ZOOL 567 Annotated Bibliography

NOTE TO READER: My annotated bibliography entries are organized by topic, and therefore relevance to different sections of my literature review on chronic pain-related behaviours in mice. First is the comparison of the use of reflexive and voluntary behaviours, primarily with evidence against the use of reflexive measures, followed by evidence for new measures of voluntary behaviours. This is followed by studies which indicate some proximate and ultimate influences on behaviours related to chronic pain.

Highlighting Overreliance on Reflexive Behaviours in Research

Evidence Against the Use of Reflexive Measures in Mouse Chronic Pain Research

Citation:

Mogil, J. S. (2009). Animal models of pain: Progress and challenges. *Nature Reviews Neuroscience, 10*, 283-294. https://doi.org/10.1038/nrn2606

Article Summary:

This review article addresses the slow progress in applying animal models to clinical pain treatments by evaluating the advantages and challenges associated with animal pain models, including mice. The author attributes this lack of translational progress to the complexity of pain experiences and lists various influential factors including sex, communication, and genetics. The difficulty associated with using transgenic mice models in pain research is discussed, and it is concluded that most pain disorders are polygenic and therefore difficult to induce and study. The author suggests that there are differences associated with chronic pain and analgesic potency (strength of pain-mediating drugs), between sexes, ages, strains, and mice with different husbandry conditions (e.g. diet, handling, bedding) which studies rarely account for.

The paper indicates four major types of pain: (1) nociceptive (reactive pain to acute stimuli), (2) inflammatory pain, (3) neuropathic pain (nerve damage), and (4) idiopathic pain (unknown origin). Pain types (1) and (2) are usually short-lived or acute pain, while (3) and (4) are considered chronic pain. Finally, the authors discuss many behavioural measures of pain, suggesting that the commonly used measures of hypersensitivity (e.g. innate behaviours like scratching and licking) are not reliable indicators of chronic pain. For example, these behaviours

are no longer evoked if motor neurons are damaged, yet the animal still experiences pain. As such, measures of spontaneous pain, rather than evoked pain, are more reliable measures of chronic pain. Negative changes in complex behaviours such as attention, memory, locomotion, and sleep better indicate chronic pain in mice because they suggest a reduction in overall quality of life. The author makes a series of suggestions to study chronic pain more accurately in animal models, such as measuring complex and voluntary behaviours rather than reflexive pain behaviours, based on the short-comings and successes of previous models.

Article Contribution:

I included this article in my review because it describes a wide array of measures used in the study of chronic pain, which gives me more background when reading about these procedures in the primary literature. This paper advanced knowledge in the field by summarizing and drawing conclusions regarding the efficacy of pain assays based on numerous previous studies. For my purposes, the main take-away of this article is that complex behaviours are more accurate in measuring chronic pain, as chronic pain disorders are typically complex in nature.

Citation:

Mogil, J. S., Graham, A. C., Ritchie, J., Hughes, S. F., Austin, J. Schorscher-Petcu, A., Langford, D. J., & Bennett, G. J. (2010). Hypolocomotion, asymmetrically directed behaviors (licking, lifting, flinching, and shaking) and dynamic weight bearing (gait) changes are not measures of neuropathic pain in mice. *Molecular Pain*, *6*(34), 1-15. https://doi.org/10.1186%2F1744-8069-6-34

Article Summary:

This article scrutinized some commonly used measures of neuropathic chronic pain (due to nerve damage) in mice to determine whether these measures accurately measure pain behaviours. The authors aimed to investigate spontaneous (non-evoked) chronic pain behaviours, in an effort to clarify controversy in the literature regarding which behaviours are representative of such pain. Two procedures were used to induce neuropathic chronic pain: (1) spared nerve injury (SNI), which constitutes transecting of two of three sciatic nerve branches in the hindlimb, and (2) chronic constriction injury (CCI), where the sciatic nerve is constricted but not cut. In terms of behavioural measures, this study looked at mechanically induced hypersensitivity,

asymmetrically directed behaviours (i.e. directed at the injured limb) such as licking, lifting, flinching, and shaking, as well as gait and activity changes such as print area and stance phase duration (how long the paw stays in contact with the floor).

The researchers reported that all measures above were in fact not appropriate for measuring spontaneous, and therefore chronic, pain in mice. They suggest numerous possible reasons for this conclusion. It is stated that it is possible that CCI and SNI do not actually produce chronic pain in mice, that potentially chronic pain is produced but the spontaneous pain measures used cannot measure that pain, or that spontaneous chronic pain in mice does not actually lead to any measurable behaviours in mice. Some of these potential reasons (specifically that nerve injury does not cause chronic pain) seem to vary from other literature regarding chronic pain in mice, and it seems that this issue will only be resolved with further and more rigorous research using high sample sizes, longitudinal models, and extensive recording and double-blinding procedures.

Article Contribution:

I included this article because it uses two different methods of inducing neuropathic chronic pain and comes to the same conclusion that hypersensitivity and asymmetric behaviours are not reliable measures of chronic pain. This conclusion is supported in the literature, supporting the trend towards measuring voluntary pain-related behaviours. This paper also presents reasons for why reflexive behaviours might not reflect the experience of chronic pain. Although some of these may not be realistic, the authors do a good job presenting what is known and unknown so that future research in the area can build upon their findings.

