Pain Sensitivity and Inflammatory Gene Expression in Young and Aged Rat Spinal Cord in Response to Surgery and Morphine

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Abstract

After surgery, older adults are particularly vulnerable to experiencing increased and poorly controlled pain (Halaszynski, 2013). Despite its common use after surgery, there is evidence that chronic morphine treatment contributes to increased pain sensitivity, or hyperalgesia (Joly et al., 2005). In young adult animals, morphine has been shown to incite an inflammatory response, evidenced by the upregulation of pro-inflammatory molecules, such as TLR4, NLRP3, NFkB, IL1B, and TNFa, in the spinal cord (Grace et al., 2016; Grace et al., 2019). Here, we examine the effect of age and morphine treatment on post-operative hyperalgesia and begin to examine a possible underlying mechanism. Aged (24 mo) and young adult (3 mo) male F344xBN F1 rats underwent either a laparotomy (exploratory abdominal surgery) or a sham surgery. After, they were either treated with saline or 2 mg/kg/ml morphine twice a day for 7 days. A Von Frey test for pain sensitivity was conducted at 2 and 8 weeks post-surgery. qPCR was run to measure mRNA gene expression of proinflammatory mediators IL1, IL6, HMGB1, TNFa, and NLRP3 in dorsal horn samples taken at L4/L5 vertebrae. In a second experiment, aged rats were treated with the inflammatory cytokine IL1 receptor antagonist (IL-1RA) immediately prior to surgery and morphine treatment; 2 weeks later rats completed a Von Frey test. At two weeks post-surgery, aged rats who underwent laparotomy and morphine treatment exhibited significantly lower pain thresholds than sham controls, saline-treated controls, or young controls. IL-1RA pre-treatment significantly improved pain sensitivity compared to saline-pretreated rats. However, pain threshold was still significantly lower than rats that had never received morphine in the first place. Preliminary gene expression data suggest a main effect of age elevating inflammatory markers, although no interaction effects with morphine were evident. The ameliorating effects of IL-1RA pre-treatment suggests that IL-1β may play a role in the observed surgery+morphine-induced enhanced pain in aged rats. Investigating post-operative inflammation in other spinal cord segments and examining genes expression of pain mediation downstream of acute inflammation may help elucidate the mechanisms underlying morphine-induced hyperalgesia in aged rats. More generally, a better understanding of how the aging body reacts to morphine and to an external insult

needs to be reached in order to mitigate unnecessary pain through appropriate pre- and post-operative care.

Introduction

Despite the common practice of prescribing opioids for both immediate and chronic pain (Manchikanti et al,. 2010; Chapman et al., 2010), long term effects of morphine and other opioids have yet to be fully understood, especially in older adults (Chou et al., 2014). Beyond problems with tolerance and abuse, studies have also found counterintuitive connections between opioid treatments and hyperalgesia (Hutchinson et al,. 2007; Hutchinson et al,. 2008). Hyperalgesia is a condition where a normally unpainful stimulus, such as touch or temperature, produces excessive pain (Campbell et al., 2006). Hyperalgesia is most commonly associated with injury to peripheral nerves, although it has also been linked to injury of the central nervous system, particularly the brainstem and gray matter within the spinal cord, and to some diseases with neuropathic aspects, as well as post-operative situations (Campbell et al., 2006 ; Doverty et al., 2001; Joly et al., 2005). This study focuses on hyperalgesia in aged rodents treated with morphine following surgery.

Post-operative pain among the elderly population presents a significant problem, as50% of all elderly individuals are estimated to undergo at least one surgical procedure(Kotekar et al., 2014; Etzioni et al., 2003) and approximately 40% of those patients will experience moderate to severe post-operative pain, with 10% of those going on to develop chronic pain (Weinbroum 2017; Halaszynski 2013). Importantly, 90% of all surgical patients are prescribed morphine for post-operative pain management (Aubrun et al., 2012; Garimella et al., 2013), despite morphine's ability to paradoxically prolong post-operative pain (CITE). Unfortunately, the mechanisms underlying this opioid-induced exaggerated pain are not fully understood.

The majority of studies examining morphine in the context of post-operative care have been conducted in young adult rodents (Hutchinson et al., 2010; Hutchinson et al., 2008; Hutchinson et al., 2012; Grace et al., 2016; Grace et al., 2019; Zissen et al., 2007). These studies have linked hyperalgesia to

an exaggerated inflammatory response in the spinal cord through greater expression of toll-like receptor 4 (TLR4), interleukin 1 beta (IL1B), nod-like receptor protein 3 (NLRP3,) nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), tumor necrosis factor (TNFa) and caspase1 (Hutchinson et al., 2010; Hutchinson et al., 2008; Hutchinson et al., 2012; Grace et al., 2016; Grace et al., 2019). This response is in part a result of morphine initiating cytokine release in the CNS through ligation and activation of the pattern recognition receptor TLR4 which can in turn trigger NLRP3 and cause the release of IL1beta, the principal proinflammatory cytokine (Grace et a., 2016; Wang et al., 2012).

