Therapeutic benefits of Tramadol and Running Exercise in a Mouse Model of Low Back Pain

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Abstract

Background: Low Back Pain (LBP) is one of the main causes of disability and one of the most frequent reasons for medical appointments. Among all the available treatments for chronic LBP management, the two most prescribed are analgesic medication and exercise. A commonly prescribed medication is tramadol, which is a weak agonist at mu-opioid receptors and plays a role in the inhibition of serotonin and noradrenaline reuptake. Many studies have also shown that being physically active reduces pain and has benefits for intervertebral discs. Currently, there is only one well-characterized mouse model for spontaneous LBP: the SPARC-null mice. These SPARC-null mice develop progressive disc degeneration as they age, similar to humans. **The main goal of this project is to investigate whether chronic use of tramadol will increase the amount of physical activity animals are willing/able to do and potentiate the analgesic effects of increased exercise.**

Hypotheses and aims: We hypothesize that prolonged tramadol treatment will increase the physical activity (running) of animals with LBP and potentiate analgesic effect of running. To address this hypothesis, we divided this study into three aims: **Aim 1**: Determine the most effective running protocol to reverse pain in SPARC-null mice, where we hypothesize that the forced treadmill running will be more effective than voluntary running to reverse the SPARC-null phenotype. **Aim 2:** Determine the dose response of tramadol in control mice and in SPARC-null mice; we hypothesize that males will be more sensitive than females and we do not expect a strain difference. **Aim 3:** Determine the benefits of the combination of tramadol and exercise in SPARC-null mice.

Methods: Aim 1: Mice underwent pain behavior tests (von Frey filaments, Acetone test, and Grip Strength) at the end of each running protocol (14 days for the treadmill and 45 days for the voluntary running). **Aim 2:** Each mouse received four doses of tramadol with a wash-out period of one week between two injections (10, 30, 60 and 100 mg/kg, intraperitoneal injection). Using the Tail Flick assay, heat sensitivity was then assessed 30min following treatment administration. **Aim 3:** Animals underwent pain behavior tests (von Frey filaments, Acetone test, Grip Strength, Tail Suspension and Counting wheels) in three different time points: baseline, 45 days running and 60 days running plus 14 days of tramadol (delivered subcutaneously by osmotic pumps).

Results: While forced Treadmill running intervention did not significantly affect behavioral assays, voluntary running SPARC-null mice showed reduced phenotype in the Grip Strength test. Tramadol efficacy was similar between the WT and the SPARC-null, and 30mg/kg showed to be effective in males. The combination of chronic tramadol administration and voluntary running did not show any advantage in any behavioral assay.

Discussion: Although the combination of exercise and tramadol is often used in patients suffering from low back pain, this approach was not beneficial in the SPARC-null mouse model of LBP. This raises questions about the real advantage of using such combination in patients. Thus, there is a need for investigating further the relevance of the combining those two treatments.

Résumé

Contexte: La lombalgie est une des principales causes d'invalidité et un des motifs les plus fréquents de consultation médicale. Parmi tous les traitements disponibles pour la prise en charge de la lombalgie chronique, les deux plus prescrits sont les médicaments analgésiques et l'exercice physique. Un médicament couramment prescrit est le tramadol, agoniste peu puissant des récepteurs mu-opioïdes et qui joue un rôle dans l'inhibition du recaptage de la sérotonine et de la noradrénaline. De nombreuses études ont également démontré l'efficacité de l'activité physique à réduire la douleur et ses effets bénéfiques sur les disques intervertébraux. Il n'existe actuellement qu'un seul modèle murin de lombalgie spontanée bien caractérisé : les souris SPARC-null. Comme les humains, ces souris SPARC-nulles développent une dégénérescence discale progressive en vieillissant. **L'objectif principal de ce projet est de déterminer si l'utilisation chronique du tramadol peut augmenter l'activité physique et donc renforcer les effets analgésiques, résultants d'une activité physique accrue.**

Hypothèses et objectifs: Nous émettons l'hypothèse qu'un traitement prolongée au tramadol augmentera l'activité physique (course) des animaux atteints de mal de dos et potentialisera l'effet analgésique de la course. Pour répondre à cette hypothèse, nous avons divisé cette étude en trois objectifs: **Objectif 1:** Déterminer le protocole de course le plus efficace pour renverser la douleur chez les souris SPARC-null, où nous postulons que la course forcée sur tapis roulant sera plus efficace que la course volontaire pour renverser le phénotype SPARC-null. **Objectif 2:** Déterminer pour le tramadol la relation dose-réponse des souris sauvages (WT) et SPARC-null; nous postulons que les mâles seront plus sensibles que les femelles et nous ne prévoyons pas de

différence entre les souches. **Objectif 3:** Déterminer les avantages de la combinaison du tramadol et de l'exercice chez les souris SPARC-null.

Méthodes: Objectif 1: le comportement face à la douleur (filaments de von Frey, test d'acétone et résistance à la traction) a été évalué à la fin de chaque protocole de course (14 jours pour le tapis roulant et 45 jours pour la course volontaire). **Objectif 2**: Chaque souris a reçu quatre doses de tramadol avec un délai d'une semaine entre deux injections (10, 30, 60 and 100 mg/kg, i.p.). À l'aide du Tail Flick, la sensibilité à la chaleur a ensuite été évaluée de 30 minutes après l'administration**. Objectif 3:** Le comportement des animaux (filaments de von Frey, test à l'acétone, résistance à la traction, suspension par la queue et roues de comptage) a été évalué à trois moments différents : avant et aprés 45 jours de course et après 60 jours de course plus 14 jours de tramadol (administré par voie sous-cutanée par des pompes osmotiques).

Résultats: Alors que l'intervention forcée sur le tapis roulant n'a pas eu d'effet significatif sur les tests comportementaux, les souris SPARC-null courant volontairement ont démontré un phénotype réduit dans le test de résistance à la traction. L'efficacité du tramadol était similaire entre les WT et le SPARC-null, et 30mg/kg se sont révélés efficaces chez les mâles. La combinaison de l'administration chronique de tramadol et de la course volontaire n'a montré aucun avantage dans aucun test de comportement.

Discussion: Bien que la combinaison de l'exercice et du tramadol soit souvent utilisée chez les patients souffrant de lombalgies, cette approche n'a pas été bénéfique dans le modèle murin de lombalgie SPARC-null. Cela soulève des questions sur le réel avantage de l'utilisation de cette

combinaison chez les patients. Il est donc nécessaire d'étudier plus avant la pertinence de la combinaison de ces deux traitements.

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Preface

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List of Abbreviations

IASP	International Association for the Study of Pain
CDC	Centers for Disease Control and Prevention
HRQoL	Health Related Quality of Life
LBP	Low Back Pain
IVD	Intervertebral discs
AF	Annulus Fibrosus
NP	Nucleus Pulposus
ECM	Extracellular Matrix
IL-1β	Interleukin 1 Beta
IL-8	Interleukin 8
IL-6	Interleukin 6
IL-17	Interleukin 17
IL-1α	Interleukin 1α
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-10	Interleukin 10
TNF-α	Tumor Necrosis Factor α
IL-1ra	interleukin-1 receptor antagonist
CNS	Central Nervous System
WHO	World Health Organization

PAG	Periaqueductal Grey
RVM	Rostral Ventromedial Medulla
SPARC	Secreted Protein, Acidic Rich in Cysteine
AERCP	Alan Edwards Centre for Research on Pain
NSAID	Nonsteroidal anti-inflammatory drug

1.Introduction

1.1 Pain

Pain is extremely common and affects people worldwide with its prevalence reaching 20% of the global population.[1] The International Association for the Study of Pain (IASP) defines pain as "*an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."*[2]

Pain can be classified according to its duration as either acute, subacute, or chronic. In humans, acute pain is defined as a period of pain that lasts less than three months and subacute pain is when it is present for at least six weeks but less than three months. This subacute pain usually overlaps with the acute pain duration. The other classification of pain is chronic pain which lasts at least three months.[3] Of these designations, chronic pain is the most costly and is an economic burden for countries around the globe. The Centers for Disease Control and Prevention (CDC) estimates $560 billion dollars spent each year in direct medical costs in the United States population alone.[4] However, this expensive cost is not limited to US, for instance in Austria one chronic pain patient is estimated to cost approximately €10,191 per year.[5]

The impact of pain on the economy has been demonstrated in many studies around the globe. Unfortunately, this impact is not only felt at the national level but also in the personal and family incomes. In developing countries, the burden of this economic impact can be

exaggerated. One study in Brazil showed that the two thirds of the population suffer from chronic pain and a higher prevalence was associated with lower levels of household income, which makes chronic pain a major concern in public health.[6] Furthermore, another study in the same country revealed that 49% of the population were dissatisfied with the chronic pain management options available.[7] Another developing country in South America, Chile, reported an annual cost of USD $1387.2 million for musculoskeletal chronic pain, with low back pain representing 31.8% of the total cost. Thus, chronic pain is a major source for the high social and financial burden in Chile.[8] Moreover, the prevalence of chronic pain has been increasing each year. It is estimated that there is a 10% increase in the number of adults diagnosed with chronic pain each year globally.[1] Across Europe the prevalence of chronic pain is estimated to be from 25 to 35% in the adult population[9] whereas in the United States, it is projected that the prevalence of chronic pain ranges from 10 to 40% of the population. Another study, this time focusing on Canada, has shown that there is an increase in chronic pain prevalence in all Canadian provinces with 21% of the general population suffering from this type of pain from 2000 to 2014.[10]

