcSh-lesioned animals.

IV.4 Effects of the neuropathic pain model on NAcSh morphology phenotype

Changes in morphology and activity in the NAc resulting from persistent pain conditions are known to occur, but to this day information remains incomplete. In this regard, the NAc was observed to have a volume decrease and a hypodopaminergic environment in chronic pain cases ^{[83], [87]}. Furthermore, the depletion of DA causes region-specific changes in MSN morphology: while NAcC MSNs are more sensible to DA decrease and respond by upregulating DA receptor expression, the NAcSh is less susceptible to MSN degeneration but present lower spine density ^{[133], [134]}. More recently, García-González and colleagues ^[135] discovered that in cases of inflammatory and neuropathic pain (SNI), neural stem cells migrate from the ventricular–subventricular zone into the NAc to mature as MSN projection neurons in adult mice. This indicates that the pain-generated environment can disturb normal morphology in the NAc area, but also promotes adult neurogenesis ^{[134], [135]}.

Through immunohistochemistry assays, the brain slices obtained from experimental groups were used to measure the integrated intensity values of the contralateral and ipsilateral hemispheres of each slice. In the present work, the ipsi-contralateral difference in GAD67⁺-expressing GABAergic neurons intensity remained unaltered between Sham and SNI-injured animals. This observation is in conformity with a previous study from Ren and colleagues ^[97], where they elegantly demonstrated that the NAcSh area did not display neuron morphology changes between groups ^[97]. Additionally, in that study they also demonstrated that dendritic morphology of direct and indirect MSNs remained unaltered, but the intrinsic excitability of indirect MSNs was increased in SNI-injured animals ^[97]. The unchanged morphology of NAcSh could be due to the proximity to the ventricular–subventricular neurogenic zone; however, it is still unclear how other components, such as glial cells, modulate this neural stem cell migration and how it sustains studies of gray matter decrease in chronic pain patients ^[87].

IV.5 Limitations of the study

Nevertheless, I must consider the limitations present in this work. Firstly, the use of male rats exclusively leads to incomplete results. Sex-related differences in WM tasks and pain assessments have been reported previously ^{[136], [137], [138]}, and the absence of female rats in this study may have led to partial results. As above mentioned, the unilateral lesions may have subsided some differences between CT and NAcSh-lesioned animals and subsequently diminished impacts on WM and pain processing mechanisms. Lastly, the recall sessions performed before the probe sessions could have also reduced any performance impairments and may be the reason why there was no significant differences, especially on day 14 probe session.

V. Conclusions and Future Perspectives

In summary, this study demonstrates that 1) neuropathic pain induced alterations in the IL-NAcSh microcircuit during WM-dependent processes, specifically during context information acquisition and processing, but did not impact significantly on behavior performance. In addition to performance values, this observation is also supported by the latency responses differences, and the positive correlations between mean centre velocity and reward zone time; 2) no motor impairments were observed for the spatial WM task, as total distance travelled, mean (centre) velocity, and zone time distribution values were similar between the experimental groups. The navigation patterns explored through the heat maps of zone allocation time, the entropy index and the instantaneous velocity plots also remained unchanged between experimental groups; 3) the SNI pain model was well established, as those animals displayed lower post-surgery mechanical noxious threshold; 4) the NAcSh GABAergic neuron density remained unchanged between Sham and SNI-treated animals.

The clear involvement of the IL-NAcSh circuitry in the WM preserving capacity and pain perception would be further understood through the modulation of the specific IL glutamatergic projections to the NAcSh. In future studies, the use of electrodes in the two brain regions could unveil functional variabilities in oscillation patterns and zone-specific activity related to this microcircuit under persistent pain conditions. Finally, a broader analysis including other NAcSh tissue markers may also help obtain a more complete view in future studies of the pain-related alterations in this region.

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VII. Supplementary information

VII.1. Results supplementary information

Table S.1 – Average number of trials performed in the eight-maze spatial alternation task during the learning phase and respective performance percentage.

Days/ Phase	Number of trials												Performance (%)	
	Total		Correct left		Correct right		Correct		Incorrect		Omission			
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	60,719	2,951	11,219	1,362	10,500	1,261	21,281	2,511	2,094	0,319	36,969	1,583	32,768	2,995
2	84,500	3,413	20,938	2,120	20,313	2,020	41,250	3,954	1,281	0,216	42,281	3,233	46,925	4,062
3	92,688	2,855	26,125	2,540	29,688	2,695	55,375	4,411	1,281	0,274	36,719	3,241	57,935	3,948
4	91,938	3,037	32,188	2,072	33,656	1,587	65,844	3,521	0,938	0,148	25,156	2,365	71,257	2,813
5	90,813	2,833	36,188	1,729	36,125	1,713	72,344	3,378	0,500	0,135	17,969	1,674	78,942	2,070
6	82,656	3,463	33,406	1,851	32,969	1,705	66,375	3,505	1,000	0,191	15,281	0,787	79,280	1,301
7	94,563	3,802	39,469	2,389	40,375	2,035	80,906	4,077	0,531	0,110	13,063	1,335	84,741	1,644
8	95,844	2,615	41,594	1,468	41,719	1,439	83,313	2,879	0,688	0,188	11,844	1,065	86,574	1,246
9	96,531	3,321	42,094	1,612	40,938	1,887	84,594	3,228	0,375	0,117	11,438	0,857	87,409	0,919
10	91,906	3,742	39,750	1,945	40,906	1,989	80,656	3,872	0,594	0,155	10,281	0,931	87,839	1,211
early	89,708	2,596	26,417	2,049	27,885	1,731	54,156	3,551	1,167	0,171	34,719	2,599	58,706	3,384
late	94,760	2,784	41,146	1,466	41,187	1,455	82,854	2,964	0,552	0,111	11,187	0,772	87,274	0,935

Table S.2 – Average number of trials performed in the eight-maze spatial alternation task during the probe sessions and respective performance percentage.

			Number of trials													
			Total		Correct left		Correct right		Correct		Incorrect		Omission		Performance (%)	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Days	Experimental Group															
14	Sham	CT	79,625	5,956	36,625	3,880	38,875	2,486	75,500	6,288	0,250	0,144	11,125	2,135	94,653	1,274
		NAcSh lesion	75,500	4,852	34,875	2,593	34,500	3,195	69,375	5,760	1,000	0,354	7,250	1,762	91,693	3,539
	SNI	CT	73,700	10,283	30,700	5,524	32,400	5,238	63,100	10,604	0,600	0,245	10,000	1,636	83,536	4,051
		NAcSh lesion	85,100	7,026	37,100	3,716	35,700	3,192	72,200	6,778	1,300	0,682	9,400	1,495	87,150	3,471
21	Sham	CT	88,000	11,051	41,750	4,918	41,750	4,512	83,750	9,534	0,375	0,125	10,500	2,010	95,613	2,051
		NAcSh lesion	84,250	5,793	37,750	4,265	37,625	4,342	75,750	8,714	0,750	0,433	11,125	3,992	89,073	4,302
	SNI	CT	93,400	4,768	40,300	3,580	42,000	3,899	82,300	7,400	0,400	0,100	10,700	2,667	88,970	2,556
		NAcSh lesion	89,600	5,860	40,900	2,657	40,200	2,909	81,100	5,460	0,200	0,122	8,300	2,251	90,592	2,249
28	Sham	CT	94,750	5,868	43,250	2,712	43,375	3,125	86,625	5,640	0,625	0,375	7,500	0,204	91,368	0,401
		NAcSh lesion	86,375	9,470	39,375	4,710	38,750	5,703	78,125	10,379	0,375	0,239	7,875	2,304	89,753	3,862
	SNI	CT	98,200	6,196	44,700	3,513	44,600	3,533	89,300	6,965	0,800	0,200	8,100	1,187	90,580	1,846
		NAcSh lesion	99,100	8,625	45,900	4,176	44,700	4,058	90,600	8,215	0,700	0,464	7,800	1,670	91,438	1,945