Morphine's ability to induce neuroinflammation and its association with post-operative hyperalgesia may have profound implications for older populations, as normal aging is associated with microglial priming (Wang et al., 2012; Fonken et al., 2016; Zhao et al., 2017). Microglia are the immune cells of the CNS and one of their primary function is threat surveillance (Kreutzberg, 1996); they are equipped with many cell surface pattern recognition receptors that allow them to detect danger- and pathogen-associated molecular patterns (Kreutzberg, 1996). TLR4 is one of these receptors. Upon detecting such threats, microglia in the young adult CNS proliferate, become rapidly activated, and produce proinflammatory cytokines in the brain and spinal cord until the threat has been resolved (Colton, 2009). Following this activated state, microglia produce and release anti-inflammatory cytokines and growth factors to facilitate repair and a return to a homeostatic state. With normal aging, microglia transition from simply being surveillant into a primed state, an intermediate state between surveillant and activated (Frank et al., 2010; Cunningham et al., 2005; Godbout et al., 2005). Primed microglia are characterized by their lower activation threshold, such that when a threat is detected, the resulting inflammatory response is exaggerated and prolonged compared to when the microglia were not primed (Combrink et al., 2002). Primed microglia also display a higher number of immune receptors, including TLR4, which aids in their exaggerated response (Zhao et al., 2017). There is a large literature showing that microglial priming is a hallmark of normal aging (Barrientos et al., 2010; Barrientos et al., 2015), thus making aging individuals more susceptible to exaggerated neuroinflammatory responses following a

variety of insults. Indeed, several studies have demonstrated that aging rodents exhibited a potentiated neuroinflammatory response following a bacterial or viral infection (Barrientos et al., 2006; Frank et al., 2006; Abraham et al., 2006), surgery(Barrientos et al., 2012), traumatic brain injury (Kumar et al., 2013), or consumption of a high-fat or high-sugar diet (Spencer et al., 2017; Tucsek et al., 2014) .

In our own lab, we have developed a rodent surgical model in which aged and young male F344xBN F1 rats undergo a laparotomy, an exploratory abdominal surgery, and receive either morphine or saline for 7 days post-operatively. We have demonstrated that aged laparotomy and morphine-treated rats show significantly higher levels of neuroinflammation in the hippocampus compared to the young laparotomy and morphine-treated group, and this was linked to robust memory impairments (Muscat et al.). In a separate group of aged animals, half were pre-treated with IL-1RA, the receptor antagonist to the inflammatory cytokine IL1, immediately prior to the surgery. Selectively blocking IL-1 signaling and thus prohibiting an exaggerated neuroinflammatory response completely prevented the surgery and morphine-induced memory impairments in aged rats, suggesting that the exaggerated response played a role in producing the observed memory impairments.

Whether similar potentiated inflammatory responses occur in the aged spinal cord following surgery and morphine, and whether this is linked to exaggerated hyperalgesia in aged rodents is not known. Furthermore, the mechanisms of inflammatory responses to morphine and surgery have never been explored in the aged dorsal horn of the spinal cord, which is a critical location for registering pain (Zhao et al., 2017). This study aimed to determine the extent to which surgery and morphine would promote hyperalgesia in the aged rodent, and to characterize the proinflammatory phenotype in the spinal cord two weeks post-surgery.

Materials and Methods

General Experimentation

Two experiments were conducted. In the first, aged and young adult male F344xBN F1 rats underwent either a laparotomy, a model for an exploratory abdominal surgery, or a sham surgery. Immediately following surgery, they were treated with either saline or 2 mg/kg/ml morphine twice a day for 7 days. At two weeks post-surgery, rats' pain sensitivity was measured using the Von Frey test. Based on previous studies (Grace et al., 2016; Hutchinson et al., 2008), rats were euthanized and dorsal horn samples were collected from L4/L5 spinal cord for examination of mRNA and protein expression of various markers of inflammation (IL1, IL6, HMGB1, TNFa, and NLRP3). In a second experiment, the contribution of the proinflammatory cytokine IL-1β in exaggerated pain sensitivity following surgery and morphine was examined. Here, aged rats were treated with a central injection of the IL1 receptor antagonist, IL-1RA, immediately prior to surgery. All other aspects of the experiment were identical to the first experiment. All following methods unless otherwise noted are further described in Barrientos et al., 2012.

Subjects

Subjects were aged (24 months old) and young (3 month old) adult male F344xBN F1 rats obtained from Charles River via National Institute on Aging. They were housed two per cage under standard conditions and in accordance with protocols from the OSU Animal Care and Use Committees.

Surgery

Laparotomies were performed with animals under isoflurane anesthesia according to (Martin et al. 2005). Animals' abdomens were shaved and wiped with 70% ethanol and surgical scrub. A 3 cm incision was made just below the ribcage on the right abdomen and the viscera and musculature were disturbed. A section of the small intestine was exteriorized and rubbed between the fingers using sterile gloves for about 30 seconds before replacing them back into the abdominal cavity. The incision to the

muscle layers was sewn with sterile chromic gut sutures and skin was closed with sterile wound clips. Polysporin was applied topically to prevent infection.

Sham animals were shaved and wiped with 70% ethanol and surgical scrub, then stayed under anesthesia for about 25 minutes, the same amount of time as the average laparotomy.