Chronic pain is more than just an economic burden, it affects peoples' lives directly, and it is debilitating for the patients.[11-13] Chronic pain has been related to poor quality of sleep,[11] in addition to a worse quality of life,[13] functionality limitations,[14] and to depression and anxiety.[12] Poor quality of sleep can exacerbate pain, lowering pain thresholds. Sleep states are also affected by the Periaqueductal Grey, which is an important area in the brain for pain modulation.[15] Chronic pain can have a major impact on the cognitive and emotional aspects

of the health-related quality of life (HRQoL). The impact of pain on quality of life is worse in chronic pain patients compared to those with acute or without pain.[13] In adolescents, the patients' quality of life was also impacted by chronic pain but studies also showed that this pain affected the quality of life of the family involved.[16] Depression can also increase as the pain worsens.[12] A Canadian study demonstrated that severe pain increases the chances for higher levels of depression and that depression becomes more prevalent for patients suffering from chronic pain. This same study demonstrated that there is a strong relationship between depressive symptoms and patients suffering from spinal pain.[17] In addition to the personal quality of life, chronic pain also has a major impact on the patients' work life.[18] A recent review discussed the effect of chronic pain in the workplace, and found that pain causes problems such as loss of efficiency and more sick leave requests, which in turn results in many patients losing their jobs.[13]

2.Background

2.1 Low Back Pain

Low Back Pain (LBP) is one of the main causes of disability and one of the most frequent reasons for medical appointments.[18 19] LBP has a major impact in the activity of peoples' daily lives, such as bathing, doing laundry, performing household chores and carrying a shopping bag, for example.[20] Despite the efforts by the health community, the number of people affected by LBP has been increasing since 1990.[19] Its prevalence ranges from 4 to 25% in the

global population, but this prevalence varies according to age. For patients aged 24 to 39 years old, the prevalence is around 4.2%, and it increases to 19.6% for those aged 40 to 59. For those above 65, the prevalence reaches 25.4% and remains stable after 70 years of age.[21] One study expressed that the prevalence of LBP in people aged 70-79 was 36%, and was strongly related to self-reported impairment in functionality.[22] Nonetheless, recent studies showed that the number of children and adolescents suffering from LBP is increasing each year;[23] which may suggest a higher prevalence of LBP in the future, since the presence of LBP in adolescence is an important risk factor for LBP in adulthood.[24]

2.1.1 Risk Factors for LBP

Several studies have reported different environmental and personal risk factors for LBP, which include low educational and economic status, smoking, being a woman, and obesity. Low educational and economic status has been shown to increase the risk of developing LBP.[21 25-27] One possible explanation for this might be the inferior living and working conditions, and the lack of access to an adequate health care program.[25 28] Furthermore, one study pointed out that the highest prevalence of LBP were among the rural workers,[27] which could be due to poorer working conditions and a heavier workload. Similarly, another study showed that the highest frequency of LBP was found among workers in agriculture and cleaning services,[28] which again are physically-demanding jobs which require a lot of bending and lifting. This can induce more stress on the spine than in many other professions.

Smoking has also been implicated as an important risk factor for LBP[26] and chronic LBP.[29] It has been demonstrated that there is a relationship between the frequency of smoking and LBP. For daily smokers the prevalence of developing LBP is 23.3%, whereas the prevalence for occasional smokers is only 17.2% and 15.7% for non-smokers.[30] Likewise, back pain has also been shown to correlate with exposure to smoking; 23.5% of non-smokers (never smoked), 31.1% of former smokers and 36.9% of present smokers presented back pain. These authors also found a relationship between back pain and the number of cigarettes smoked daily.[31] Some papers have sought to explore the biological explanations for this relationship. One hypothesis is that smoking reduces blood flow and increases hypoxia, which may lead to reduced muscle strength and disc degeneration. Additionally, nicotine has an excitatory effect on the central nervous system, which might alter the perception and pain thresholds of smokers.[32] Smoking has also been associated with disc degeneration. This relationship has been explored in pre-clinical studies where smoking was shown to cause histological changes in the intervertebral discs (IVD) and gene expression, the latter especially in the expression of collagens I and IX.[33] Besides this, passive smoking was also found to be related to apoptosis of the cartilaginous endplate of the IVDs, which, according to the authors, could cause chondrocyte apoptosis and the reduction of the extracellular matrix through blood flow cause resulting in disc degeneration.[34] Another important effect of smoking is that it can increase pro-inflammatory cytokine in the blood, which can lead to pain intensification.[35]

Multiple studies have shown that there is a higher prevalence of LBP in woman compared to men.[6 26 27 29] This sex difference could be due to the higher frequency of concomitant

diseases and fluctuation of hormone levels present in women.[36] Other authors have argued that this sex difference could also be due to gynecological problems and childbirth.[26] Furthermore, women often have double workday with both paid work and domestic tasks, and as a result, their musculoskeletal systems suffer more due to pregnancy and child care.[21]

Body weight and obesity have been shown to be important risk factors for LBP.[21 25-27 29] Some studies have demonstrated that there is a relationship between obesity and LBP especially for women,[29 37] which was inferred to be hormone-related.[37] Other authors have illustrated that obesity can overload the structures of the spine, which can in turn lead to a predisposition to disc degeneration.[28] Moreover, it has been shown that obesity was related to changes in the spine, with an increase of disc degeneration, some vertebral endplate changes and decreased spinal mobility. Other alterations due to metabolic syndrome, for example hypertension and dyslipidemia, can cause a change in the normal body physiology leading to LBP.[37] Surgeries resulting in pronounced weight loss point to an improvement and possible disappearance of LBP.[38] In addition, obesity and metabolic syndrome are correlated with a sedentary lifestyle, and it has been demonstrated that a sedentary lifestyle increases the chances of developing recurrent LBP by a factor of 3.5.[39] Similarly, lower levels of physical activities combined with longer times spent sitting was also associated with a higher risk of developing LBP for those over 50 years old.[40]

2.2 Low Back Pain and Intervertebral Disc Alterations

The intervertebral discs (IVD) are structures that connect adjacent vertebrae. They are composed of the annulus fibrosus (AF), and an inner part called the nucleus pulposus (NP). The AF has intersecting bundles of type I collagen and fibrocartilage, which is also rich in collagen of types I and II. The NP has a high concentration of proteoglycans, type II collagen and water. The discs are separated from the adjacent vertebrae by the cartilage endplate, a thin layer of hyaline cartilaginous tissue.[41]

The main functions of the IVDs are to support compressive loads and to provide flexibility to the spinal column. The NP is responsible for the resistance to compressive axial forces and pressure on the spine, while the AF provides resistance to transverse expansion of the IVD during spinal loading. However, when these structures start losing their natural configuration, they start to present signs of disc degeneration. These degenerated discs become more compressed and lose not only their flexibility but also the property of shock-absorption.[42] One meta-analysis pointed out that disc degeneration has been strongly associated to LBP in 50% of all cases.[43] Although this correlation exists, disc degeneration also occurs naturally due to ageing, from 30 to 95% being asymptomatic depending on the age group. [43] Similarly, the absence in association between disc degeneration and low back pain was shown in 56% of patients without LBP.[44] Disc herniations are another common LBP initiator involving alterations in the IVDs. One meta-analysis study showed that 40% of the symptomatic patients presented disc protrusion, 5-10% disc extrusion and, surprisingly, a strong

correlation was found between disc bulging and low back pain, with 43% of the symptomatic population presenting bulging discs in the MRI scans.[43]

The reasons why disc degeneration may drive discogenic pain remains unclear. Inflammation that originates in the IVDs has been in the spotlight of many studies as a possible cause, but whether this is the cause, or the consequence is still under debate. The natural ageing process can cause changes in the extracellular matrix (ECM), such as a decrease in the native cell population, matrix breakdown and calcification. These alterations can trigger the inflammatory response by the IVD cells, macrophages, T cells, and neutrophils, which promote the production of pro-inflammatory cytokines (IL-1 β, IL-8, IL-6, IL-17, IL-1α, IL-2, IL-4, IL-10, TNF- α, interferon-γ, and various chemokines and prostaglandin), and can result in autophagy, senescence or apoptosis, culminating with disc degeneration.[45] [46] Moreover, addressing this imbalance of pro-inflammatory cytokines has been shown to be an effective treatment for LBP.[47-49] The use of TNF inhibitors has shown to be efficient in a group of eighty patients with low back and radicular pain.[47] The use of TNF-α suppression medication also proved to be effective in the treatment of acute and severe sciatica, with decreasing pain as well as a decrease in the number of necessary spinal surgeries, in the short term[49] and three-years follow up.[50] Similar results were also found with the use of IL-6 receptor antibody at three days, along with one, two, and four weeks after infiltration.[48]

2.3 Treatments for Low Back Pain

Although there are many different guidelines for LBP management, a majority of them consider a multimodal approach to be the most effective intervention. These approaches include surgeries, drug therapy and non-medical interventions, ranging from the use of muscle relaxants to acupuncture. Regarding medical prescriptions, the guidelines vary according to the class of medication as well as the appropriate time to start a different class of medication.[51 52] Although the guidelines for LBP vary greatly, there are some similarities, like the fact that patients should avoid bed rest and should return to normal activities as fast as possible.[51 52]

2.3.1 Exercise

Exercise is often pointed out as an effective therapy for prevention of LBP. Two reviews investigated the use of exercise as a form of prevention of back problems related to work.[53 54] The study by Bigos et al.[53] 2009 concluded that exercise was the only effective therapy to prevent work-related LBP. Furthermore, exercise was more effective than other interventions analyzed in the study such as ergonomic/back education alone. Exercise was also correlated to less work absence and an increase of back and abdominal muscle strength.[53] The study by Bell and Burnett[54] 2009 claimed that exercise is effective in preventing recurrent LBP in the workplace despite being unclear about the type, intensity and frequency of exercise. An average exercise period of 10min/day showed to have significant improvements in this study.[54]

Similarly, it is almost a consensus in the literature that exercise therapy should be adopted for pain management in chronic LBP. However, there is little consistency about the type of

exercise that is the best for chronic LBP. Some suggestions include sports rehabilitation, as much physical activity as the patient can tolerate, strength training, motor control exercise, yoga, Tai Chi, and aerobic exercise. [51] The amount of exercise is also a topic for discussion, but according to one review, the recommended frequency and duration is a maximum of eight sessions of exercise in general over the course of 12 weeks.[52]

Exercise as a form of treatment for pain has been studied in rodent models. The running paradigm can be explored through forced running (treadmill), or voluntary exercise (voluntary running wheels). According to one review, more than 80% of the studies favored working with the forced running paradigm.[55] But even in these pre-clinical studies regarding exercise as a treatment, there is no consistency for the intensity or the time spent exercising. The mechanisms behind the beneficial effect of exercise are still being investigated. One study shows that exercise can increase the glycosaminoglycan content in the IVD.[56] Glycosaminoglycan loss has a known correlation to LBP.[57] Many other molecular changes are induced by exercise, such as changes in the levels of cytokines, improvement in the neurotrophic receptor signaling and, in the Central Nervous System (CNS), enhancement of opioid receptor activity in the pain descending pathway.[55]

While there are multiple hypotheses for the effect of exercise in pain management, one of the most accepted and studied is the activation of endogenous system, through the action of opioid system and anti-inflammatory cytokines. The action by the endogenous opioid system involves opioid peptides (enkephalins, endorphins, dynorphins) which bind to opioid receptors.