VII.2. Data analysis supplementary information – MATLAB scripts

The following custom MATLAB scripts were used throughout the data analysis presented in the present work.

```

1 % Compilation of MATLAB scripts
2 %
3 % =====
4 % Variables =====
5 % zone_x_xx; Data regarding zone location in each point extracted directly
6 % from txt file (OpenControl)
7 % zonexxx; Filtered zones, containing only sequence travelled without
8 % consecutive repetition
9 % L1-L2-L3; Sequence of areas travelled (1 trial)
10 % Correct_R; Total number of correct trials travelled to right feeder
11 % Correct_L; Total number of correct trials travelled to left feeder
12 % Incorrect_Total; Total number of incorrect trials
13 % Omission_Total; Total number of omissions
14 % Performance_onlyInc; Total number of correct trials divided by sum of
15 % correct+incorrect trials only
16 % Performance; Total number of correct trials divided by sum of
17 % correct+incorrect+omission trials
18 % Total_Trials; Total number of trials extracted
19 % bodyX000up; Updated variable of bodyX_cm_0_00 (extracted directly with
20 % OpenControl) with X limited to coordinates within the defined maze
21 % - also referred to as: axis_x and x_coord
22 % bodyY000up; Updated variable of bodyY_cm_0_00 (extracted directly with
23 % OpenControl) with Y limited to coordinates within the defined maze
24 % - also referred to as: axis_y and y_coord
25 % Area0_Count; Total number of points within the defined area
26 % Area0_Perc; Percentage of Area0_Count divided by sum of all areas' counts
27 % Distance_000; Calculate distance between each 2 ponits
28 % TotalDist_000; Sum of all calculated distances
29 % data_track; Combined matrix of x coordinates (column1), y coordinates
30 % (column 2), and instantaneous velocity values in pixels/bin (column 3)
31 % meanVel; Mean velocity values
32 % loc_time_0_00; Normalized time spent per zone, divided by 12x16 grid
33 % Bonsai_total_sec_10_32; Total seconds in Bonsai video recording (15 frames
34 % per sec)
35 % Time_diff_BonsaiOpenControl_10_32; Time difference between start Bonsai -
36 % start OpenControl /IF Bonsai stopped later then OpenControl
37 % Columns_Exclude_Bonsai_10_32; Number of columns to exclude from early
38 % Bonsai recording
39 % X_0_00up or Y_0_00up; Updated matrix of x/y coordinates extracted from
40 % Bonsai recording, matching to the OpenControl recording start
41 % Feeders_Bonsai_000; Manually extracted timestamps of feeder activation,
42 % extracted through Bonsai video recording
43 % Feeders_Converted_000; Converted Bonsai feeder activation time to match
44 % OpenControl values
45 % Actuator_Status_0_00; Feeder actuator status (on/off) extracted from
46 % OpenControl
47 % x_coord; x coordinates extracted from OpenControl (updated)
48 % y_coord; y coordinates extracted from OpenControl (updated)
49 % Choice_count; Time correspondent to when the animal is in the selected
50 % area (choice zone + feeder)
51 % - also referred to as zone_test and zone_test_f
52 % Nav_choice_start; Timestamps of entrance in the selected area
53 % Nav_choice_end; Timestamps of exit of the selected area
54 % Nav_Choice_Int; Combination matrix of Nav_choice_start (column 1) and
55 % Nav_choice_end (column 2)
56 % FINAL_Latency_data_2832; Matrix comprising of Nav_choice_start (column 1),
57 % Nav_choice_end (column 2), feeder activation within the interval
58 % (column 3), latency responses (column 4) and feeder timestamp (column 5)
59 % Latency_data_12TimeBlocks_282_0_60; Latency responses in the min selected
60 % (0-60 sec as an example)
61 % Latency_data_12TimeBlocks_282_Mean_0_60; Mean latency responses per min
62 % Latency_Cumulative_12TimeBlocks_282; Cumulative latency responses in the
63 % 12 min testing session
64 % Latency_MeanCumulative_12TimeBlocks_282; Cumulative latency responses per
65 % animal, averaged for the 2 daily 12 min testing sessions
66 % zonetime2832_2; Combined matrix of zonexxx (column 2) and the timestamp
67 % of zone entrance (column 1)
68 % zone2832_2_0_60; Filtered zones, containing only sequence travelled without
69 % consecutive repetition within the time interval selected
70 % mean_heatmaps_day_XXX; Mean time/space values per probe session per animal
71 % mean_heatmaps_day_ExpGroup; Mean time/space values per experimental group
72
73
74 % =====
75 % Function to obtain sequence of areas =====
76
77 zone2825_2=reshape(zone_28_25_2,1,[1]);
78 zone2825_2=zone2825_2([true diff(zone2825_2)~=0]);
79 zone2825_2=reshape(zone2825_2,[1],1);
80
81 % =====
82 % Funtion to calculate trial outcome and performance =====
83
84 % Area Code
85 % Area 1: Delay Left
86 % Area 2: Feeder Left
87 % Area 3: Out of Maze
88 % Area 4: Centre + Choice (T zone)
89 % Area 5: Delay Right
90 % Area 6: Feeder Right
91
92 clear all
93
94 zone2832_2 = load('zone2832_2.txt','-ascii'); %clear filename
95
96 x=length(zone2832_2); %Full sequence length
97 L1=zeros(1,1);
98 L2=zeros(1,1);
99 L3=zeros(1,1);
100 Correct_R=zeros(1,1);
101 Correct_L=zeros(1,1);
102 Correct_Total=zeros(1,1);
103 Incorrect_Total=zeros(1,1);
104 Omission_Total=zeros(1,1);
105
106 % for x=1:length(zone2832_2)