Drug Administration

Rats were injected post-operatively with either morphine or saline. Morphine was injected into the intraperitoneal cavity at 2mg/kg/ml twice a day for seven days following either laparotomy or sham surgery, based on previous studies (Morgan et al, 2006, Hutchinson et al, 2009, Hutchinson et al, 2010, Grace et al, 2019). Morphine was gifted by the NIDA drug repository. Morphine is reported as free base concentrations and wae diluted in sterile saline (0.9%). The human morphine dose equivalency to the rat dose used in this study is 45 mg/day, falling within the recommended dose for managing post-operative pain for opioid-naïve patients of 30-60 mg/day (Reagan-Shaw et al., 2008, MD Anderson Cancer Center, 2018). Respective equivolumes of sterile saline were administered to control rats.

IL1-receptor antagonist obtained from Kineret, was administered intra-cisterna magna at 112 µg/3 µl immediately before surgery. During these injections, rats were under isoflurane anesthesia. The dorsal aspect of the skull was first shaved and swabbed with 70% EtOH. A 27-gauge needle attached via PE50 tubing to a 25 µl Hamilton syringe was inserted into the cisterna magna. To verify entry into the cisterna magna, ~2 µl of clear cerebral spinal fluid was drawn up and gently pushed back in and then 3 µl total volume of IL-1RA was administered over 30 sec. An equal volume of sterile saline was injected (icm) into vehicle control rats.

von Frey Tests

Rats were allowed to habituate to the testing environment and testers were blind to group assignments. At the 2 week mark after either sham or laparotomy procedure, the von Frey test (Chaplan et al., 1994) was performed at the distal region of the heel in the hind paws (Chacur et al., 2001; Milligan et

al., 2001). This involved applying a logarithmic series of 10 Semmes-Weinstein monofilaments (von Frey hairs; Stoelting, Wood Dale, IL) ranging in stiffness from 3.61 (0.40 g) to 5.18 (15.14 g) to a hind paw at increasing stiffnesses until the rat withdrew the paw. 50% probability of response was calculated with a Gaussian integral psychometric function using a maximum-likelihood fitting method (Harvey, 1986; Treutwein and Strasburger, 1999), as described previously (Milligan et al., 2000; Milligan et al., 2001).

Tissue Dissection

Rats were given a lethal dose (50 mg/kg) of sodium pentobarbital (Fatal Plus) before being transcardially perfused with cold saline for three minutes. L4/L5 section of the dorsal spinal cords were dissected and frozen in liquid nitrogen and stored at -80C until processing.

qPCR

The following genes were quantified through qPCR: HMGB1, NLRP3, IL-1, IL-6, IkBa, toll-like receptor 4, and tumor necrosis factor. IL-1, IL-6, and TNFa are all inflammatory cytokines. TLR4 and NLRP3 are immune receptor proteins and have been associated with CNS inflammation post-morphine exposure (Grace et al., 2016; Grace et al., 2019; Zissen et al., 2007). HMGB1 is an endogenous danger signal. qPRC was run as described in (Barrientos et al. 2012). Briefly, total RNA was first isolated with standard procedure previously put forth (Chomczynski and Sacchi, 1987). cDNA amplification was done through Quantitect SYBR Green PCR kit (Qiagen, Valenica, CA) in iCycler iQ on a MyiQ single Color RealTime PCR Detection System (Bio-Rad) (Primers; Invitrogen). Samples were completed in duplicate using the MyiQ single Color Real-Time PCR Detection System (Bio-Rad). B actin used as the designated housekeeping gene. Gene sequences can be found in Table 1 Appendix 1.

Statistical Analysis

Statistics were completed through Statview v.5 and Prism v.7 software. Two-way ANOVAs were used for von Frey tests and three way ANOVAs and Tukey's tests were used for qPCR analysis. Alpha was set to 0.05.

Results

Effects of Morphine, Peripheral Insult, and Age: Sensitivity Threshold

First, the effects of laparotomy and morphine treatment on pain sensitivity across age were compared at the two-week timepoint (n=6-7/group). A two-way ANOVA showed significant age ($F_{(1,44)}$ = 51.35, $p < 0.0001$) and condition interactions ($F_{(3,44)} = 36.88$, $p < 0.0001$). *Post hoc* analysis revealed that the aged, laparotomy and morphine-treated group had a significantly lower threshold than the young, laparotomy and morphine-treated group, the aged, laparotomy and saline-treated group, and the aged, sham and morphine-treated group ($p < 0.0001$, Fig. 1).

To determine the duration of this decreased threshold, the sensitivity of a subset of aged animals was tested again at the eight-week timepoint. A two-way ANOVA resulted in significant morphine ($F_{(1,4)}$ = 15.05, $p < 0.05$) and surgery interactions ($F_{(1,4)} = 12.01$, $p < 0.05$). A *post hoc* multiple comparisons test showed significant differences between morphine and saline treatments after laparotomy, as well as significant differences between laparotomy and sham groups with morphine treatment ($p<0.05$, Fig. 2).