There are three G-protein coupled opioid receptors that mediate analgesia: mu, delta and kappa receptors.[58] Two structures from the central nervous system involved in the descending pain-modulating pathway system related to the analgesia caused by exercise are the periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM). Recent studies show that exercise can increase endorphin and met-enkephalin content in these two regions,[59] contributing to post-exercise analgesia. Additionally, aerobic exercise with an intensity of 85% of the maximum heart rate was shown to increase plasma levels of β-endorphins.[60] Likewise, another study using a progressive exercise protocol demonstrated that with the increase of intensity there is an increase of β-endorphins measured in plasma.[61] Resistance training was also showed to increase β-endorphins levels.[62] This relationship between analgesia and the opioid system is supported by multiple studies, which demonstrates that the analgesic effect of exercise driven by the influence of the opioid system can be blocked by naloxone, a well-known opioid-antagonist.[59 63]

In addition to the opioid system, cytokines are also involved in the exercise analgesic effect. Treadmill protocols in pre-clinical studies showed that exercise can decrease in pro-inflammatory cytokines like IL-6, IL-1β and TNF- α,[64-68] while increasing anti-inflammatory cytokines such as IL-10, IL-1ra, IL-5 and IL-4,[64 68 69] and these changes were related to better performance in pain-evoked test in rodents. Interestingly, one study showed that exercise analgesia was IL-4-dependent as the IL-4 knockout mouse did not show exercise induced analgesia after the treadmill protocol.[70]

2.3.2 Tramadol

Medication is another option for pain management, especially for chronic pain. The first line of treatment includes nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol/acetaminophen, and sometimes antidepressants.[51] Moreover, some physicians prescribes weak opioids for acute cases of LBP. According to the World Health Organization (WHO), the analgesic ladder consists in three steps: 1) NSAIDs and acetaminophen/paracetamol with or without adjuvant therapy; 2) weak opioids with or without non-opioid medication, with or without adjuvant therapy; and 3) strong opioids with or without non-opioid medication, with or without adjuvant therapy.[71]

Although there are controversies about opioid prescriptions, many physicians still prescribe opioids.[51] The number of opioid prescriptions is increasing and, among all regular opioids, the leaders in prescription growth are oxycodone, fentanyl, and morphine, with increases in growth of 50%, 150%, and 60%, respectively.[72] The prevalence of opioids prescriptions for LBP was described to be 32.7% in the United Kingdom,[73] 21.2% in the United States,[74] and 7.1% in Canada.[75] Furthermore, there is a tendency for doctors to prescribe higher doses of opioids for LBP compared to other common pain diagnoses.[76] These high-doses of opioids were related to poorer outcomes in patients with chronic musculoskeletal diseases[77], compromising the treatment and leading to a long-term use of opioids for pain management.[78]

The efficacy of opioids in LBP management is a topic for discussion. Opioids have been shown to be effective in the acute phase, but long term-use studies are scarce.[79] In addition, their

efficacy in the acute phase is not a consensus in the literature. Some authors have claimed that the use of opioids in the acute phase of LBP can lead to prolonged disabilities, higher risks for future surgeries, and continued use of opioids.[74] These findings could diminish the use of opioids for acute LBP. Moreover, there are studies showing the comorbidities associated to use of opioids, like opiate abuse,[80] opioid-induced hyperalgesia,[81] abuse of alcohol and other substances.[76] Another problem regarding the use of opioids are sex differences. One study showed that men presented higher risk to develop opioid dose escalation.[82] Sex differences might also impact opioid efficacy. Morphine has higher efficacy and potency in males, which is hypothesized to be due to different sensitivity to opioids in the CNS.[83]

One commonly used opioid for LBP management is tramadol,[84] which is a weak mu-opioid receptor agonist, and presents fewer side effects, including a reduction in incidence of constipation, respiratory depression, and overdose compared to strong opioids such as morphine and fentanyl. Most importantly, it also presents with lower addiction rates compared to other opioids.[85] According to the WHO, tramadol is a suitable drug for the second step of their analgesic ladder.[71] Moreover, a Cochrane review showed that from 15 RCTs for chronic LBP management, 5 investigating the use of tramadol showed that it alleviated pain and improved functional outcomes.[84]

Opioids act by simulating the action of endogenous opioid peptides in the central and peripheral nervous system. Some of the areas related to this analgesic effect from opioids are the PAG and RVM. This class of medication acts on these areas to induce analgesia, and the mu-

opioid receptors mediate this process.[86] These receptors are G-protein coupled receptors that inhibit the opening of Ca^{+2} channels and stimulate the opening of K^{+} channels, resulting in inhibition of synaptic excitability. Normally, the neurons from the PAG are constantly inhibited by GABAergic interneurons within the PAG. However, when the mu-opioid receptor is activated by the opioid medication, this inhibition is weakened and the PAG neurons can signal to the RVM.[87] From the RVM, different classes of neurons connect to the spinal cord. The ON and OFF cells from RVM project specifically to laminae I, II and V of the dorsal horn.[88] The opioid medication acts by hyperpolarizing the ON cells that project to the dorsal horn through the increase of K^{+} conductance, therefore contributing to the process of analgesia.[88] Additionally, tramadol presents a second action, which is the inhibition of norepinephrine and serotonin re-uptake through a blockade of their transporters. These two neurotransmitters are also involved in the modulation of the descending pain pathway on the PAG and the RVM.[89]

2.4 Animal Model for Spontaneous Low Back Pain: SPARC-null mice

Our lab has been using SPARC-null mouse as a model for spontaneous LBP. SPARC is the acronym for Secreted Protein, Acidic Rich in Cysteine, a secreted Ca^{+2-} binding glycoprotein. It is a matricellular protein highly expressed in remodeling tissues, which binds many extracellular matrix components like collagen type V and fibrillar collagens (I,II,III and V).[90] It has been shown that SPARC-null mice present with impaired wound healing,[90] accelerated tumor development,[91] osteopenia, and decreased bone formation.[92]

SPARC is also present in human IVDs, and has been shown to be decreased with aging and disc degeneration.[93] Similarly, Gruber et al.[94] 2005 demonstrated that SPARC-null disc fibrils presented irregular formation and variable sizes consistent with disc degeneration. Furthermore, in our lab, Millecamps et al.[95] 2012 showed that ageing SPARC-null mice start developing disc degeneration with presentation of lumbar disc wedging and a loss of disc height compared to age-matched WT mice. One DNA methylation study compared lumbar discs between aging mice and LBP patients and revealed that the SPARC promoter gene was hypermethylated in both subject groups, suggesting the SPARC-null mouse mimics natural age-dependent disc degeneration in humans.[96] This animal model was also explored for behavioral signs of pain, showing hypersensitivity to cold stimuli in both lower back and hindpaws; aversion to stretching in the Tail Suspension test and reduced resistive force in the Grip test are also observed in the SPARC-null mice when compared to WT, which are hypothesized to be driven by disc degeneration. On the other hand, this model did not show any sign of motor impairment or altered sensitivity to mechanical stimuli.[95 97]

3. Experimental Rational and Initial Hypothesis

The prescription of medication combined with exercise for LBP treatment is common in clinical practices. One of the most prescribed medications is tramadol, which is a weak agonist at mu-opioid receptors that also plays a role in the inhibition of serotonin and noradrenaline reuptake. Concerning exercise, studies have shown the effectiveness of being physically active on analgesia and reduction of disc degeneration. **The main goal of this project is to investigate**

whether chronic use of tramadol will increase the physical activity performed by mice, thus potentiating the analgesic effects of increased exercise.

To investigate this the following aims were explored:

Aim 1: Determine the most effective running protocol to reverse pain in SPARC-null mice. Based on protocols already well-established in the literature showing the efficacy of forced running exercise in neuropathic animal models, we hypothesized that Treadmill running will be more effective than voluntary running to reverse the SPARC-null phenotype.

Aim 2: Determine the dose response of tramadol in control mice and in SPARC-null mice. Tramadol and other opioids have proven to be more potent and effective in males than in females, thus we hypothesized that males will be more sensitive to tramadol and there will be no strain effect.

Aim 3: Determine the benefits of the combination of tramadol and exercise in SPARC-null mice. Exercise has been shown by others to increase opioid receptor density, therefore we hypothesized that chronic use of tramadol will increase physical activity and potentiate running analgesic effect.

4.Materials and Methods

4.1 Animals

SPARC-null mice backcrossed on a C57B16 strain and bred in McGill Animal Resources Centre were used for these experiments, along with sex- and age-matched Wild-Type (WT) controls. All mice in this study were housed in groups of 2 to 4 per cage in temperature-controlled room in polycarbonate cages on a 12h light/dark cycle. Mice received food (2092X Global Soy Protein-Free Extruded Rodent Diet, Irradiated) and water *ad libitum*. All experiments were approved by the Animal Care Committee at McGill University and the rules from the ethical guidelines of the Canadian Council on Animal Care Committee for Research and Ethical Issues were followed.