```

```

107     flag=1;
108     for b=1:length(zone2832_2)-2
109         L1=zone2832_2(b,1); %1 or 5
110         L2=zone2832_2(b+1,1); %4
111         L3=zone2832_2(b+2,1); %6 or 2
112         if (L1==1) && (L2==4) && (L3==6); %Right reward
113             Correct_R=Correct_R+1;
114             disp('R');
115         elseif (L1==5) && (L2==4) && (L3==2); %Left reward
116             Correct_L=Correct_L+1;
117             disp('L');
118         elseif (L1==1) && (L2==4) && (L3==2) || (L1==5) && (L2==4) && (L3==6);
119             Incorrect_Total=Incorrect_Total+1;
120             disp('I');
121         elseif (L1==4) && (L2==1) && (L3==2) || (L1==2) && (L2==4) && (L3==5) || (L1==4) && (L2==5) && (L3==6) || (L1==6) &&
122             (L2==4) && (L3==1) || (L1==2) && (L2==4) && (L3==6) || (L1==6) && (L2==4) && (L3==2);
123             Omission_Total=Omission_Total+1;
124             disp('O');
125         flag=flag+3; %Jump 3 positions after identified trial
126     end
127 end; clear b
128
129 Correct_Total = Correct_L+Correct_R; %Total Correct
130 Performance_onlyInc = Correct_Total / (Correct_Total + Incorrect_Total);
131 Performance = Correct_Total / (Correct_Total + Incorrect_Total + Omission_Total);
132 Total_Trials = Correct_Total + Incorrect_Total + Omission_Total;
133
134 fprintf('Performance with only Incorrect: %d\n',Performance_onlyInc) %Display Performance Percentage
135 fprintf('Performance Incorrect + Omissions: %d\n',Performance) %Display Performance Percentage
136
137 % =====
138 % Function to limit the X values within the maze perimeter, substituting
139 % the ones that are outside the perimeter with the previous one inside the
140 % maze =====
141
142 % X limits: 100 < X < 380
143
144 ii1=zeros(1,1);
145 ii2=zeros(1,1);
146 ii3=zeros(1,1);
147 ii4=zeros(1,1);
148 bodyX000up = bodyX_cm_0_00;
149
150 % lower limit
151 while any(bodyX000up(:)<100)
152     ii1=(bodyX000up<100);
153     ii2=circshift(ii1,[-1 0]);
154     bodyX000up(ii1)=bodyX000up(ii2);
155 end
156
157 % upper limit
158 while any(bodyX000up(:)>380)
159     ii3=(bodyX000up>380);
160     ii4=circshift(ii3,[1 0]);
161     bodyX000up(ii3)=bodyX000up(ii4);
162 end
163
164 clear ii1, clear ii2, clear ii3, clear ii4;
165 save('bodyX000up.txt', 'bodyX000up', '-ascii');
166
167 % =====
168 % Function to limit the Y values within the maze perimeter, substituting
169 % the ones that are outside the perimeter with the previous one inside the
170 % maze =====
171
172 % Y limits: 50 < Y < 445
173
174 jj1=zeros(1,1);
175 jj2=zeros(1,1);
176 jj3=zeros(1,1);
177 jj4=zeros(1,1);
178 bodyY000up=bodyY_cm_0_00;
179
180 % lower limit
181 while any(bodyY000up(:)<50)
182     jj1=(bodyY000up<50);
183     jj2=circshift(jj1,[-1 0]);
184     bodyY000up(jj1)=bodyY000up(jj2);
185 end
186
187 % upper limit
188 while any(bodyY000up(:)>445)
189     jj3=(bodyY000up>445);
190     jj4=circshift(jj3,[1 0]);
191     bodyY000up(jj3)=bodyY000up(jj4);
192 end
193
194 clear jj1, clear jj2, clear jj3, clear jj4;
195 save('bodyY000up.txt', 'bodyY000up', '-ascii');
196
197 % =====
198 % Function to separate points in designated areas in the maze =====
199 %
200 % Area 1: Delay Left
201 % Area 2: Feeder Left
202 % Area 3: Out of Maze
203 % Area 4: Centre + Choice (T)
204 % Area 5: Delay Right
205 % Area 6: Feeder Right
206
207 % Get file
208 [filename, pathname] = uigetfile({'bodyX*.txt'}) % Busca ficheiro X
209 bodyX = load(filename, '-ascii'); clear filename
210 [filename, pathname] = uigetfile({'bodyY*.txt'}) % Busca ficheiro Y
211 bodyY = load(filename, '-ascii'); clear filename

```

```

212
213 b=length(bodyX); % Full sequence length
214
215 Area1_Count=0;
216 Area2_Count=0;
217 Area3_Count=0;
218 Area4_Count=0;
219 Area5_Count=0;
220 Area6_Count=0;
221 Area4_Choice_Count=0;
222 Area4_Centre_Count=0;
223
224 Area1_Perc=0;
225 Area2_Perc=0;
226 Area3_Perc=0;
227 Area4_Perc=0;
228 Area5_Perc=0;
229 Area6_Perc=0;
230 Area4_Choice_Perc=0;
231 Area4_Centre_Perc=0;
232
233 % Define areas and count
234
235 for a=1:b
236     x=bodyX(a,1);
237     y=bodyY(a,1);
238
239     if (x>=100) && (x<277) && (y>=50) && (y<=80) || (x>=100) && (x<=135) && (y>80) && (y<183) % 2 rectangles (1.1 + 1.2)
240         Area1_Count=Area1_Count+1;
241     elseif (x>=277) && (x<=380) && (y>=50) && (y<=80) || (x>300) && (x<=380) && (y>80) && (y<142) % 2 rectangles (2.1 + 2.2)
242         Area2_Count=Area2_Count+1;
243     elseif (x>135) && (x<300) && (y>80) && (y<183) || (x>130) && (x<295) && (y>295) && (y<400) % Zonas de plataforma - salto
244         Area3_Count=Area3_Count+1;
245     elseif (x>=300) && (x<=380) && (y>=142) && (y<183) || (x>=100) && (x<=380) && (y>=183) && (y<320) || (x>=295) && (x<=380)
246         && (y>=320) && (y<357) % 3 rectangles (4.1 + 4.2 + 4.3)
247         Area4_Count=Area4_Count+1;
248         if (x>=295) && (x<=380) && (y>=125) && (y<=375)
249             Area4_Choice_Count=Area4_Choice_Count+1;
250         else
251             Area4_Centre_Count=Area4_Centre_Count+1;
252         end
253     elseif (x>=100) && (x<=130) && (y>=320) && (y<400) || (x>=100) && (x<280) && (y>=400) && (y<=445) % 2 rectangles (5.1 +
254         5.2)
255         Area5_Count=Area5_Count+1;
256     elseif (x>=295) && (x<=380) && (y>=357) && (y<400) || (x>=280) && (x<=380) && (y>=400) && (y<=445) % 2 rectangles (6.1 +
257         6.2)
258         Area6_Count=Area6_Count+1;
259     end
260
261     Area1_Perc=Area1_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
262     Area2_Perc=Area2_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
263     Area3_Perc=Area3_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
264     Area4_Perc=Area4_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
265     Area4_Choice_Perc=Area4_Choice_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
266     Area4_Centre_Perc=Area4_Centre_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
267     Area5_Perc=Area5_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
268     Area6_Perc=Area6_Count/(Area1_Count+Area2_Count+Area3_Count+Area4_Count+Area5_Count+Area6_Count);
269 end; clear b;
270
271 % Export area data in .txt
272 % Control: correct number of frames analysed
273
274 clear filename; clear pathname;
275 output = zeros(17,1);
276 output(1,1)=Area1_Count;
277 output(2,1)=Area2_Count;
278 output(3,1)=Area3_Count;
279 output(4,1)=Area4_Count;
280 output(5,1)=Area5_Count;
281 output(6,1)=Area6_Count;
282 output(7,1)=Area1_Perc;
283 output(8,1)=Area2_Perc;
284 output(9,1)=Area3_Perc;
285 output(10,1)=Area4_Perc;
286 output(11,1)=Area5_Perc;
287 output(12,1)=Area6_Perc;
288 output(14,1)=Area4_Choice_Count;
289 output(15,1)=Area4_Centre_Count;
290 output(16,1)=Area4_Choice_Perc;
291 output(17,1)=Area4_Centre_Perc;
292
293 disp('==CONTROLO CALCULO==');
294 disp('Num frames'); length(bodyX)
295 disp('Total calculado'); output(1,1)+output(2,1)+output(3,1)+output(4,1)+output(5,1)+output(6,1)
296
297 [filename, pathname] = uigetfile('*.txt', 'Save file - Output name');
298 save(filename, 'output', '-ascii');
299 disp('-- fim --');
300
301 % =====
302 % Function to calculate total distance travelled in the maze (12 min) =====
303
304 [filename, pathname] = uigetfile({'bodyX*.txt'}) % Busca ficheiro X
305 bodyX = load(filename, '-ascii'); clear filename
306 [filename, pathname] = uigetfile({'bodyY*.txt'}) % Busca ficheiro Y
307 bodyY = load(filename, '-ascii'); clear filename
308
309 Distance_000 = hypot(diff(bodyX000up), diff(bodyY000up));
310 TotalDist_000 = sum(Distance_000) * 0.2415 % Conversion pixel-cm
311
312 save('TotalDist_000.txt', 'TotalDist_000', '-ascii');
313
314 % =====
315 % Function to illustrate instantaneous velocity, navigation and time
316 % allocated per zone