Effects of Morphine, Peripheral Insult, and Age: Pro-inflammatory Gene Expression

Given both the evidence connecting morphine treatment with an exaggerated pro-inflammatory response in young animals and the potentiated neuroimmune response seen in aged rodents, pro-inflammatory mRNA was analyzed (Hutchinson et al., 2010; Hutchinson et al., 2008; Hutchinson et al., 2012; Grace et al., 2016; Grace et al., 2019; Zissen et al., 2007; Combrink et al., 2002; Barrientos et al., 2010; Barrientos et al., 2015). A three way ANOVA with age, surgery, and treatment indicated significant age interactions (HMGB1 $F_{(1,44)} = 29.19$, $p < 0.0001$, IL1 $F_{(1,41)} = 7.212$, $p < 0.05$, IL6 $F_{(1,44)} = 7.276$, $p < 0.05$, NLRP3 $F_{(1,45)} = 79.94$, $p < 0.0001$, TLR4 $F_{(1,43)} = 4.108$, $p < 0.05$, TNFa $F_{(1,42)} = 20.31$, $p < 0.0001$), along with significant treatment interactions for NLRP3 ($F_{(3,45)} = 3.257$, $p < 0.05$). The remaining mRNA varied in response to surgery and morphine (Fig.3). There are possible reasons why clear trends were not seen in regards to surgery or treatment, and will be discussed below.

Pro-inflammatory Antagonist with Peripheral insult and Morphine: Sensitivity Threshold

Pain sensitivity was again examined in aged rats with exposure to surgery and morphine, this time following pre-treatment with the pro-inflammatory IL1beta receptor antagonist, IL-1RA. von Frey testing was completed two weeks following surgery. A two way ANOVA showed significant morphine ($F_{(1,26)}$ = 93.48, $p < 0.0001$) and IL-1RA treatment ($F_{(1,26)} = 8.544$, $p < 0.01$) interactions. *Post hoc* comparisons indicated significant differences between morphine and saline post-surgery-treated animals among those that received pre-operative saline, between morphine and saline post-surgery-treated animals among those that received pre-operative IL-1RA treatment, and importantly, between IL-1RA and saline pre-surgery-treated animals among those that received morphine post-operatively ($p<0.05$, Fig.4).

Discussion

The aged rats who underwent laparotomy and morphine treatment exhibited significantly lower absolute thresholds compared to the sham or morphine control groups, or the young animals that had surgery and morphine. The lower threshold suggests that these animals are experiencing a higher sensitivity to pain. A smaller difference can be seen between young and aged animals across all groups, which supports the idea that the aged body may react differently to peripheral insults compared to young animals (Fig. 1). It is important to note that this drastically reduced threshold only occurred with the combination of aging, surgery, and morphine treatment. This increase in sensitivity in conjunction with surgery and morphine agrees with previous studies conducted in young rats (Grace et al., 2016; Grace et al., 2019).

At 8 weeks post-surgery, aged animals that underwent laparotomy and morphine continued to exhibit a far lower threshold compared to the sham and saline treated animals. This both reinforces the findings in Figure 1 and indicates that the heightened sensitivity to pain persists at least eight weeks post-surgery.

Pro-inflammatory genes were chosen to be examined due to their key roles in inflammation, and, in the cases of NLRP3, IL1, TNFa, and TLR4, due to their upregulation in young animals with injury and morphine treatment (Grace et al., 2016). Overall, the resulting data did not follow any clear trends. Age was found to be a significant factor, along with the morphine condition for NLRP3. However, in the cases of HMGB1 and Il-1, young animals exhibited higher relative amounts of the mRNA than the aged animals. With TLR4, an innate immune receptor expressed robustly in primed microglia and to which morphine is known to bind, the group with the highest expression was that of aged+surgery+morphine. The TLR4 expression in other groups were lower and fairly consistent, though not significantly different.

One particularly compelling explanation for this collection of results is the location of the samples retrieved. These samples were taken at the L4-L5 levels of the spinal cord, which while consistent with the focus of other pain sensitivity studies such as (Grace et al., 2016) at the time of the experiment, it has since been found that the innervation of the area affected by the laparotomy is slightly lower, at sacral 1-2 (Lemos and Possover, 2015). This means that damage or changes to nerves following laparotomy, which may contribute to the Von Frey behavioral test results, might not be accurately represented by gene expression in the L4-L5 region, as presented here.

In the second experiment, the Von Frey test, which was conducted with aged animals two weeks after IL-1RA or saline pre-treatment and laparotomy, indicates a correlation between IL-1 activation, morphine treatment, and pain sensitivity. Both morphine groups displayed higher sensitivities compared to the saline groups. However, the group that received the IL-1 receptor antagonist showed a significantly higher threshold than the saline treated morphine group, meaning they are likely experiencing less hyperalgesia. These results suggest that blocking inflammatory pathways in aged rats just prior to surgery and morphine-treatment results in reduced post-operative hyperalgesia. These results suggest that neuroinflammation may play a critical role in mediating the effects of surgery and morphine-treatment on hyperalgesia in aged rats.

Another factor that may play into the extent of the recovery of the pain threshold could be the method of administration of the IL-1RA. It was given through an intra-cisterna magna injection, which is still some distance from the primary affected region of S1-S2 (Frank et al., 2012). Since the degradation rate of IL-1RA in the body is quite high peripherally, at roughly 90 minutes, but potentially closer to a day (Cawthorn et al., 2011), if the injection was given even closer to S1-S2, it is possible that the effects of IL-1RA would raise the sensitivity threshold further in the morphine + IL-1RA group.