4.2 Experimental Design

Three cohorts of animals were used for the experiments in this thesis. Twenty-four males (7 to 8 months old) with 16 SPARC-null and 8 WT genotypes were used for the treadmill experiment. Twenty-four males and 24 females (4 to 7 months old) with 12 WT and 12 SPARC-null genotypes in each gender were used for studies evaluating the dose response of tramadol. The combination of exercise and drug intervention was studied in 35 males (2 to 5 months old) 9 WT and 26 SPARC-null. Thus, 131 animals were used in total for this thesis.

In the treadmill experiment the animals were divided in groups after the behavior baselines were assessed. In this first cohort, the 16 SPARC-null were divided into ‘Runners” and

"Sedentary" groups (n=8/group), and 8 WT were used as controls; the WT controls were all "Sedentary" (n=8/group).

In the drug experiments the mice were divided into 4 groups according to the dose randomization **(Figure 1)**. Males were assessed in the hot water tail immersion assay one day prior to the females.

In the voluntary running and drug experiment, mice were divided in 5 groups: 9 WT sedentary, 6 SPARC-null sedentary saline, 6 SPARC-null run saline, 7 SPARC-null sedentary Tramadol and 7 SPARC-null run Tramadol. **(Figure 2)** The timeline for this experiment can be seen on **Figure 3.**

4.3 Exercise Interventions

4.3.1 Treadmill Protocol

The male treadmill running protocol (Exer 3/6, Columbus Instruments, Columbus, Ohio, USA) consisted of two stages. The first stage was the familiarization with the treadmill, where all mice were exposed to the treadmill for 6 days, 10min/day at a speed of 10m/min with no inclination. After the familiarization, the exercise group ran for 30min (10m/min, 5days/week, with no inclination, for 2 weeks).[98] The sedentary groups were exposed to the treadmill for the same amount of time as the running group but without motion.

4.3.2 Voluntary Running

The animals were housed in groups of 2 to 4 per cage in a temperature-controlled room on a 12h light/dark cycle. The plastic wheels were made of two parts, the bottom, which has an igloo format favoring nesting, and the top spinning part has a disc shape and it is attached to the bottom (InnoDome™ and InnoWheel™, bio-serve). The animals randomly assigned for the running group had free access to the running wheels for 60 days, while the sedentary groups received fixed wheels for the same period. The wheels were replaced with clean ones every week. Before each behavior assay, all the wheels were taken out of the cages during the one-hour habituation period and put it back after at the end of each day of experiments.

4.4 Pharmacological Interventions

4.4.1 – Information about the drug – Tramadol (Medisca Inc., Montreal, Quebec) was used in two different parts of this thesis: in the second and in the third aim.

4.4.2 – Acute injections for dose response effect: For the second aim, each mouse received four doses of tramadol with a wash-out period of one week between two injections (10, 30, 60 and 100 mg/kg, intraperitoneal injection). By the end of the four weeks each mouse had received all the doses randomly assigned. **(Figure 1)**

4.4.3 Subcutaneous delivery by osmotic minipump - For the third aim, osmotic mini pumps (#1004, 0.11 µL/h, Durect Corporation, Cupertino, CA, USA) were implanted in the mice to reduce the stress caused by the constant injections, otherwise necessary for this study. Under

deep isoflurane anesthesia, confirmed by the lack of peripheral reflexes, the animals had the surgical area shaved and disinfected with Povidone-Iodine Swabstick (Professional Disposable International - PDI, NY, USA). An incision was made on the lateral-posterior part of the back, and a small pocket was made subcutaneously using a hemostat. The pumps then were implanted, with the valve positioned far from the incision, and subsequently the incision was closed using 4-0 silk suture. The pumps delivered a constant systemic administration of 30mg/kg/day of tramadol suspended in saline (dose selected based by the experiment for aim 2). The control groups underwent the same surgical procedure for the pump implantation, but these pumps were filled with only saline, with same speed of infusion (0.11 µL/h).

4.5 Behavior Assays

Prior to each behavior experiment, the mice spent one hour in the experimental room for habituation with the environment. All the behavior experiments were conducted in the same room and they were performed at the same time of the day to avoid confounding effects on the circadian rhythms. The testing room was provided by the Alan Edwards Centre for Research on Pain (AERCP) and the temperature and humidity were kept constant throughout all experimental days.

4.5.1 Mechanical Sensitivity: von Frey Filaments

Mice were placed in individual spaces on the von Frey rack (5.5x10x7 cm platform with a mesh floor) for one-hour habituation to the rack where the test was performed to decrease the

possible stress effect that could be evoked by the new environment. The test consisted of the application of thin filaments on the plantar surface of the left hind paw. Measurements were taken using the up-and-down method with a series of filaments (0.07, 0.16, 0.4, 0.6, 1, 1.4, 2 and 4g).[99] The complete withdrawal of the paw was considered a positive behavior response. The 50% withdrawal threshold in grams was calculated as described previously by Chaplan et al.[99] Significant decreases in the 50% withdrawal threshold compared to the baseline was interpreted mechanical hypersensitivity, while increases in the withdrawal threshold compared to baseline was interpreted analgesia.

4.5.2 Cold Sensitivity: Acetone Evoked Behavior

Acetone test was performed right after the von Frey test, while the mice were on the von Frey rack. A drop (25µl) of acetone was applied using a 1mL syringe on the plantar surface of the left hindpaw and the time of the evoked behavior (licking, biting, scratching and shaking) over 1 minute was measured with a stopwatch. Increase time of acetone evoked behavior was interpreted as indicative of cold hypersensitivity.

4.5.3 Heat Sensitivity: Tail Flick

Mice were gently held by their body and their tail was then immersed in a hot water bath (50°C). Using a stopwatch, the time until they withdraw their tail was measured. A cutoff point of 12 seconds was established to avoid skin damage. The increased time compared to the baseline for tail withdrawal is a measure of analgesic efficacy of the drug.

4.5.4 Axial Discomfort: Grip Force

Mice gripped a metal bar with their forepaws on the Grip Strength Meter (Stoelting Co., Wood Dale, IL) while the experimenter gently pulled them by the tail. Two peaks of force in grams were assessed, and the average was calculated. If there was a difference higher than 30% between the two values, the mice were assessed a third time and the two closest values were chosen to calculate the average. On this test more force means less stretching avoidance behavior.

4.5.5 Axial Discomfort: Tail Suspension

Mice were suspended two at a time by the tail underneath a platform. Between these mice a barrier was positioned to avoid them seeing each other. Adhesive tape was used to attach these animals to the platform, and a 3min video was recorded. The time spent in immobility (hanging but not moving), rearing (trying to reach the platform), stretching (trying to reach the ground), and supported (holding the base of the tail or the platform) was analyzed using AnyMaze program (Wood Dale, IL, USA). One week before the baselines, each animal was habituated to the test by a 3 minutes long suspension. More time spent in immobility means less aversion to back stretching.

4.6 Voluntary Running: Counting Wheels

Each mouse was placed in an individual cage containing a low-profile wireless running wheel with a radiofrequency emitter (ENV- 047, Med Associates Inc, USA), and the total of revolutions were recorded over 1h period.

4.7 Tissue Extraction

One day after the final time point for the behavior assays, animals were anesthetized with isoflurane and euthanized by cervical dislocation. Subsequently, lumbar spinal cord (dorsal and ventral), and lumbar intervertebral discs were harvested and stored in a -80°C freezer for future analysis.

4.8 Statistical Analysis

Statistical analysis was performed using Graph Pad Prism® software (San Diego, USA). All data were plotted as mean ±S.E.M. The effect of running on both the treadmill and voluntary running protocols was assessed by comparing groups using one-way ANOVA between experimental groups (WT/sedentary, SPARC-null/sedentary and SPARC-null/runners) followed by Tukey's test for multiple comparisons.

The dose effect in both males and females on Tail flick test 30min after injection was analyzed using Two-Way ANOVA with experimental group (WT and SPARC-null) and dose

(10,30,60 and 100mg/kg) as the two factors followed by Bonferroni method for multiple comparisons.

For the behavior assessment, where multiple groups were analyzed over different time points, the following procedures were used: 1) in order to confirm the SPARC-null phenotype during the baselines, a comparison between the WT and the SPARC-null was conducted using a one tailed t-test; 2) to confirm the effect of voluntary running, the groups (WT/sedentary, SPARC-null/sedentary and SPARC-null/runners) were compared by one-way ANOVA followed by Tukey's test for multiple comparisons; 3) for the combination of exercise and drug, two-way ANOVA was performed, with groups (SPARC-null sedentary and SPARC-null run) and drug intervention (saline or tramadol) as the two factors, followed by Tukey's test for multiple comparisons.

5. Results

5.1 Voluntary running x forced treadmill running

The effect of treadmill running on pain-related behavior was examined using the mechanical sensitivity assessed with von Frey filaments, cold sensitivity assessed with Acetone test and axial discomfort assessed with Grip Strength. After two weeks of forced treadmill running no significant difference was observed between the WT and the two groups of SPARC-null mice (sedentary and running) in any of the behavior assays performed. **(Figures 4A,4C,5A).**

In contrast, 45 days of voluntary running showed significant effect in resistance force to stretching in Grip Strength test ($F_{(2, 32)} = 3.419$; $p < .05$). Following pairwise comparison, conducted using Tukey's indicated that SPARC-null sedentary (66.81±4.506g) had a reduced grip force strength compared to SPARC-null run (79.85±3.238, p= 0.0549) **(Figure 5B)**, which we interpret as consequence of reduced muscle tone and stretching avoidance behavior. No other pairwise comparison was significant (von Frey and Acetone test) (p>.05) **(Figures 4B and 4D).**

5.2 Tramadol dose effect

The effect of tramadol on nociceptive behavior was examined by Tail Flick test. The tail withdrawal response was expressed as percentage of maximum possible effect (%MPE). %MPE was calculated as $[(T_{30}-T_0)/(T_2-T_0)] \times 100$. T_{30}, T_0, and T_2 are the latency 30min after injection, the latency of the baseline, and the cutoff point, respectively. The dose effect showed no significant interaction between strains (WT x SPARC-null) and doses (10,30,60,100mg/kg) in either males ($F_{(3, 88)} = 0.2301$) or females ($F_{(3, 75)} = 0.9762$), but showed simple effects of dose in both males ($F_{(3, 88)} = 3.860$; $p<.05$) and females ($F_{(3, 75)} = 5.747$; $p<.01$) in the two-factor within subject ANOVA in the Tail Flick test. A pairwise comparison showed no significant difference between strains of the four different doses in both males and females (p>.05) **(Figure 6A and 6B)**