```

```

315
316 clear all
317
318 % load data
319 [filename, pathname] = uigetfile({'bodyX*.txt'}); % Busca ficheiro coord_X
320 axis_x = load(filename, '-ascii'); clear filename
321 [filename, pathname] = uigetfile({'bodyY*.txt'}); % Busca ficheiro coord_Y
322 axis_y = load(filename, '-ascii'); clear filename
323
324 temp = length(axis_x);
325 data_track = zeros(temp, 3);
326
327
328 % XX | YY | dist/motion
329
330 data_track(:,1)=axis_x;
331 data_track(:,2)=axis_y;
332
333 % Euclidian distance equation
334
335 for i=1:length(data_track(:,1))-1
336
337     dist = sqrt(((data_track(i+1,1) - data_track(i,1)) ^ 2) + ((data_track(i+1,2) - data_track(i,2)) ^ 2));
338
339     data_track(i,3) = dist;
340
341 end
342 meanVel = mean(data_track(:,3));
343
344 disp('Averaged velocity (in pixels/bin):'); meanVel
345
346 meanVel = (meanVel * 0.2145)/0.05;
347
348 disp('Averaged velocity (in cm/s):'); meanVel
349
350 % NOTE: please change bin per time unit;
351 data_track(:,3) = (data_track(:,3) * 0.2145)/0.05; % Instantaneous velocity
352 % in cm/s
353
354 % Normalization of the motion velocity vector
355 % step = 5 bins
356 motion = max(data_track(:,3));
357
358 for j=1:length(data_track)
359     data_track(j,3) = data_track(j,3)/motion;
360 end
361
362 for i=1:length(data_track)-5
363     data_track(i,3)=mean(data_track(i:i+5,3));
364 end
365
366 % Scatter instantaneous velocity
367
368 figure(1);
369 hold on
370 grid on
371 set(gca, 'clim', [0 1]);
372 set(gca, 'xlim', [0 640]);
373 set(gca, 'ylim', [0 480]);
374 c = data_track(:,3);
375
376 [sortDist sortIndexes] = sort(c(:,1));
377
378 x = data_track(:,1); xs= x(sortIndexes);
379 y = data_track(:,2); ys= y(sortIndexes);
380
381 cmap = jet(length(c));
382 shading interp;
383
384 colorbar;
385 scatter(xs, ys, 10, cmap, 'filled');
386 xlabel('Pixels'); ylabel('Pixels');
387 title('Normalized instantaneous velocity')
388 hold off
389
390 % Total navigation trace
391 figure(2);
392 hold on
393 grid on
394 set(gca, 'clim', [0 1]);
395 set(gca, 'xlim', [0 640]);
396 set(gca, 'ylim', [0 480]);
397 c = data_track(:,3);
398
399 [sortDist sortIndexes] = sort(c(:,1));
400
401 x = data_track(:,1); xs= x(sortIndexes);
402 y = data_track(:,2); ys= y(sortIndexes);
403
404 cmap = jet(length(c));
405 colorbar;
406 scatter(xs, ys, 1, 'filled'); line(x,y, 'color','bl');
407 xlabel('Pixels'); ylabel('Pixels'); title('Navigation trace')
408 hold off
409
410 L = findobj(1, 'type','line');
411 copyobj(L, findobj(2,'type','axes'));
412
413 % Estimation of the total time spent per location in maze
414 % bin resolution 40 x 40 dpi; 640x480
415
416 x_bin = 640/40; y_bin = 480/40;
417
418 loc time = zeros(x_bin, y_bin); % counting output array
419 data = data_track(:,1:2);
420