Future Work

As shown in Figure 4, there is evidence to suggest that inflammation plays a role in producing the hyperalgesia seen acutely in the aged+morphine+laparotomy group. Due to TLR4's involvement with both aging and morphine, it is a key target of future studies (Colton, 2009; Combrink et al., 2002; Barrientos et al., 2010; Barrientos et al., 2015). In addition to binding to TLR4, morphine also binds to the mu-opioid receptor (Pathan and Williams, 2012). In order to parse out the effects of the mu-opioid receptor versus TLR4, a number of approaches could be taken. First, a mu-opioid receptor antagonist can be used to determine the specific role of morphine on the mu-opioid receptor. Second, an alternate morphine isoform, the (+)morphine homolog, which does not bind to the mu-opioid receptor but does bind to TLR4, can be used to determine the role of TLR4. The effects of TLR4 can be further elucidated through the administration of a TLR4-specific antagonist. Additionally, the impact of a pre-surgery IL-1RA injection could be examined with a change in injection site. Giving the drug intrathecally may be more effective as it would be administered closer to the affected region.

Appendix 1

Table 1. PCR Primer Description and Sequences

Gene	Primer Sequence: 5' -> 3'	Function
β-Actin	F: TTCCTTCCTGGGTATGGAAT R: GAGGAGCAATGATCTTGATC	Cytoskeletal protein (housekeeping gene)
IL-1β	F: CCTTGTGCAAGTGTCTGAAG R: GGGCTTGGAAGCAATCCTTA	Pro-inflammatory cytokine
IL-6	F: AGAAAAGAGTTGTGCAATGGCA R: GGCAAATTTCCTGGTTATATCC	Pro-inflammatory cytokine
TNFα	F: CAAGGAGGAGAAGTTCCCA R: TTGGTGGTTTGCTACGACG	Pro-inflammatory cytokine
HMGB1	F: GAGGTGGAAGACCATGTCTG R: AAGAAGAAGGCCGAAGGAGG	Endogenous danger signal
NLRP3	F: AGAAGCTGGGGTTGGTGAATT R: GTTGTCTAACTCCAGCATCTG	IL-1 Inflammasome
TLR4	F: TCCCTGCATAGAGGTACTTC R: CACACCTGGATAAATCCAGC	PRR for motifs of gram-negative bacteria

Table 1. Abbreviations: IL: interleukin, TNF (tumor necrosis factor alpha); HMGB1: high mobility group box 1, NLRP3: nod-like receptor protein 3, TLR (Toll-like receptor).

Appendix 2: Figures and Figure Legends

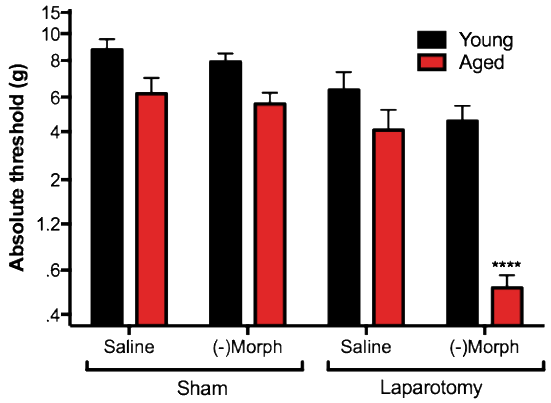

Figure 1: Two weeks post-surgery, aged rats given morphine twice daily for 7 days showed a significantly decreased pain threshold, using a Von Frey assay, compared to aged rats not exposed to morphine, younger animals, or sham controls. (n=6-7/group) Error bars represent SEM; ****$p < 0.001$.

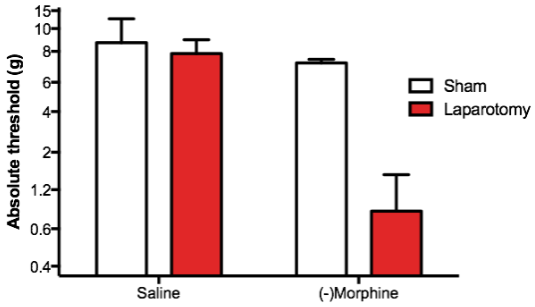

Figure 2: Eight weeks post-surgery, aged rats given morphine twice daily for 7 days post laparotomy still showed a significant decrease in pain threshold compared to aged rats exposed to only one of these insults. (n=3/group) Error bars represent SEM