5.3 Chronic use of Tramadol and voluntary running

At baseline assessment, SPARC-null mice exhibited greater acetone evoked behavior ($t=3.608$, $df=33$; $p<.001$; one tailed t-test) as a result of hypersensitivity to cold, as compared to

the control group. **(Figure 7D)** They also exhibited less force in the Grip Strength (83.84±2.896; t=3.452, df=33; p<.001, one tailed t-test) compared to the WT (101.7±2.503) as a result of decreased muscle tone and stretching avoidance behavior **(Figure 8A).** No significant differences were observed in the other behavior assays performed at baseline (von Frey, Tail Suspension and Counting wheels) **(7A, 8D and 9A).**

Following 45 days of voluntary running, the SPARC-null phenotype was reversed in the Grip Strength test (one-way ANOVA; $F_{(2, 32)} = 3.419$; $p < .05$). Multiple comparisons conducted using Tukey's test indicated that SPARC-null sedentary (66.81±4.506) showed reduced grip force compared to SPARC-null runners (79.85±3.238, p= 0.0549) **(Figure 8B).** No other pairwise contrast was found significant (von Frey, Acetone test, Tail suspension and Counting wheels) (p>.05) **(Figure 7B, 7E, 8E and 9B).**

The chronic use of tramadol (14 days) in addition to the voluntary running (60 days) did not show any significant difference between groups (SPARC-null sed/saline, SPARC-null sed/tramadol, SPARC-null run/saline, and SPARC-null run/tramadol) in any behavior assay analyzed (von Frey, Acetone test, Grip Strength, Tail Suspension, and Counting wheels). **(Figures 7C, 7F, 8C, 8F and 9C)**

6. Discussion

6.1 Summary of results

Voluntary running was more efficient at reversing the SPARC-null phenotype than forced running on the treadmill. Tramadol efficacy was similar between the WT and the SPARC-null, and 30mg/kg was the most efficacious of the doses tested for the final experiment in males. In SPARC-null sedentary and runner groups, tramadol did not have any significant effect on the behavior assays after 14 days of treatment. Moreover, the combination of running and tramadol did not present any beneficial effect on the cohort analyzed.

6.2 Voluntary running x forced treadmill running

There is strong evidence showing the beneficial effect of running to prevent and to manage pain in patients suffering from LBP.[51 53 54] Exercise was also shown to improve disc hydration in humans[100] and animals.[56] In order to understand the mechanisms responsible for the effects of exercises, people are exploring exercise protocols in animal models so that we can study this pre-clinically. Most of these protocols use a forced exercise paradigm (treadmill) with proven efficacy. Although all these studies reported efficacy of their protocol, the great majority of them use neuropathic pain animal models, and running protocols begin around day 3 following the neuropathic pain induced surgeries,[67 70 101] which we consider an acute phase of pain. In contrast, our SPARC-null model develops spontaneous disc degeneration and pain over multiple months, a difference that must be considered when interpreting results. This likely contributed

to the fact that forced running did not yield comparable results, as compared to those found in neuropathic pain models. We believe however, that our model is more clinically relevant as the course of the disease is comparable to what is observed in patients. The difference between acute and chronic pain phases involves physiological changes which might impact the efficacy of a treatment. Topham et al. 2020[102] showed differences in of Pre-Frontal Cortex DNA methylation in a mouse model of neuropathic pain at four different time points (1 day, 2 weeks, 6 months and 1 year). This suggests that the definition of acute and chronic pain is not arbitrary and there are physiological changes that take place. Thus, it is reasonable to assume that different treatments are required to manage acute and chronic pain. Moreover, as each chronic pain patient may experience unique irregularities in central pain modulation, exercise as a treatment must be chosen with care in regards to the type of exercise, its duration and its intensity.[103]

In spite of the difference in duration between the two protocols in our experiments, the treadmill protocol was also tested in a different cohort, a female cohort. These females were at the same age as the males' treadmill experiment, they ran for 5 weeks instead of two, and the results were similar to the males. The treadmill protocol did not show any improvement in either cold sensitivity (Acetone test) or axial discomfort (Grip test) after five weeks of running **(Figure 10)**. This is comparable to the amount of time mice on in the voluntary running protocol spent running. Moreover, it was already demonstrated that voluntary running needs more than two weeks to show efficacy, even in neuropathic pain models.[104] Furthermore, there is no consensus in the literature regarding the most effective duration for exercise, as protocols have varied the length from 2 to 8 weeks, and all of them using treadmill exercise.[70 105 106]

6.3 Dose efficacy

Tramadol is an efficient drug for pain management and is considered a low risk-drug for drug-dependency.[85] Thus, it is one of the main drugs prescribed for LBP management.[84] The dose range of tramadol can be from 100 to 400mg/day in adults.[107] A large range in dose can also be seen in pre-clinical studies, where many studies work from 10-100mg/kg to determine the most effective dose.[108 109] The majority of these pre-clinical studies have shown that doses around 30 to 50mg/kg are effective in the test performed, and higher doses can cause motor impairment or sedation.[110 111] Saynok et al.[110] 2013, demonstrated that 50mg/kg is sufficient to cause sedation and Nagakura et al.[111] 2003, pointed out that 80mg/kg caused reduction in motion.

Importantly, our data showed that there is no difference in tramadol efficacy between strains (WT and SPARC-null). This provides useful information for future experiments as a standard dose will be equally efficient in WT and SPARC-null, and thus does not need to be tailored to the experiment.

6.4 The combination of voluntary running and Tramadol

Chronic use of tramadol, with or without running did not improve the pain-related behavior in the assays tested. This could be due to the fact that the model is not responsive; or the drug does not work, at least at that dose and in those assays; or that the chronic use of opioids resulted in Opioid-Induced Hyperalgesia (OIH).

The OIH is the possible explanation we decided to focus on this thesis. This has been observed in patients, and results in increased sensitivity and the gradual increase in pain as a consequence of opioid therapy and compensation in pronociceptive pathways.[112] According to Lee et al.[112] 2013, OIH can occur under 3 circumstances: 1) due to maintenance and withdrawal; 2) very high doses and/or escalating doses; and, 3) ultra-low doses. Furthermore, they argued that long-term tramadol use can cause OIH, which could in part explain our results after 14 days of constant administration of tramadol. Similar results have been described where it was demonstrated that analgesia was present in the first two days after the pump implantation, but after six or seven days there was a significant decrease in the effect of the drug.[113] In this study, the authors suggested that the cause of this drop in efficacy might be due to the exhaustion of tramadol supply in the pump reservoir; however, we can exclude this possibility from our work as our pumps were programmed for 24 days of continuous and constant dose. Thus, our results are consistent with the idea that long term use of tramadol results in a decrease in analgesic effect and potentially chronic use can cause hyperalgesia.

An interesting observation from our study is tramadol's effect on axial discomfort (grip test and tail suspension) and ineffective on radiating pain (von Frey filaments and Acetone test). In other words, on inflammatory pain but not neuropathic pain. This aligns with current evidence that opioids may be ineffective to treat neuropathic pain. This remains an area of controversy in the literature; however, it has been observed that opioids show a lower efficacy for treating neuropathic pain[114]. On the other hand, recent studies have demonstrated a peripheral action of the opioid system, involving the management of inflammatory cytokines and chemokines,[115]

decreasing sensory neuron excitability.[116] This modulation in the immune system induced by opioids is also influenced by CNS and hypothalamic-pituitary-adrenal (HPA) axis.[117] We hypothesize that these alterations in the inflammatory response can modulate the inflammatory reaction caused by disc alterations, present SPARC-null, leading to beneficial effects in the axial discomfort.

6.5 Limitations

The author acknowledges limitations in the study. Regarding the comparison between treadmill and voluntary running, the protocols had different duration. However, as noted above, there was an experiment with females, lasting 5 weeks (approximate the same length as the voluntary running) where the treadmill protocol was also not effective. Furthermore, this comparison involved different age groups which might have influenced the final results. However, our lab had shown in previous studies (unpublished) that voluntary running was effective in older animals (7-8 months old).

In Aim 3, tramadol in combination with running experiments has some important limitations to be highlighted. First, after the pump implantation surgeries, it was necessary to wait 6 days after surgeries before returning the wheels in their home cages. Due to the slow healing process already mentioned in this thesis, SPARC-null mice needed to have their sutures redone for 4-5 days after the surgeries, which might have affected the running and its analgesic effect.

In addition, the fact that the combination of exercise and tramadol was only explored in males. The author is aware that sex differences have been highlighted in the present study and the work of others and thus warrant further investigation; however, lack of resources limited the number of animals used in this experiment.

Finally, the sample size was small was too small to be sufficiently powered for an experiment with two different interventions and two different strains. However, this work served as an exploratory investigation and provided useful preliminary results to guide future experiments with a larger sample size.

6.6 Future Directions

As a general future direction all work will be replicated in animals of both sexes to ensure a sex difference is not overlooked. This is particularly important as we have observed that males were more sensitive to tramadol as compared to females. This could have implications for dosing in the clinic.

In order to best treat patients in the clinic a comprehensive understanding of what is prescribed is always useful and allows for a more personalized approach to medicine. Along those lines, it is important to assess the effect of tramadol on the tissues of interest in LBP i.e., intervertebral discs and spinal cord. As highlighted above, ongoing work in the field is investigating the effect of opioids on the periphery, changes in the periphery may result in changes in healing in the central nervous system REF. Investigating this further could shed some

light on our observation of a selective effect of tramadol on axial discomfort and not radiating pain assays.