```

```

421 for t=1:length(data_track(:,1));
422
423 % col (1)
424 lim_down = 0;
425 lim_up = 40;
426 col = 1;
427 for yy=1:16 % move in col
428     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>0 & data(t,2)<40)
429         loc_time(yy,col) = loc_time(yy,col) + 1;
430         lim_down = lim_up; lim_up = lim_up + 40;
431     else
432         loc_time(yy,col) = loc_time(yy,col);
433         lim_down = lim_up; lim_up = lim_up + 40;
434     end;
435 end; clear lim_down; clear lim_up; clear yy
436
437 % col (2)
438 lim_down = 0;
439 lim_up = 40;
440 col = 2;
441 for yy=1:16 % move in col
442     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>40 & data(t,2)<80)
443         loc_time(yy,col) = loc_time(yy,col) + 1;
444         lim_down = lim_up; lim_up = lim_up + 40;
445     else
446         loc_time(yy,col) = loc_time(yy,col);
447         lim_down = lim_up; lim_up = lim_up + 40;
448     end;
449 end; clear lim_down; clear lim_up; clear yy
450
451 % col (3)
452 lim_down = 0;
453 lim_up = 40;
454 col = 3;
455 for yy=1:16 % move in col
456     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>80 & data(t,2)<120)
457         loc_time(yy,col) = loc_time(yy,col) + 1;
458         lim_down = lim_up; lim_up = lim_up + 40;
459     else
460         loc_time(yy,col) = loc_time(yy,col);
461         lim_down = lim_up; lim_up = lim_up + 40;
462     end;
463 end; clear lim_down; clear lim_up; clear yy
464
465 % col (4)
466 lim_down = 0;
467 lim_up = 40;
468 col = 4;
469 for yy=1:16 % move in col
470     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>120 & data(t,2)<160)
471         loc_time(yy,col) = loc_time(yy,col) + 1;
472         lim_down = lim_up; lim_up = lim_up + 40;
473     else
474         loc_time(yy,col) = loc_time(yy,col);
475         lim_down = lim_up; lim_up = lim_up + 40;
476     end;
477 end; clear lim_down; clear lim_up; clear yy
478
479 % col (5)
480 lim_down = 0;
481 lim_up = 40;
482 col = 5;
483 for yy=1:16 % move in col
484     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>160 & data(t,2)<200)
485         loc_time(yy,col) = loc_time(yy,col) + 1;
486         lim_down = lim_up; lim_up = lim_up + 40;
487     else
488         loc_time(yy,col) = loc_time(yy,col);
489         lim_down = lim_up; lim_up = lim_up + 40;
490     end;
491 end; clear lim_down; clear lim_up; clear yy
492
493 % col (6)
494 lim_down = 0;
495 lim_up = 40;
496 col = 6;
497 for yy=1:16 % move in col
498     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>200 & data(t,2)<240)
499         loc_time(yy,col) = loc_time(yy,col) + 1;
500         lim_down = lim_up; lim_up = lim_up + 40;
501     else
502         loc_time(yy,col) = loc_time(yy,col);
503         lim_down = lim_up; lim_up = lim_up + 40;
504     end;
505 end; clear lim_down; clear lim_up; clear yy
506
507 % col (7)
508 lim_down = 0;
509 lim_up = 40;
510 col = 7;
511 for yy=1:16 % move in col
512     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>240 & data(t,2)<280)
513         loc_time(yy,col) = loc_time(yy,col) + 1;
514         lim_down = lim_up; lim_up = lim_up + 40;
515     else
516         loc_time(yy,col) = loc_time(yy,col);
517         lim_down = lim_up; lim_up = lim_up + 40;
518     end;
519 end; clear lim_down; clear lim_up; clear yy
520
521 % col (8)
522 lim_down = 0;
523 lim_up = 40;
524 col = 8;
525 for yy=1:16 % move in col
526     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>280 & data(t,2)<320)

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```

527         loc_time(yy,col) = loc_time(yy,col) + 1;
528         lim_down = lim_up; lim_up = lim_up + 40;
529     else
530         loc_time(yy,col) = loc_time(yy,col);
531         lim_down = lim_up; lim_up = lim_up + 40;
532     end;
533 end; clear lim_down; clear lim_up; clear yy
534
535 % col (9)
536 lim_down = 0;
537 lim_up = 40;
538 col = 9;
539 for yy=1:16 % move in col
540     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>320 & data(t,2)<360)
541         loc_time(yy,col) = loc_time(yy,col) + 1;
542         lim_down = lim_up; lim_up = lim_up + 40;
543     else
544         loc_time(yy,col) = loc_time(yy,col);
545         lim_down = lim_up; lim_up = lim_up + 40;
546     end;
547 end; clear lim_down; clear lim_up; clear yy
548
549 % col (10)
550 lim_down = 0;
551 lim_up = 40;
552 col = 10;
553 for yy=1:16 % move in col
554     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>360 & data(t,2)<400)
555         loc_time(yy,col) = loc_time(yy,col) + 1;
556         lim_down = lim_up; lim_up = lim_up + 40;
557     else
558         loc_time(yy,col) = loc_time(yy,col);
559         lim_down = lim_up; lim_up = lim_up + 40;
560     end;
561 end; clear lim_down; clear lim_up; clear yy
562
563 % col (11)
564 lim_down = 0;
565 lim_up = 40;
566 col = 11;
567 for yy=1:16 % move in col
568     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>400 & data(t,2)<440)
569         loc_time(yy,col) = loc_time(yy,col) + 1;
570         lim_down = lim_up; lim_up = lim_up + 40;
571     else
572         loc_time(yy,col) = loc_time(yy,col);
573         lim_down = lim_up; lim_up = lim_up + 40;
574     end;
575 end; clear lim_down; clear lim_up; clear yy
576
577 % col (12)
578 lim_down = 0;
579 lim_up = 40;
580 col = 12;
581 for yy=1:16 % move in col
582     if (data(t,1)>lim_down & data(t,1)<lim_up) && (data(t,2)>440 & data(t,2)<480)
583         loc_time(yy,col) = loc_time(yy,col) + 1;
584         lim_down = lim_up; lim_up = lim_up + 40;
585     else
586         loc_time(yy,col) = loc_time(yy,col);
587         lim_down = lim_up; lim_up = lim_up + 40;
588     end;
589 end; clear lim_down; clear lim_up; clear yy
590
591 end
592
593 % Heatmap time/space
594 disp('image')
595 loc_time = log10(loc_time)'; % 12x16
596 max_loc_time = max(loc_time(:));
597
598 for q=1:12
599     loc_time(q,:) = loc_time(q,:)/max_loc_time;
600 end
601
602 loc_time_21_22_21 = zeros(12,16);
603 loc_time_21_22_21 = loc_time;
604 save('heatmap_2821.txt', 'loc_time_21_22_21', '-ascii');
605
606 figure(3);
607 hold on
608 grid off
609 set(gca, 'clim', [0 1]);
610 set(gca, 'xlim', [0 16]);
611 set(gca, 'ylim', [0 13]); % Changed from 12 to 13 to avoid cropping
612 colormap(jet)
613 imagesc(loc_time); colorbar off
614 axis off
615 xlabel('Bin (40 pixels/block)');
616 ylabel('Bin (40 pixels/block)');
617 title('Normalized time spent per zone')
618 hold off
619
620 % Export figuras as a TIF image
621 saveas(figure(1), 'Insta_vel_21_22_2.tif');
622 saveas(figure(2), 'Nav_trace_9_1.tif');
623 saveas(figure(3), 'Time_zone_21_22_2.tif');
624 disp('Figures exported as TIF format.')
625
626 disp(' - End of computations - ')
627
628 % =====
629 % Function to convert time difference between Bonsai and OpenControl start
630 % and exclude extra columns in Bonsai file =====
631
632 Bonsai_total_sec_10_32 = length(X_10_32)/15; % Total seconds in Bonsai