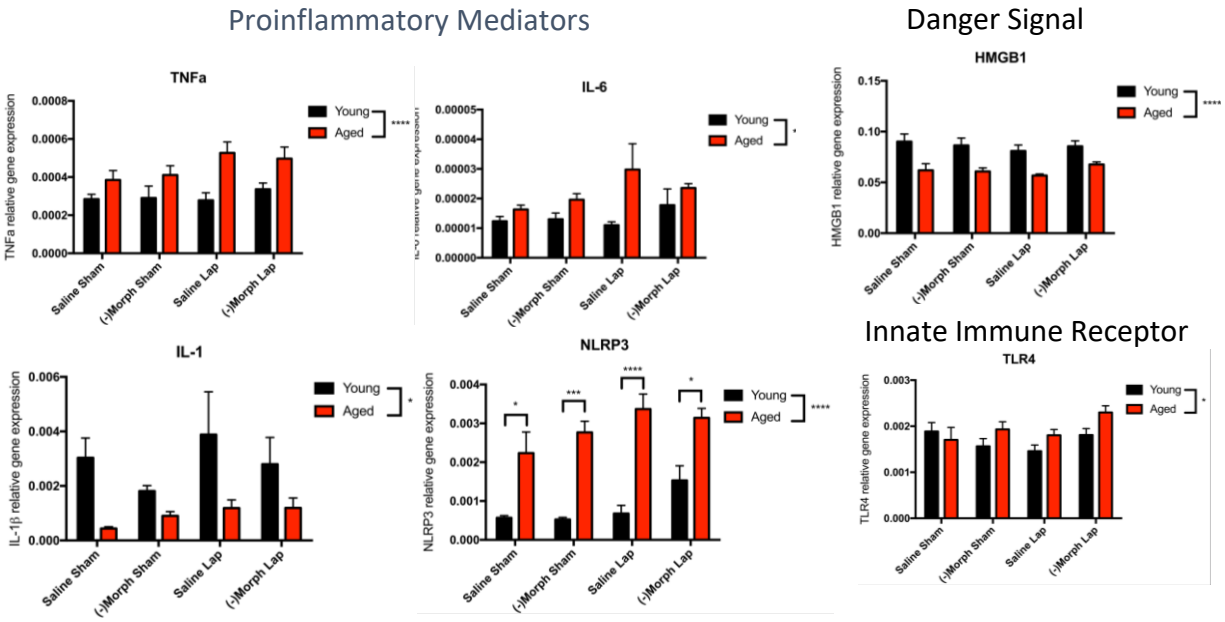

Figure 3: Aged rats showed significantly higher concentrations of TNFa, HMGB1, IL1, IL6, TLR4, and NLRP3 mRNA. Rats given morphine also showed significantly higher NLRP3 mRNA. Error bars represent SEM; *p < 0.05; ***p<0.01; ****p < 0.001.

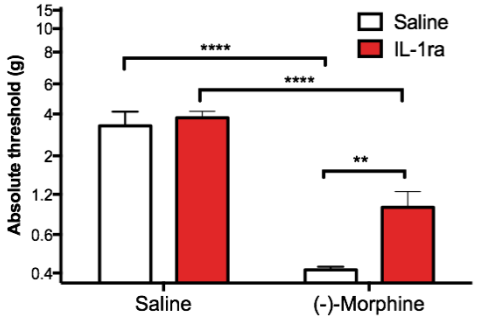

Figure 4: Aged rats treated with a single dose of IL-1ra immediately prior to surgery and followed by 7 days of twice daily morphine injections showed a significant improvement in pain threshold compared to aged animals only given saline but a significantly decreased threshold compared to those not given morphine. (n=6-7/group) Error bars represent S.E.M; **p < 0.01; ****p < 0.001.

References

Abraham, J., Jang, S., Godbout, J.P., Chen, J., Kelley, K.W., Dantzer, R., Johnson, R.W., 2008. Aging sensitizes mice to behavioral deficits induced by central HIV-1 gp120. Neurobiol Aging 29, 614-621.

Aubrun, F., J.X. Mazoit, and B. Riou, Postoperative intravenous morphine titration. Br J Anaesth, 2012. 108(2): p. 193-201.

Barrientos, R.M., Hein, A.M., Frank, M.G., Watkins, L.R., Maier, S.F., 2012. Intracisternal interleukin-1 receptor antagonist prevents postoperative cognitive decline and neuroinflammatory response in aged rats. J Neurosci 32, 14641-14648.

Barrientos, R.M., Frank, M.G., Watkins, L.R., Maier, S.F., 2010. Memory impairments in healthy aging: Role of aging-induced microglial sensitization. Aging Dis 1, 212-231.

Barrientos, R.M., Kitt, M.M., Watkins, L.R., Maier, S.F., 2015. Neuroinflammation in the normal aging hippocampus. Neuroscience 309, 84-99.

Barrientos, R.M., Higgins, E.A., Biedenkapp, J.C., Sprunger, D.B., Wright-Hardesty, K.J., Watkins, L.R., Rudy, J.W., Maier, S.F., 2006. Peripheral infection and aging interact to impair hippocampal memory consolidation. Neurobiol Aging 27, 723-732.

Campbell JN, Meyer RA. Mechanisms of neuropathic pain. Neuron. 2006;52(1):77-92. doi:10.1016/j.neuron.2006.09.021

Cawthorne C, Prenant C, Smigova A, et al. Biodistribution, pharmacokinetics and metabolism of interleukin-1 receptor antagonist (IL-1RA) using [^{18}F]-IL1RA and PET imaging in rats. Br J Pharmacol. 2011;162(3):659-672. doi:10.1111/j.1476-5381.2010.01068.x

Chacur,M.,Milligan,E.D.,Gazda,L.S., Armstrong,C., Wang,H., Tracey,K.J., Maier,S.F.,Watkins,L.R., A new model of sciatic inflammatory neuritis (SIN): Induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain, 94 (2001), pp. 231-244

Chaplan S.R., Bach F.W., Pogrel J.W., Chung J.M., Yaksh T. L., Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Meth, 53 (1994), pp. 55-63.