The SPARC-null model used in this study depletes the SPARC protein in the entire body, a useful future direction for the advancement of the field would be to develop a selective SPARC-KO mouse model targeted to intervertebral discs. This would be particularly useful in overcoming the slow healing process and its complications after pump implantation as observed in this study. Avoiding this would be beneficial as the slow healing process itself may trigger an inflammatory response confounding the results of the experiment.

6.7. Conclusion

Our results suggest novel aspects to be considered when treating LBP, beginning with the choice of physical activity. This decision must be made with caution; as already mentioned by other studies[103], the efficacy of exercise might differ between intensities, time spent participating in activities, and between patients. The same can be said for different mice models. We used a well-established forced running exercise protocol and it was shown to be ineffective in our model, which raises the question how many ineffective exercise protocols in pre-clinical research have not been published, and how important these reports are as not all individuals will respond equally to the same treatment.

Another point that should be better explored is if tramadol can cause hypersensitivity with continuous use. The combination of exercise and tramadol is common in patients suffering

from LBP, but this approach did not show any significant benefit in the well-controlled animal model exploited. If this result is confirmed in a larger study, it might raise concerns about the efficacy of this treatment combination in patients. It is always optimal to minimize the use of medication in patients if it is not necessary. Therefore, if exercise done properly is as effective of an analgesic as tramadol, or in combination with tramadol, then perhaps the combination treatment in not necessary in clinic. This warrants further investigation into the efficacy of the combined use of these two treatments, especially regarding the chronic use of this medication and its impact on exercise in the long term.

Figures

Figure 1 - Drug Randomization for Aim 2

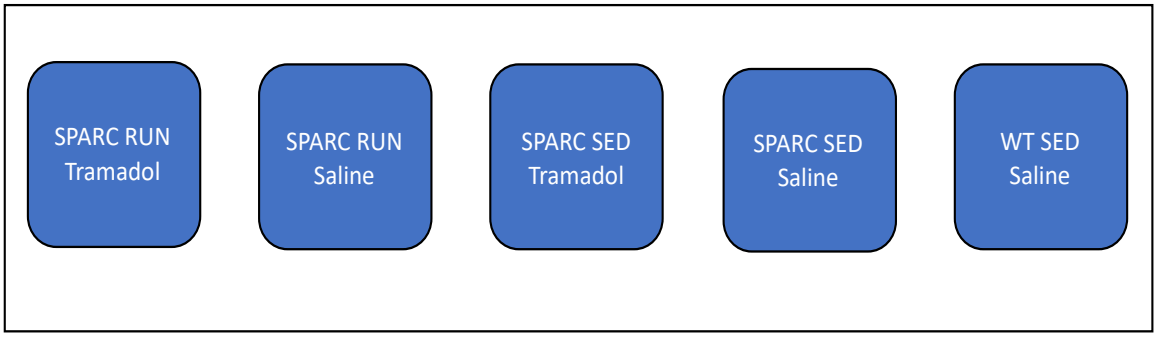
Mouse	Line	TT 1	TT 2	TT 3	TT 4
	1	A	D	B	C
	2	B	D	C	A
	3	C	D	B	A
	4	D	C	B	A
	5	D	A	C	B
	6	C	A	B	D
	7	B	A	C	D
	8	A	C	B	D
	9	D	A	B	C
	10	C	B	A	D
	11	B	C	A	D
	12	A	B	C	D
	13	C	D	A	B
	14	D	C	A	B
	15	A	B	D	C
	16	C	B	D	A
	17	D	B	C	A
	18	B	A	D	C
	19	A	D	C	B
	20	D	B	A	C
	21	C	A	D	B
	22	B	D	A	C
	23	A	C	D	B
	24	B	C	D	A

Males	
A	10mg/kg
B	30mg/kg
C	100mg/kg
D	60mg/kg

Females	
A	30mg/kg
B	60mg/kg
C	10mg/kg
D	100mg/kg

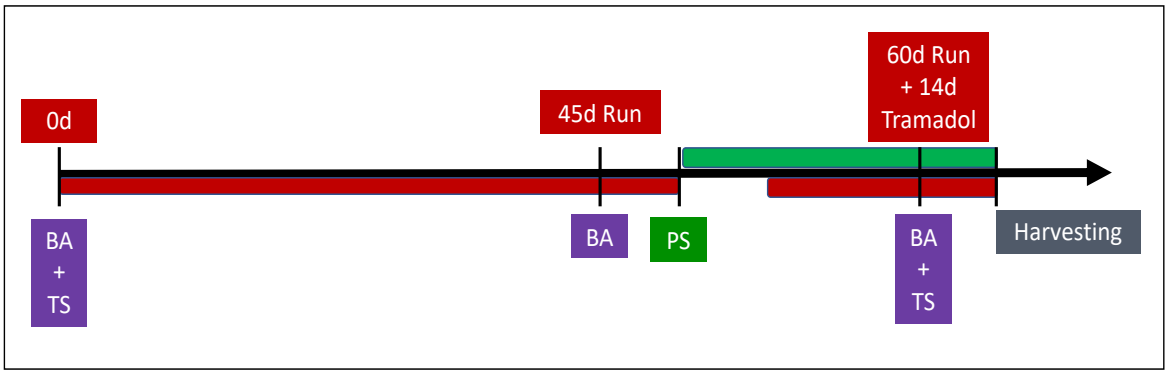
TT1: first week of treatment; TT2: second week of treatment; TT3: third week of treatment; TT4: fourth week of treatment.

Figure 2 - Intervention Groups Aim 3



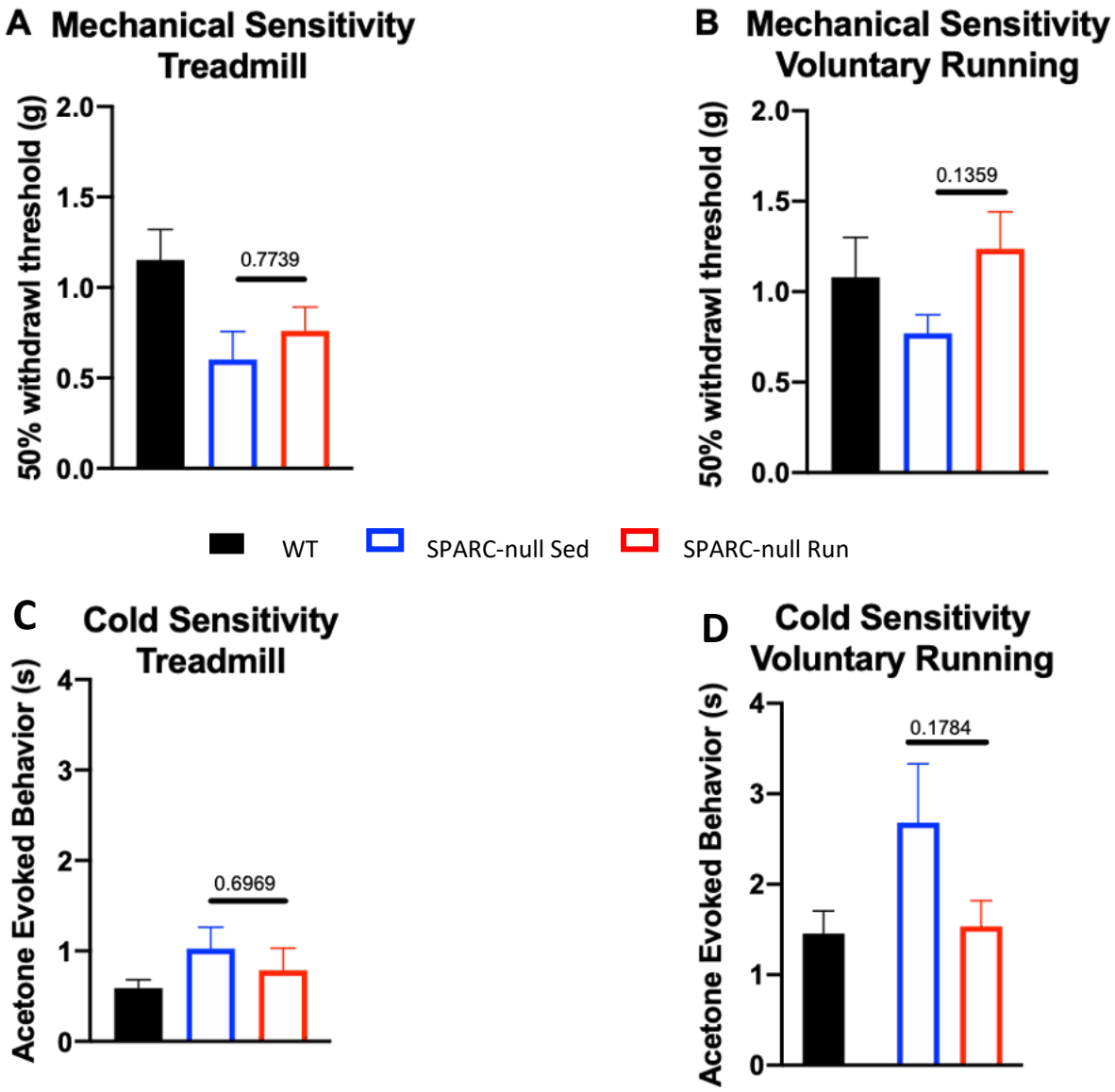
Schematic showing the five intervention groups for Aim 3