```

```

633 % recording
634 Time_dif_BonsaiOpenControl_10_32 = Bonsai_total_sec_10_32 - time_s_10_32(end,1); % Time difference: start Bonsai - start
OpenControl
635 Columns_Exclude_Bonsai_10_32 = Time_dif_BonsaiOpenControl_10_32*15;
636 % Number of columns to exclude correspondent to the extra seconds
637
638 X_10_32up = X_10_32(COLUMNS_Exclude_Bonsai_10_32:end,1);
639
640 % Function to convert feeder activation time values (done from Bonsai
641 % video) to the correspondent scale in OpenControl =====
642 % - Bonsai .txt: 15 fps
643 % - OpenControl .txt: 20 fps
644
645 x=length(Feeders_Bonsai_1025); %Values extracted from Bonsai video
646 for b=1:x
647     value=Feeders_Bonsai_1025(b,1);
648     Feeders_Converted_1025up(b,1)=((value/(length(X_10_25up)/20))*(length(X_10_25up)/15)); %Convert to real time in Bonsai
(20-15 fps)
649     Feeders_Converted_1025up(b,1)=Feeders_Converted_1025up(b,1)-((length(X_10_25up)/15)-(length(Actuator_Status_10_25)/20));
650     %Subtract time differences between running Bonsai - OpenControl
651 end
652 clear x, clear b, clear value
653 save('Feeders_Converted_1025up.txt', 'Feeders_Converted_1025up', '-ascii');
654
655 % =====
656 % Function to calculate latency response from the decision point to the
657 % reward zone =====
658
659 bodyX = load('bodyX2832up.txt','-ascii'); %clear filename
660 bodyY = load('bodyY2832up.txt','-ascii'); %clear filename
661
662 % Navigation zones limits
663
664 num_coord_points = length(bodyX); % Full sequence length
665 Choice_count=zeros(1,1);
666 z=zeros(1,1);
667
668 for a=1:num_coord_points
669     x_coord = bodyX(a,1); % Ler linha a linha no x
670     y_coord = bodyY(a,1); % Ler linha a linha no y
671
672     if (x_coord >= 295) && (x_coord <= 380) && (y_coord >= 50) &&
673         (y_coord <= 445) % Choice + feeder zones
674         w = time_s_28_32(a,1);
675         else
676         w=0;
677     end
678
679     if (x_coord >= 295) && (x_coord <= 380) && (y_coord >= 125) &&
680         (y_coord <= 375) % Choice zone
681         z = time_s_28_32(a,1);
682         else
683         z=0;
684     end
685     Choice_count(a,1) = w;
686
687 end; clear a; clear b; clear x_coord; clear y_coord; clear w; clear z;
688 clear num_coord_points
689
690 % Clear output array zeros | only continuous timestamps
691 Choice_count = nonzeros(Choice_count); % choice + feeder
692 disp('Navigation time sequence - choice + feeder zones: extracted')
693
694 % Navigation zone start timepoint
695
696 zone_test = Choice_count;
697 output_zone_test = zeros(length(zone_test),1);
698 output_zone_test(1,1) = zone_test(1,1); % time(1)
699
700 for i=2:length(zone_test)
701
702     if zone_test(i,1) > (zone_test(i-1,1) + 0.5);
703         output_zone_test(i,1) = zone_test(i,1);
704     else
705         output_zone_test(i,1) = 0;
706     end
707
708 end; clear i;
709 output_zone_test = nonzeros(output_zone_test);
710 Nav_choice_start = output_zone_test; clear output_zone_test
711
712 % Navigation zone end timepoint
713
714 zone_test_f = Choice_count;
715 output_zone_test_f = zeros(length(zone_test_f), 1); temp = length(zone_test_f);
716 output_zone_test_f(temp,1) = zone_test_f(temp, 1);
717
718 for i=1:length(zone_test_f)-1
719
720     if (zone_test_f(i,1) + 1) > (zone_test_f(i+1, 1))
721         % time threshold 100 msec
722         output_zone_test_f(i,1) = 0;
723     else
724         output_zone_test_f(i,1) = zone_test_f(i, 1);
725     end
726
727 end; clear i;
728 output_zone_test_f = nonzeros(output_zone_test_f);
729 Nav_choice_end = output_zone_test_f; clear output_zone_test_f;
730 clear temp
731
732 % Navigation interval
733
734 for a=1:length(Nav_choice_start)-1
735     if Nav_choice_start(a+1,1) > Nav_choice_end(a,1)

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```

737     Nav_choice_start(a+1,1) = Nav_choice_start(a+1,1);
738 elseif Nav_choice_start(a+1,1) < Nav_choice_end(a,1)
739     Nav_choice_start(a+1:end,1) = Nav_choice_start(a+2:end,1);
740 end
741 end;
742
743 Nav_Choice_Int = zeros(length(Nav_choice_start),2);
744 Nav_Choice_Int(:,1) = Nav_choice_start; Nav_Choice_Int(:,2) = Nav_choice_end;
745
746 % Final outputs
747 % Int | out | O/1 | latency | Feeder ON
748
749 FINAL_Latency_data_2832 = zeros(length(Nav_choice_start), 5);
750 FINAL_Latency_data_2832(:,1) = Nav_choice_start;
751 FINAL_Latency_data_2832(:,2) = Nav_choice_end;
752
753 for h = 1:length(Nav_Choice_Int) % latency responses
754     for j = 1:length(Feeders_Converted_2832up);
755         if (Feeders_Converted_2832up(j,1) > FINAL_Latency_data_2832(h,1)) && (Feeders_Converted_2832up(j,1) <
756             FINAL_Latency_data_2832(h,2))
757             FINAL_Latency_data_2832(h,3) = 1;
758             FINAL_Latency_data_2832(h,4) = Feeders_Converted_2832up(j,1) - FINAL_Latency_data_2832(h,1);
759             FINAL_Latency_data_2832(h,5) = Feeders_Converted_2832up(j,1);
760         else
761             FINAL_Latency_data_2832(h,3) = 0;
762             FINAL_Latency_data_2832(h,4) = 0;
763             FINAL_Latency_data_2832(h,5) = 0;
764         end
765     end
766 end
767
768 % Results Summary
769 disp('=====')
770 disp('Number of correct trials:'); length(Feeders_Converted_2832up)
771 disp('Correct overlap:'); sum(FINAL_Latency_data_2832(:,3))
772 disp('Mean response latency:'); sum(FINAL_Latency_data_2832(:,4))/sum(FINAL_Latency_data_2832(:,3))
773 Mean_Latency_28_32 = sum(FINAL_Latency_data_2832(:,4))/sum(FINAL_Latency_data_2832(:,3));
774 disp('Max response latency:'); max(FINAL_Latency_data_2832(:,4))
775
776 disp('=====')
777
778 save('Mean_Latency_28_32.txt', 'Mean_Latency_28_32', '-ascii');
779 save('FINAL_Latency_data_2832.txt', 'FINAL_Latency_data_2832', '-ascii');
780
781 % =====
782 % Function to calculate the mean central velocity =====
783
784 bodyX = load('bodyX2825up.txt','-ascii'); %coordinates of the maze
785 bodyY = load('bodyY2825up.txt','-ascii'); %coordinates of the maze
786 FINAL_Latency_data_2825 = load('FINAL_Latency_data_2825.txt','-ascii');
787 Feeders_Converted_2825up = load('Feeders_Converted_2825up.txt','-ascii');
788 time_s_28_25 = load('time_s_28_25.txt','-ascii'); %time
789 FINAL_Areal_data_2825 = zeros(1,1);
790
791 % Navigation zones limits
792
793 num_coord_points = length(bodyX); % Full sequence length
794 Area4_centre_count=zeros(1,1);
795 z=zeros(1,1);
796 w=length(bodyX);
797 x=length(bodyX);
798 y=length(bodyY);
799
800 for a=1:num_coord_points
801     x_coord = bodyX(a,1); % Ler linha a linha no x
802     y_coord = bodyY(a,1); % Ler linha a linha no y
803
804     if ((x_coord>=90) && (x_coord<=295) && (y_coord>=183) && (y_coord<320))
805         % Centre area
806         w = time_s_28_25(a,1);
807         x = bodyX(a,1);
808         y = bodyY(a,1);
809
810     else
811         w=0;
812         x=NaN;
813         y=NaN;
814     end
815     Area4_centre_count(a,1) = w;
816     axis_Area4_centre_x(a,1) = x;
817     axis_Area4_centre_y(a,1) = y;
818
819 end; clear a; clear b; clear x_coord; clear y_coord; clear w; clear z;
820 clear num_coord_points
821
822 % Clear output array zeros | only continuous timestamps
823 Area4_centre_count = nonzeros(Area4_centre_count); % centre area time
824 x_nan = ~isnan(axis_Area4_centre_x); axis_Area4_centre_x = axis_Area4_centre_x(x_nan); % x coordinate in centre area
825 y_nan = ~isnan(axis_Area4_centre_y); axis_Area4_centre_y = axis_Area4_centre_y(y_nan); % y coordinate in centre area
826
827 Area4_centre_coord_time_data = zeros((length(Area4_centre_count)),3);
828 Area4_centre_coord_time_data(:,1) = axis_Area4_centre_x;
829 Area4_centre_coord_time_data(:,2) = axis_Area4_centre_y;
830 Area4_centre_coord_time_data(:,3) = Area4_centre_count;
831
832
833 disp('Navigation time sequence - area 4 centre zone: extracted')
834
835 % Navigation zone start timepoint
836
837 zone_test = Area4_centre_count;
838 output_zone_test = zeros(length(zone_test),1);
839 output_zone_test(1,1) = zone_test(1,1);
840
841 x_coord = zeros(length(Area4_centre_coord_time_data),1);

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842 y_coord = zeros(length(Area4_centre_coord_time_data),1);
843 meanVel_entrance_data_x = zeros(length(Area4_centre_coord_time_data),1);
844 meanVel_entrance_data_y = zeros(length(Area4_centre_coord_time_data),1);
845 x_coord(1,1) = Area4_centre_coord_time_data(1,1);
846 y_coord(1,1) = Area4_centre_coord_time_data(1,2);
847
848 for i=2:length(zone_test)
849
850     if zone_test(i,1) > (zone_test(i-1,1) + 0.5);
851         output_zone_test(i,1) = zone_test(i,1);
852         x_coord(i,1) = Area4_centre_coord_time_data(i,1);
853         y_coord(i,1) = Area4_centre_coord_time_data(i,2);
854     else
855         output_zone_test(i,1) = 0;
856         x_coord(i,1) = NaN;
857         y_coord(i,1) = NaN;
858     end
859
860 end; clear i;
861
862 output_zone_test = nonzeros(output_zone_test);
863 x_coord(isnan(x_coord)) = [];
864 y_coord(isnan(y_coord)) = [];
865
866 Nav_centre_start = output_zone_test; clear output_zone_test
867 %entrance in central area
868 meanVel_entrance_data_x = x_coord; clear x_coord %entrance x coordinate
869 meanVel_entrance_data_y = y_coord; clear y_coord %entrance y coordinate
870
871
872 % Navigation zone end timepoint
873
874 zone_test_f = Area4_centre_count;
875 output_zone_test_f = zeros(length(zone_test_f), 1); temp = length(zone_test_f);
876 output_zone_test_f(temp,1) = zone_test_f(temp, 1);
877
878 x_coord = zeros(length(Area4_centre_coord_time_data),1);
879 y_coord = zeros(length(Area4_centre_coord_time_data),1);
880 meanVel_end_data_x = zeros(length(Area4_centre_coord_time_data),1);
881 meanVel_end_data_y = zeros(length(Area4_centre_coord_time_data),1);
882 x_coord(temp,1) = Area4_centre_coord_time_data(temp,1);
883 y_coord(temp,1) = Area4_centre_coord_time_data(temp,2);
884
885 for i=1:length(zone_test_f)-1
886
887     if (zone_test_f(i,1) + 1) > (zone_test_f(i+1,1)) % time threshold
888         %100 msec
889         output_zone_test_f(i,1) = 0;
890         x_coord(i,1) = NaN;
891         y_coord(i,1) = NaN;
892     else
893         output_zone_test_f(i,1) = zone_test_f(i,1);
894         x_coord(i,1) = Area4_centre_coord_time_data(i,1);
895         y_coord(i,1) = Area4_centre_coord_time_data(i,2);
896     end
897
898 end
899
900 end; clear i;
901
902
903 output_zone_test_f = nonzeros(output_zone_test_f);
904 x_coord(isnan(x_coord)) = [];
905 y_coord(isnan(y_coord)) = [];
906
907 Nav_centre_end = output_zone_test_f; clear output_zone_test_f;
908 meanVel_end_data_x = x_coord; clear x_coord %entrance x coordinate
909 meanVel_end_data_y = y_coord; clear y_coord %entrance y coordinate
910
911 % Navigation interval
912
913 for a=2:length(Nav_centre_start)
914     if (Nav_centre_start(a-1,1) < Nav_centre_end(a-1,1)) &&
915         (Nav_centre_start(a,1) > Nav_centre_end(a-1,1))
916         Nav_centre_start(a,1) = Nav_centre_start(a,1);
917         meanVel_entrance_data_x(a,1) = meanVel_entrance_data_x(a,1);
918         meanVel_entrance_data_y(a,1) = meanVel_entrance_data_y(a,1);
919         meanVel_end_data_x(a,1) = meanVel_end_data_x(a,1);
920         meanVel_end_data_y(a,1) = meanVel_end_data_y(a,1);
921     elseif (Nav_centre_start(a-1,1) > Nav_centre_end(a-1,1)) ||
922         (Nav_centre_start(a,1) < Nav_centre_end(a-1,1))
923         Nav_centre_end(Nav_centre_start(a-1,1) > Nav_centre_end(a-1,1),:) = [];
924         Nav_centre_start((Nav_centre_start(a,1) < Nav_centre_end(a-1,1)),:) = [];
925         meanVel_entrance_data_x(a-1,1) = [];
926         meanVel_entrance_data_y(a-1,1) = [];
927         meanVel_end_data_x(a-1,1) = [];
928         meanVel_end_data_y(a-1,1) = [];
929     end
930 end;
931
932 Nav_Centre_Int = zeros(length(Nav_centre_start),2);
933 Nav_Centre_Int(:,1) = Nav_centre_start; Nav_Centre_Int(:,2) = Nav_centre_end;
934
935 meanVel_entrance = zeros(length(meanVel_entrance_data_x),2);
936 meanVel_end = zeros(length(meanVel_end_data_x),2);
937 meanVel_entrance(:,1) = meanVel_entrance_data_x; meanVel_entrance(:,2) = meanVel_entrance_data_y;
938 meanVel_end(:,1) = meanVel_end_data_x; meanVel_end(:,2) = meanVel_end_data_y;
939
940
941 % Final outputs
942 % Centre entrance | Centre exit | Time difference | Feeder Activation |
943 % Distance Travelled
944
945 FINAL_Area4_centre_data_2825 = zeros(length(Nav_centre_start), 5);
946 FINAL_Area4_centre_data_2825(:,1) = Nav_centre_start;
947 FINAL_Area4_centre_data_2825(:,2) = Nav_centre_end;