Chapman CR, Lipschitz DL, Angst MS, et al. Opioid pharmacotherapy for chronic non-cancer pain in the United States: A research guideline for developing an evidence-base. J Pain. 2010;11:807-829.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.

Chou R, et al. (2014) The Effectiveness and Risks of Long-Term Opioid Treatment of Chronic Pain. (Agency for Healthcare Research and Quality, Rockville, MD).

Colton, C.A., 2009. Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 4, 399-418.

Combrinck, M.I., Perry, V.H., Cunningham, C., 2002. Peripheral infection evokes exaggerated sickness behaviour in pre-clinical murine prion disease. Neuroscience 112, 7-11.

Cunningham, C., Wilcockson, D.C., Campion, S., Lunnon, K., Perry, V.H., 2005. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. J Neurosci 25, 9275-9284.

Doverty M, White JM, Somogyi AA, et al. Hyperalgesic responses in methadone maintenance patients. Pain. 2001;90:91–96.

Etzioni, D.A., et al., The aging population and its impact on the surgery workforce. Ann Surg, 2003. 238(2): p. 170-7.

Fonken LK, Frank MG, Kitt MM, D'Angelo HM, Norden DM, Weber MD, Barrientos RM, Godbout JP, Watkins LR, Maier SF. The Alarmin HMGB1 mediates age-induced Neuroinflammatory priming. J Neurosci. 2016;36(30):7946–7956. doi: 10.1523/JNEUROSCI.1161-16.2016.

Frank, M.G., Barrientos, R.M., Biedenkapp, J.C., Rudy, J.W., Watkins, L.R., Maier, S.F., 2006. mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiol Aging 27, 717-722.

Frank, M.G., Barrientos, R.M., Watkins, L.R., Maier, S.F., 2010. Aging sensitizes rapidly isolated hippocampal microglia to LPS ex vivo. J Neuroimmunol 226, 181-184.

Frank MG, Barrientos RM, Thompson BM, Weber MD, Watkins LR, Maier SF. IL-1RA injected intra-cisterna magna confers extended prophylaxis against lipopolysaccharide-induced neuroinflammatory and sickness responses. J Neuroimmunol. 2012;252(1-2):33-39. doi:10.1016/j.jneuroim.2012.07.010

Garimella, V. and C. Cellini, Postoperative pain control. Clin Colon Rectal Surg, 2013. 26(3): p. 191-6.

Godbout, J.P., Chen, J., Abraham, J., Richwine, A.F., Berg, B.M., Kelley, K.W., Johnson, R.W., 2005. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. Faseb J 19, 1329-1331.

Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, et al. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. Proc Natl Acad Sci U S A. 2016;113(24):E3441-50.

Grace PM, Galer EL, Strand KA, Corrigan K, Berkelhammer D, Maier SF, Watkins LR. 2019. Repeated morphine prolongs postoperative pain in male rats. Anesth Analg. 128, 161–167.

Halaszynski T. Influences of the aging process on acute perioperative pain management in elderly and cognitively impaired patients. Ochsner J. 2013;13(2):228-247

Harvey, L.O.J.,Efficient estimation of sensory thresholds. Behav Res Methods Instrum Comput, 18 (1986), pp. 623-632

Hutchinson, M.R., Zhang, Y., Brown, K., Coats, B.D., Shridhar, M., Sholar, P.W., Patel, S.J., Crysdale, N.Y., Harrison, J.A., Maier, S.F., Rice, K.C., Watkins, L.R., 2008. Non-stereoselective reversal of

neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). Eur J Neurosci 28, 20-29.

Hutchinson, M.R., Bland, S.T., Johnson, K.W., Rice, K.C., Maier, S.F., Watkins, L.R., 2007. Opioid-induced glial activation: mechanisms of activation and implications for opioid analgesia, dependence, and reward. ScientificWorldJournal 7, 98-111.

Hutchinson, M.R., Zhang, Y., Shridhar, M., Evans, J.H., Buchanan, M.M., Zhao, T.X., Slivka, P.F., Coats, B.D., Rezvani, N., Wieseler, J., Hughes, T.S., Landgraf, K.E., Chan, S., Fong, S., Phipps, S., Falke, J.J., Leinwand, L.A., Maier, S.F., Yin, H., Rice, K.C., Watkins, L.R., 2010. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. Brain Behav Immun 24, 83-95.

Hutchinson, M.R., Northcutt, A.L., Hiranita, T., Wang, X., Lewis, S.S., Thomas, J., van Steeg, K., Kopajtic, T.A., Loram, L.C., Sfregola, C., Galer, E., Miles, N.E., Bland, S.T., Amat, J., Rozeske, R.R., Maslanik, T., Chapman, T.R., Strand, K.A., Fleshner, M., Bachtell, R.K., Somogyi, A.A., Yin, H., Katz, J.L., Rice, K.C., Maier, S.F., Watkins, L.R., 2012. Opioid activation of toll-like receptor 4 contributes to drug reinforcement. J Neurosci 32, 11187-11200.