Figure 3 - Overall timeline for Aim *3*



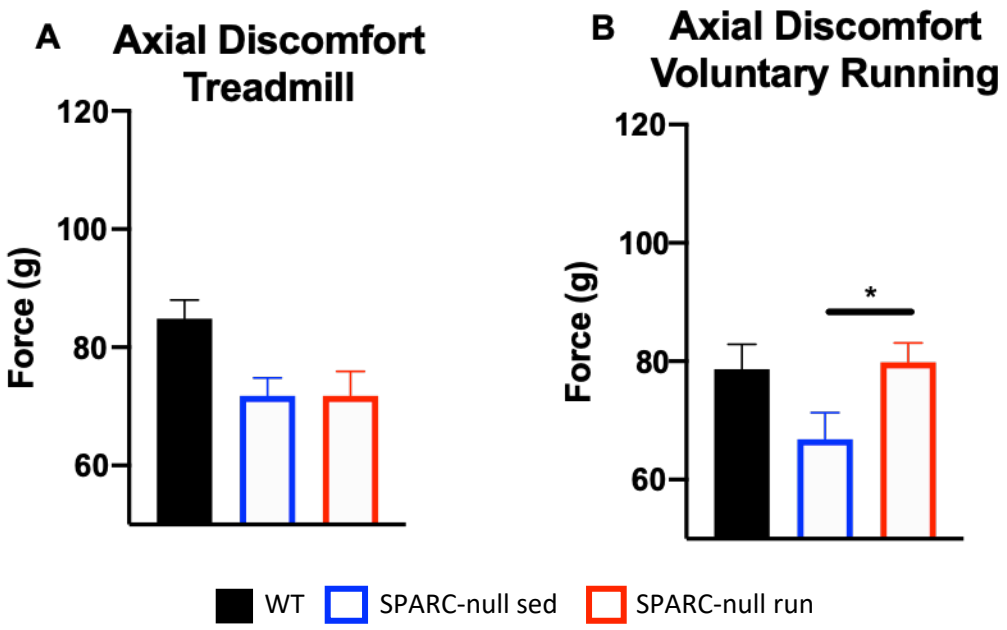
0d: Baseline; BA: behavior assays (von Frey filaments, Acetone test, Grip Strength) and counting wheels; TS: Tail Suspension; PS: Pumps surgeries.

Figure 4 - Behavior Assessment of Radiating Pain after Treadmill and Voluntary Running Protocols



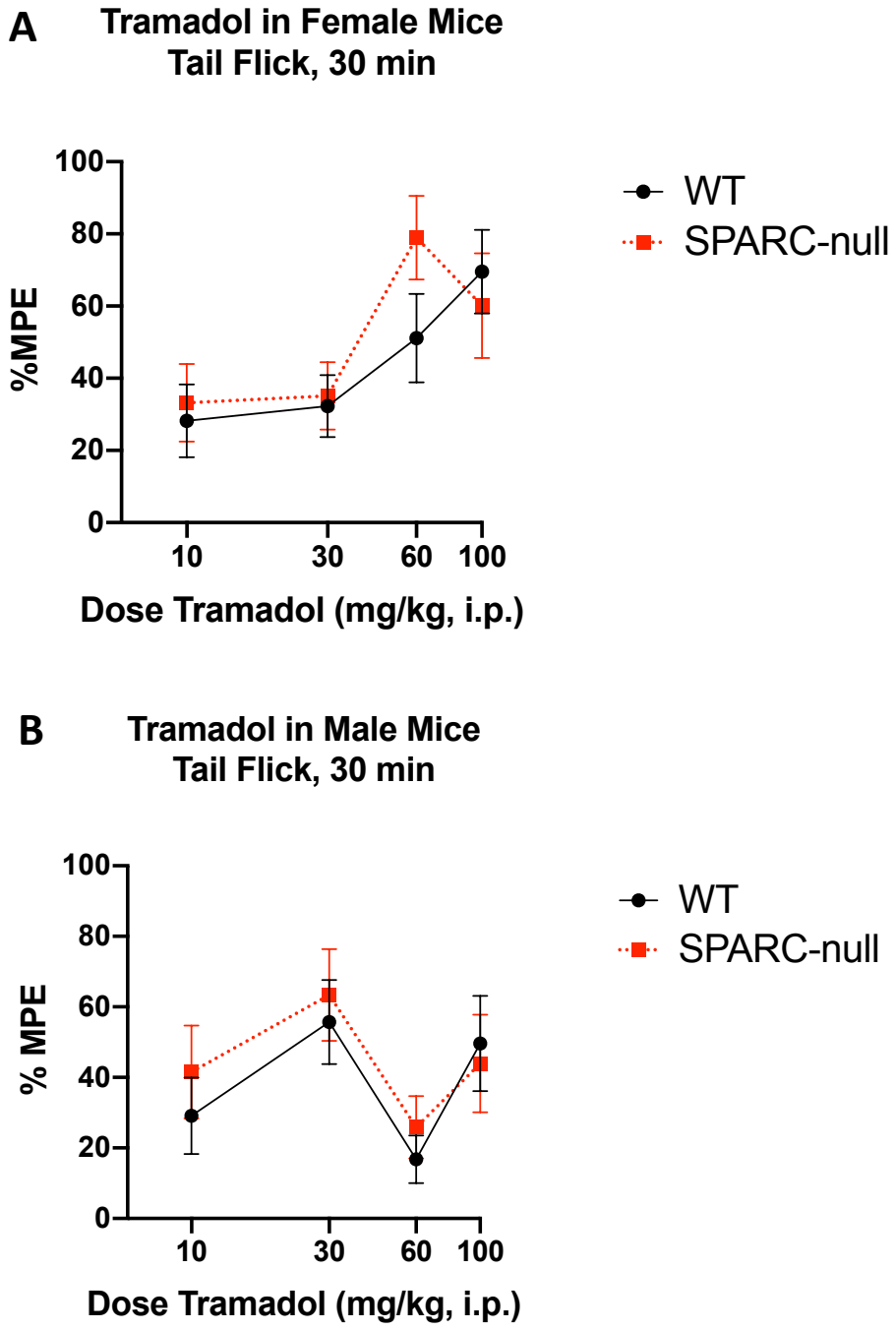
A. Mechanical sensitivity was not altered after 14 days of treadmill running in SPARC-null mice. **B.** Mechanical sensitivity after 45 days of voluntary running with no significant effect of running. **C.** Cold sensitivity was not altered after 14 days of treadmill running in SPARC-null mice. **D.** Cold sensitivity after 45 days of voluntary running with no significant trend after running.

Figure 5 - Behavior Assessment of Axial Discomfort after Treadmill and Voluntary running protocols



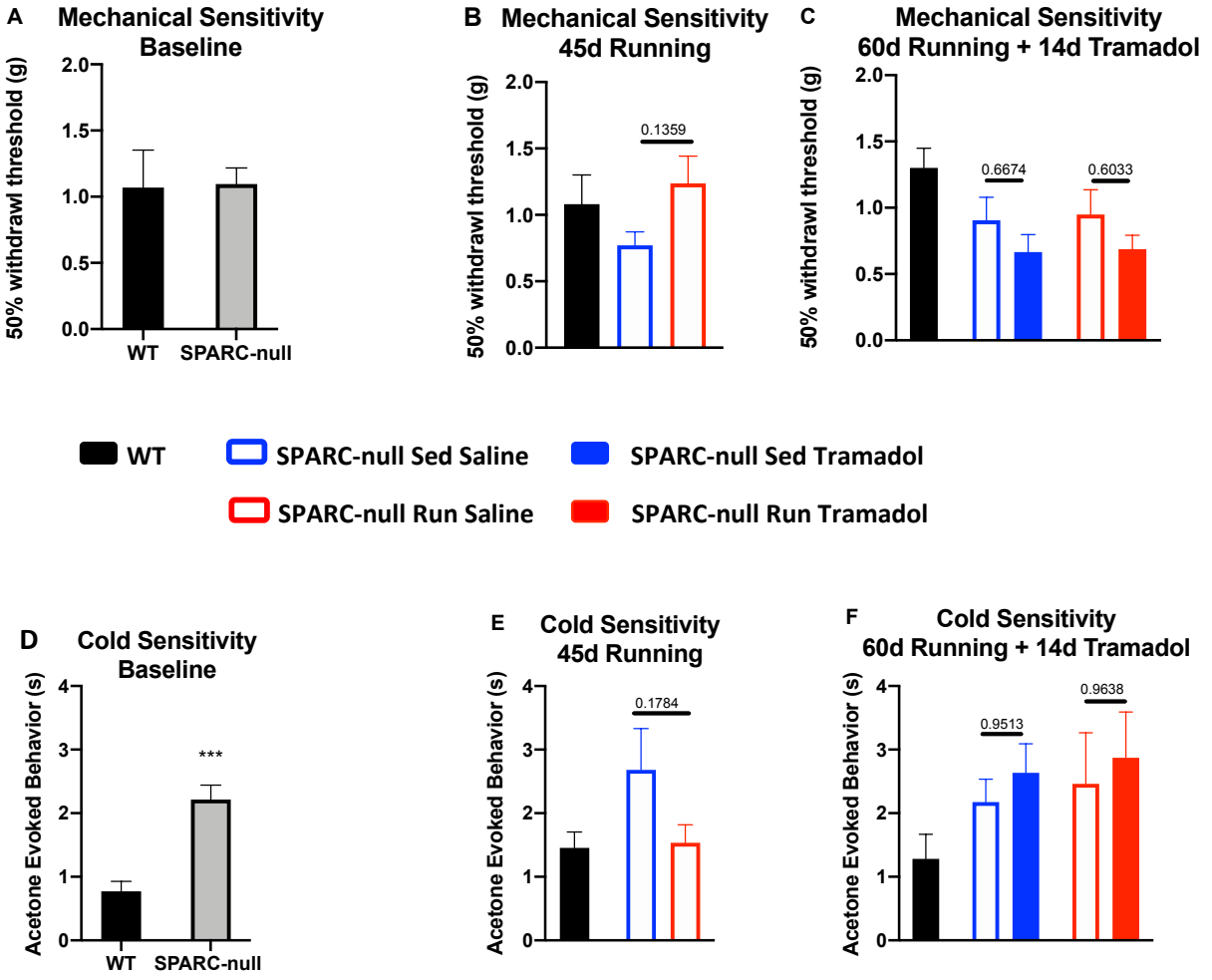
A. Axial discomfort as measured by grip strength was not altered after 14 days of treadmill running in SPARC-null mice. **B.** Voluntary running resulted in a reversal of the SPARC-null phenotype on grip strength. *= p<.05