```

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948
949 % Compare intervals with latency (centre+feeders intervals)
950
951 for h = 1:length(Nav_Centre_Int) % latency responses
952     for j = 1:length(Feeders_Converted_2825up);
953         FINAL_Area4_centre_data_2825(h,3) = Nav_centre_end(h,1) - Nav_centre_start(h,1);
954         FINAL_Area4_centre_data_2825(h,5) = sqrt(((meanVel_end(h,1) - meanVel_entrance(h,1)) ^ 2) + ((meanVel_end(h,2) -
          meanVel_entrance(h,2)) ^ 2));
955         if (Feeders_Converted_2825up(j,1) >= Nav_Centre_Int(h,1)) &&
956             (Feeders_Converted_2825up(j,1) <= Nav_Centre_Int(h,2) + max(FINAL_Latency_data_2825(:,4))) % max latency
957             FINAL_Area4_centre_data_2825(h,4) = Feeders_Converted_2825up(j,1);
958         end
959     end
960 end
961
962 % Mean velocity in centre
963
964 Vel_Area4_centre = zeros(length(meanVel_entrance), 1);
965
966 for i=1:length(FINAL_Area4_centre_data_2825)
967     Vel_Area4_centre(i,1) = FINAL_Area4_centre_data_2825(i,5) / FINAL_Area4_centre_data_2825(i,3); % inst velocity in pixels/s
968     Vel_Area4_centre(i,1) = (Vel_Area4_centre(i,1) * 0.2145); % instant velocity in cm/s
969 end
970
971 Vel_Area4_centre(isnan(Vel_Area4_centre)) = 0;
972
973 meanVel_Area4_centre = mean(Vel_Area4_centre(:,1));
974 disp('Averaged velocity (in cm/s):'); meanVel_Area4_centre
975
976 % =====
977 % Function to calculate the latency responses in the 12 min testing session
978
979 FINAL_Latency_data_282 = load('FINAL_Latency_data_282.txt', '-ascii');
980 % Load Latency data session #1
981 FINAL_Latency_data_282_2 = load('FINAL_Latency_data_282_2.txt', '-ascii');
982 % Load Latency data session #2
983
984 % Divide the latency responses per min (0-60 as an example)
985 Latency_data_12TimeBlocks_282_0_60 = zeros(length(FINAL_Latency_data_282),1);
986
987 e=zeros(1,1);
988
989 for a=1:length(FINAL_Latency_data_282)
990     if (FINAL_Latency_data_282(a,1) > 0) && (FINAL_Latency_data_282(a,1) <= 60)
991         e = FINAL_Latency_data_282(a,4);
992     else
993         e=0;
994     end
995
996     Latency_data_12TimeBlocks_282_0_60(a,1) = e;
997 end
998
999 Latency_data_12TimeBlocks_282_0_60 = nonzeros(Latency_data_12TimeBlocks_282_0_60);
1000 Latency_data_12TimeBlocks_282_Mean_0_60 = mean(Latency_data_12TimeBlocks_282_0_60);
1001 % repeat for the each minute
1002
1003 % Extract mean latency responses per min
1004 output = zeros(12,1);
1005 output(1,1)=Latency_data_12TimeBlocks_282_Mean_0_60;
1006 output(2,1)=Latency_data_12TimeBlocks_282_Mean_60_120;
1007 output(3,1)=Latency_data_12TimeBlocks_282_Mean_120_180;
1008 output(4,1)=Latency_data_12TimeBlocks_282_Mean_180_240;
1009 output(5,1)=Latency_data_12TimeBlocks_282_Mean_240_300;
1010 output(6,1)=Latency_data_12TimeBlocks_282_Mean_300_360;
1011 output(7,1)=Latency_data_12TimeBlocks_282_Mean_360_420;
1012 output(8,1)=Latency_data_12TimeBlocks_282_Mean_420_480;
1013 output(9,1)=Latency_data_12TimeBlocks_282_Mean_480_540;
1014 output(10,1)=Latency_data_12TimeBlocks_282_Mean_540_600;
1015 output(11,1)=Latency_data_12TimeBlocks_282_Mean_600_660;
1016 output(12,1)=Latency_data_12TimeBlocks_282_Mean_660_720;
1017
1018 Latency_12TimeBlocks_282 = output;
1019 save('Latency_12TimeBlocks_282.txt', 'Latency_12TimeBlocks_282', '-ascii');
1020
1021 % =====
1022 % Function to calculate the cumulative latency responses in the 12 min
1023 % testing session, and averaged per animal (2 daily sessions)
1024
1025 Latency_Cumulative_12TimeBlocks_282 = zeros(12,1);
1026
1027 Latency_Cumulative_12TimeBlocks_282(1,1) = Latency_12TimeBlocks_282(1,1);
1028 for a=2:length(Latency_12TimeBlocks_282)
1029     Latency_Cumulative_12TimeBlocks_282(a,1) = (Latency_Cumulative_12TimeBlocks_282(a-1,1)) + (Latency_12TimeBlocks_282(a,1));
1030 end
1031
1032 plot(Latency_Cumulative_12TimeBlocks_282); % confirm
1033
1034 Latency_MeanCumulative_12TimeBlocks_282 = (Latency_Cumulative_12TimeBlocks_282(:,1) +
Latency_Cumulative_12TimeBlocks_282_2(:,1)) / 2;
1035 save('Latency_MeanCumulative_12TimeBlocks_282.txt', 'Latency_MeanCumulative_12TimeBlocks_282', '-ascii');
1036
1037 % =====
1038 % Function to calculate the number of trials per min for each testing
1039 % session
1040
1041 % 0-60 time interval as an example
1042 crop0_60=zeros(1,1);
1043 zone2832_2_0_60 = zeros(length(zonetime2832_2),1);
1044
1045 disp('=====');
1046 disp('No. of animal: 2832_2');
1047 disp('=====');
1048
1049 for a=1:length(zonetime2832_2)
1050     if (zonetime2832_2(a,1)>=0) && (zonetime2832_2(a,1)<=60)
1051         crop0_60=zonetime2832_2(a,2);