Hutchinson MR, Lewis SS, Coats BD, Skyba DA, Crysdale NY, Berkelhammer DL, Brzeski A, Northcutt A, Vietz CM, Judd CM, Maier SF, Watkins LR, Johnson KW (2009) Reduction of opioid withdrawal and potentiation of acute opioid analgesia by systemic AV411 (ibudilast). Brain Behav Immun 23:240-250.

Hutchinson MR, Lewis SS, Coats BD, Rezvani N, Zhang Y, Wieseler JL, Somogyi AA, Yin H, Maier SF, Rice KC, Watkins LR (2010) Possible involvement of toll-like receptor 4/myeloid differentiation factor-2 activity of opioid inactive isomers causes spinal proinflammation and related behavioral consequences. Neuroscience 167:880-893.

Joly V, Richebe P, Guignard B, et al. Remifentanil-induced postoperative hyperalgesia and its prevention with small-dose ketamine. Anesthesiology. 2005;103:147–155.

Kotekar, N., C.S. Kuruvilla, and V. Murthy, Post-operative cognitive dysfunction in the elderly: A prospective clinical study. Indian J Anaesth, 2014. 58(3): p. 263-8.

Kreutzberg, G.W., 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19, 312-318

Kumar, A., Stoica, B.A., Sabirzhanov, B., Burns, M.P., Faden, A.I., Loane, D.J., 2013. Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. Neurobiol Aging 34, 1397-1411.

Lemos N, Possover M. Laparoscopic approach to intrapelvic nerve entrapments. J Hip Preserv Surg. 2015;2(2):92-98. doi:10.1093/jhps/hnv030

Manchikanti L, Fellows B, Ailinani H, Pampati V (2010) Therapeutic use, abuse, and nonmedical use of opioids: A ten-year perspective. Pain Physician 13(5):401–435.

Martin TJ, Kahn WR, Eisenach JC (2005) Abdominal surgery decreases food-reinforced operant responding in rats: relevance of incisional pain. Anesthesiology 103:629-637.

Martin,D., Maier, S.F.,Watkins,L.R., Intrathecal HIV-1 envelope glycoprotein gp120 enhanced pain states mediated by spinal cord proinflammatory cytokines. J Neurosci, 21 (2001), pp. 2808-2819

Milligan,E.D.,Mehmert, K.K,Hinde,J.L., Harvey,L.O.J., Martin,D., Tracey,K.J., Maier,S.F., Watkins,L.R.,Thermal hyperalgesia and mechanical allodynia produced by intrathecal administration of the human immunodeficiency virus-1 (HIV-1) envelope glycoprotein, gp120. Brain Res, 861 (2000), pp. 105-116

Morgan MM, Fossum EN, Stalding BM, King MM (2006) Morphine antinociceptive potency on chemical, mechanical, and thermal nociceptive tests in the rat. J Pain 7:358-366.

Muscat, S.M., Deems, N.P., D'Angelo, H., Kitt, M.M., Grace, P.M., Andersen, N.D., Silverman, S.N., Rice, K.C., Watkins, L.R., Maier, S.F., Barrientos, R.M., in press. Post-operative cognitive dysfunction is made persistent with morphine treatment in aged rats. Neurobiol Aging.

Pathan H, Williams J. Basic opioid pharmacology: an update. Br J Pain. 2012;6(1):11-16. doi:10.1177/2049463712438493

Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22:659-661.

Spencer, S.J., D'Angelo, H., Soch, A., Watkins, L.R., Maier, S.F., Barrientos, R.M., 2017. High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory. Neurobiol Aging 58, 88-101.

Treutwein, B. and Strasburger, H., Fitting the psychometric function. Percept Psychophys, 61 (1999), pp. 87-8106

Tucsek, Z., Toth, P., Tarantini, S., Sosnowska, D., Gautam, T., Warrington, J.P., Giles, C.B., Wren, J.D., Koller, A., Ballabh, P., Sonntag, W.E., Ungvari, Z., Csiszar, A., 2014. Aging exacerbates obesity-induced cerebromicrovascular rarefaction, neurovascular uncoupling, and cognitive decline in mice. J Gerontol A Biol Sci Med Sci 69, 1339-1352.

Wang X, Loram LC, Ramos K, de Jesus AJ, Thomas J, Cheng K, et al. . Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc Natl Acad Sci USA. (2012) 109:6325–30. 10.1073/pnas.1200130109

Weinbroum AA. Postoperative hyperalgesia—A clinically applicable narrative review. Pharmacological Research. 2017;120:188-205. doi:10.1016/j.phrs.2017.02.012

Zhao, A. Alam, Q. Chen, M. A. Eusman, A. Pal, S. Eguchi, L. Wu, D. Ma, The role of microglia in the pathobiology of neuropathic pain development: what do we know?, BJA: British Journal of Anaesthesia, Volume 118, Issue 4, April 2017, Pages 504–516, https://doi.org/10.1093/bja/aex006

Zissen MH, Zhang G, McKelvy A, Propst JT, Kendig JJ, Sweitzer SM. Tolerance, opioid-induced allodynia and withdrawal associated allodynia in infant and young rats. Neuroscience. 2007;144(1):247-262. doi:10.1016/j.neuroscience.2006.08.078