Figure 6- Acute Tramadol antinociceptive response on Tail Flick test



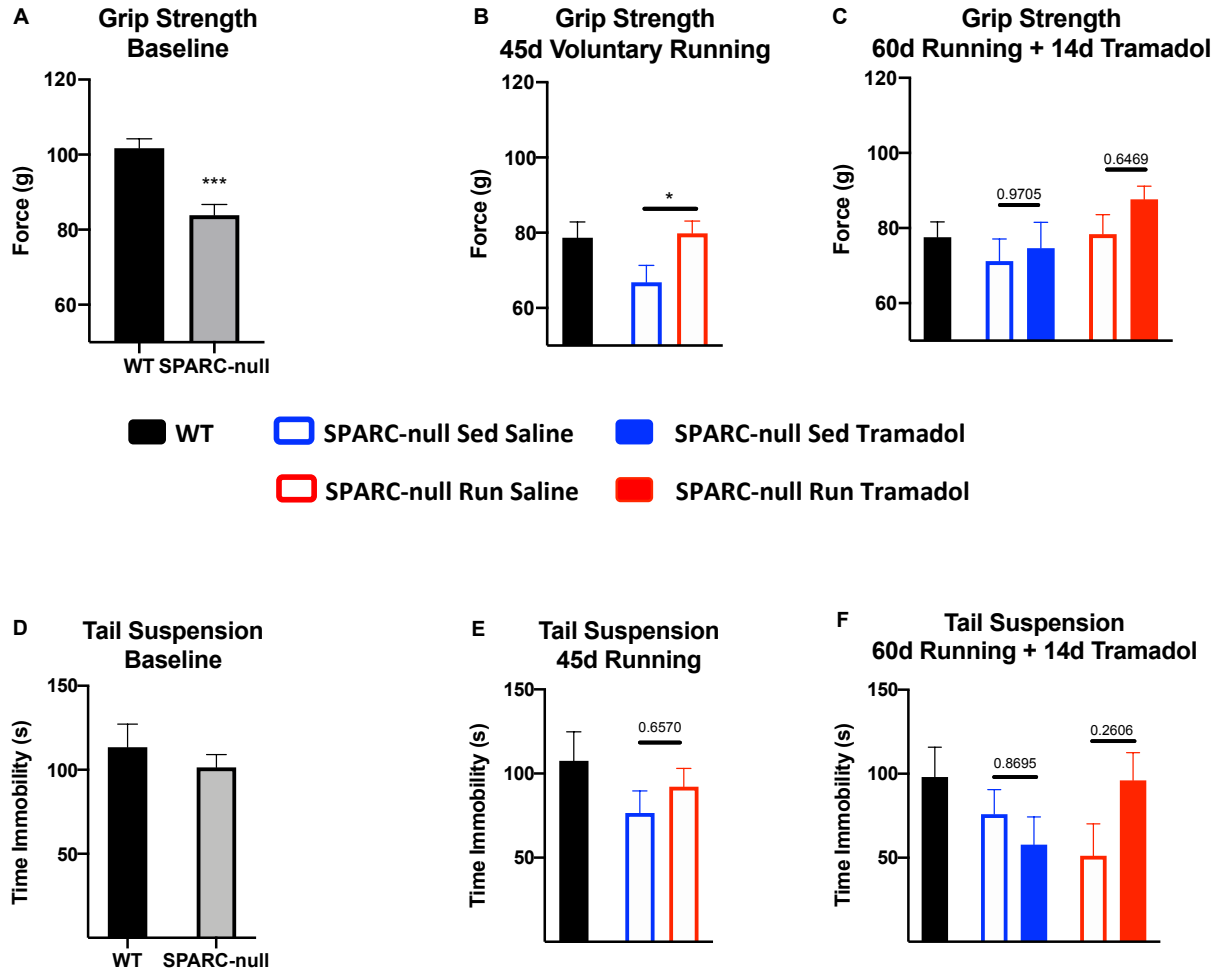
Antinociceptive effect to heat of tramadol presented as %MPE (Maximum Possible Effect) in females **(A)** and males **(B)**.

Figure 7 - Behavior Assessment for Radiating Pain at 3 time-points for Drug and Voluntary Running



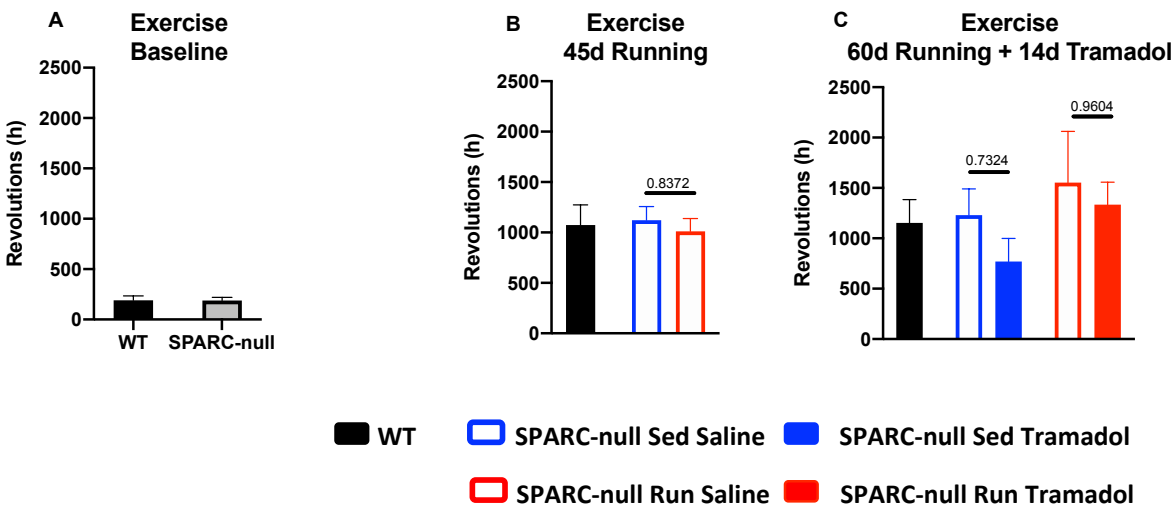
A. Mechanical sensitivity at baseline; **B.** Mechanical sensitivity after 45 days of running; **C.** Mechanical sensitivity after 60 days of running and chronic use of tramadol. **D.** Acetone test baseline showing increased cold hypersensitivity on SPARC-null; **E.** Cold sensitivity after 45 days of running with no significant reversal on the SPARC-null phenotype; **F.** Cold sensitivity after 60 days of running plus 14 days of tramadol with no effect of the drug in neither SPARC-null sedentary nor SPARC-null runners. ***= p<.001

Figure 8- Behavior Assessment for Axial Discomfort at 3 time-points for Drug and Voluntary Running



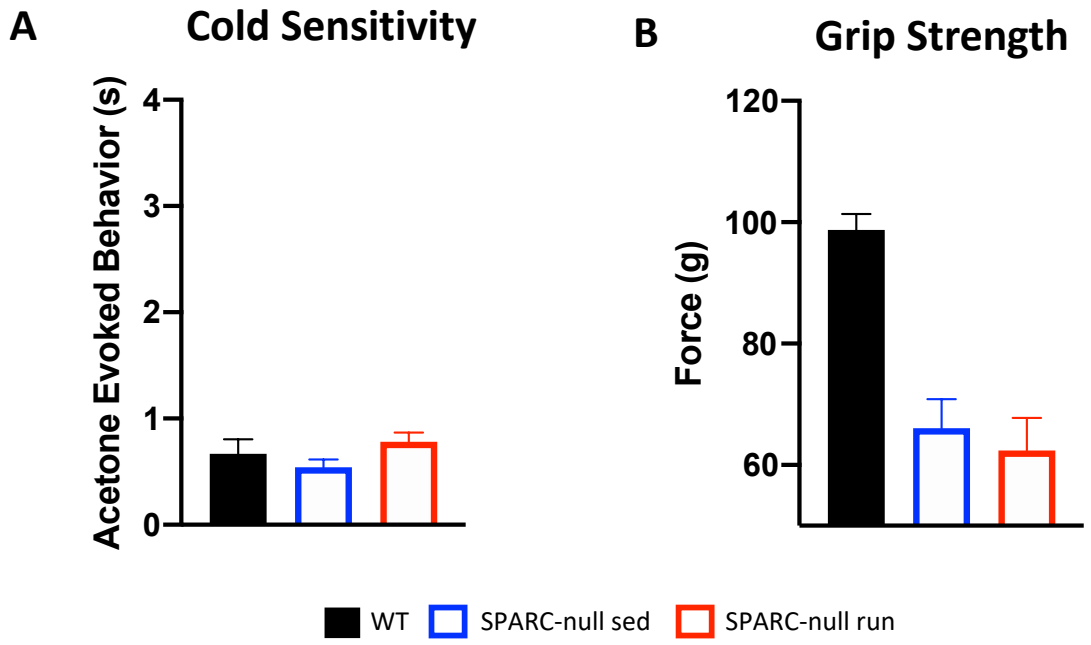
A. Grip strength baseline showing a strong reduction in force on SPARC-null compared to WT; **B.** 45 of running reversed the SPARC-null phenotype on Grip Strength; **C.**Tramadol showed no significant effect neither in SPARC-null sedentary nor Runners; **D.** SPARC-null did not expressed the expected tail suspension phenotype at Baseline; **E.** there is no significant effect of 45 running in Tail Suspension; **F.** Tramadol did not have any significant effect on Tail Suspension neither on SPARC-null sedentary nor runners. *= p<.05; ***= p<.001

Figure 9 - Exercise on the 3 time-points for Drug and Running



A. No difference was observed between strains in the baseline; **B.** there was no significant difference in physical activity after 45 days of running; **C.** Tramadol did not have any effect on SPARC-null neither sedentary nor runners.

Figure 10 - Behavior Assessments after 5 weeks of Treadmill running on Females



A. There is no significant effect of 5 weeks forced Treadmill running on Acetone test in SPARC-null females; **B.** There is no significant effect on Grip Strength in SPARC-null females after 5 weeks of forced running exercise.

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