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```

1052     else
1053         crop0_60=0;
1054     end
1055     zone2832_2_0_60(a,1)=crop0_60;
1056 end; clear crop0_60;
1057
1058 zone2832_2_0_60=nonzeros(zone2832_2_0_60);
1059
1060 L1=zeros(1,1);
1061 L2=zeros(1,1);
1062 L3=zeros(1,1);
1063 Correct_R_0_60=zeros(1,1);
1064 Correct_L_0_60=zeros(1,1);
1065 Correct_Total_0_60=zeros(1,1);
1066 Incorrect_Total_0_60=zeros(1,1);
1067 Omission_Total_0_60=zeros(1,1);
1068
1069 flag=1;
1070 for b=1:length(zone2832_2_0_60)-2 %Full sequence length
1071     L1=zone2832_2_0_60(b,1); %1 or 5
1072     L2=zone2832_2_0_60(b+1,1); %4
1073     L3=zone2832_2_0_60(b+2,1); %6 or 2
1074     if (L1==1) && (L2==4) && (L3==6); %Right reward
1075         Correct_R_0_60=Correct_R_0_60+1;
1076         disp('R');
1077     elseif (L1==5) && (L2==4) && (L3==2); %Left reward
1078         Correct_L_0_60=Correct_L_0_60+1;
1079         disp('L');
1080     elseif (L1==1) && (L2==4) && (L3==2) || (L1==5) && (L2==4) && (L3==6);
1081         Incorrect_Total_0_60=Incorrect_Total_0_60+1;
1082         disp('I');
1083     elseif (L1==4) && (L2==1) && (L3==2) || (L1==2) && (L2==4) && (L3==5) || (L1==4) && (L2==5) && (L3==6) || (L1==6) &&
1084         (L2==4) && (L3==1) ||
1085         (L1==2) && (L2==4) && (L3==6) || (L1==6) && (L2==4) && (L3==2);
1086         Omission_Total_0_60=Omission_Total_0_60+1;
1087         disp('O');
1088     flag=flag+3; %Jump 3 positions after identified trial
1089 end,clear b
1090
1091 Correct_Total_0_60 = Correct_L_0_60+Correct_R_0_60; %Total Correct
1092 Performance_onlyInc_0_60 = Correct_Total_0_60 / (Correct_Total_0_60 + Incorrect_Total_0_60);
1093 Performance_0_60 = Correct_Total_0_60 / (Correct_Total_0_60 + Incorrect_Total_0_60 + Omission_Total_0_60);
1094 Total_Trials_0_60 = Correct_Total_0_60 + Incorrect_Total_0_60 + Omission_Total_0_60;
1095
1096 disp('=====');
1097 disp('Time interval: 0-60');
1098 fprintf('Correct Left: %d\n',Correct_L_0_60) %Display correct left trials
1099 fprintf('Correct Right: %d\n',Correct_R_0_60) %Display correct right
1100 % trials
1101 fprintf('Correct Total: %d\n',Correct_Total_0_60) %Display total correct
1102 % trials
1103 fprintf('Incorrect: %d\n',Incorrect_Total_0_60) %Display total incorrect
1104 % trials
1105 fprintf('Omissions: %d\n',Omission_Total_0_60) %Display total omission
1106 % trials
1107 fprintf('Total Number of Trials: %d\n',Total_Trials_0_60) %Display total
1108 % trials
1109 fprintf('Performance with only Incorrect: %d\n',Performance_onlyInc_0_60)
1110 % Display Performance Percentage
1111 fprintf('Performance Incorrect + Omissions: %d\n',Performance_0_60)
1112 % Display Performance Percentage
1113 disp('=====');
1114
1115 % =====
1116 % Function to average time zone grid matrices =====
1117
1118 % Load all files
1119 files = dir('*.txt');
1120 for a=1:length(files)
1121     load(files(a).name, '-ascii');
1122 end
1123
1124 % Average per animal (2 daily sessions)
1125
1126 % Example: Day 14, Sham/CT group
1127 mean_heatmaps_141 = (heatmap_141 + heatmap_141_2)/2.;
1128 mean_heatmaps_142 = (heatmap_142 + heatmap_142_2)/2.;
1129 mean_heatmaps_143 = (heatmap_143 + heatmap_143_2)/2.;
1130 mean_heatmaps_144 = (heatmap_144 + heatmap_144_2)/2.;
1131
1132 mean_heatmaps_14_ShamCT = (mean_heatmaps_141 + mean_heatmaps_142 + mean_heatmaps_143 + mean_heatmaps_144)/4;
1133
1134 figure(1);
1135 hold on
1136 grid off
1137 set(gca, 'clim', [0 1]);
1138 set(gca, 'xlim', [0 16]);
1139 set(gca, 'ylim', [0 13]); % Changed from 12 to 13 to avoid cropping
1140 colormap(jet)
1141 imagesc(mean_heatmaps_14_ShamCT); colorbar
1142 xlabel('Bin (40 pixels/block)');
1143 ylabel('Bin (40 pixels/block)');
1144 title('Normalized time spent per zone')
1145 hold off
1146
1147 saveas(figure(1), 'Time_zone_14_ShamCT.tif');

```