Citation:

Pitzer, C., Kuner, R., & Tappe-Theodor, A. (2016). Voluntary and evoked behavioural correlates in neuropathic pain states under different social housing conditions. *Molecular Pain, 12*, 1-15. https://doi.org/10.1177%2F1744806916656635

Article Summary:

The authors note that chronic pain can be severely debilitating, but the typical methods used to assess this pain in mice are based on stimulus-evoked or “reflexive” behaviours, rather

than voluntary behaviours that may better indicate changes in quality of life. This study's objective was to compare classical stimulus-evoked and voluntary behaviours in relation to pain in a spared nerve injured (SNI, part of the sciatic nerve is cut to produce neuropathic pain) mouse model. This study included exposing SNI and sham mice to aversive temperature and mechanical stimuli (classical measures), as well as gait changes (using two different recording systems), voluntary wheel running, and cage activity (such as climbing). Furthermore, these were measured longitudinally over 12 weeks and in both grouped and individually housed animals in order to investigate the temporal and social influences on chronic pain-related behaviours.

As expected, SNI mice demonstrated more hypersensitivity to temperature and mechanical stimuli in both grouped and isolated mice, as has been shown in many previous studies. Based on gait changes, such as reduced stride length in SNI mice, the authors suggest gait analysis can be used as a reliable measure of neuropathic pain behaviours in this model – a conclusion with both support and disagreement in the literature. Conversely, voluntary wheel running was not found to be a valid pain behavioural measurement, which was based on mixed data likely confounded by handling stress, age, and/or functional changes in the animals over time. Finally, in monitoring animals' unrestricted behaviour in a cage, it appeared that SNI mice engaged in climbing behaviour less frequently than did controls. Overall, this study makes important points about the reliability of evoked pain measures used in mouse studies, and proposes potential voluntary behaviour alternatives for use in the field.

Article Contribution:

I included this article because, by using a longitudinal model, and many evoked and voluntary behavioural measures, this study was able to do many internal comparisons. It thus adds to my review by including the methodology for a variety of behavioural measures of pain. Finally, it supports some previous studies in that it contains evidence against measuring only evoked behaviours to measure chronic pain but disagrees with other by suggesting gait variable may be reliable measures of chronic pain. It thus indicates the need for further research delineating the relationship between gait and chronic pain.

Voluntary Behavioural Measures in Mouse Chronic Pain Research

Citation:

Cobos, E. J., Ghasemlou, N., Araldi, D., Segal, D., Duong, K., & Woolf, C. J. (2012). Inflammation-induced decrease in voluntary wheel running in mice: A nonreflexive test for evaluating inflammatory pain and analgesia. *Pain, 153*, 876-884. http://dx.doi.org/10.1016/j.pain.2012.01.016

Article Summary:

This study aimed to investigate a new measure of inflammatory pain in mice using the voluntary behaviour of wheel running. The authors note the frequent use, and potential inaccuracy, of reflexive measures of pain, therefore favouring models that use voluntary behaviours which could provide a more accurate measure of the animals' pain-related discomfort. They performed bilateral complete Freund's Adjunct (CFA) injections, which involves injecting CFA into the intraplantar surface of the hind paw, resulting in chronic inflammation and pain. The researchers then allowed these mice free access to a wheel for a period of time in order to assess if inflammatory chronic pain caused a reduction in voluntary wheel running behaviour – a behaviour typical in healthy mice.

This study found that inflammatory pain did significantly reduce the distance ran by injured mice. This effect, however, only lasted approximately three days and therefore is likely not a measure of chronic inflammatory pain, and suggests a potential issue in using CFA to induce inflammatory chronic pain. In contrast, mechanical sensitivity (a reflexive measure) was also recorded after injection, and hypersensitivity was still found up to seven days following injury. As the voluntary activity was not hindered by this apparent hypersensitivity, the authors suggest that traditional reflexive measures are not accurate measures of the experience of inflammatory pain.

Article Contribution:

I included this article because it provides even more evidence that reflexive behaviours cannot and should not be used to measure mice's experience of chronic pain. It appears that hypersensitivity can persist even after the functional limitations of pain have ceased. This result is supported by previous literature, and contributes to the field by focusing solely on a strongly maintained voluntary behaviour typically seen in laboratory mice. As such, it provides voluntary

behavioural measure for chronic pain to be used in future studies, despite that it may be more laborious to record than simple reflexive measures.

Citation:

Guo, W., Zou, S., Mohammad, Z., Wang, S., Yang, J., Li, H., Dubner, R., Wei, F., Chung, M., Ro, J. Y., & Ren, K. (2019). Voluntary biting behavior as a functional measure of orofacial pain in mice. *Physiology & Behavior*, *204*, 129-139. https://doi.org/10.1016/j.physbeh.2019.02.024

Article Summary:

This study investigated biting behaviour in mice with tendon ligation (TL) of the masseter muscle, which involves cutting the tendon and fixing the cut ends together, and results in prolonged pain of the facial muscles. Previous studies suggest that voluntary behaviours may be most indicative of chronic pain, but few have investigated bite force as one of these measures. The TL method was employed to test its efficacy over complete Freund's adjunct (CFA), which is commonly used in chronic pain studies and involves injecting CFA into the area of interest to induce inflammatory pain. Compared to measures of hypersensitivity, such as avoidance of mechanical stimulation of an injury, a change in bite force associated with TL was shorter than expected. In other words, reflexive behaviours that indicate hypersensitivity persist much longer than voluntary behaviours, such as biting, suggesting they may not actually be indicative of chronic pain. This suggests that immediate change in bite force does indicate myofascial pain but may not be useful as a long-term measure of chronic pain.

The researchers also looked at conditioned place avoidance behaviour using a chamber with one brightly lit side and one dark side in which mice are subject to mechanical stimulation of the injured side of the face. They found that mice spent significantly more time in the light (aversive) chamber to avoid mechanical stimulation of their injury. This result indicates awareness of injury and sensitivity, but not necessarily spontaneous (un-evoked) pain. They also measured grimacing, which is a typical reflexive pain-related behaviour. The grimace scale showed that injured mice grimaced significantly more, but this behaviour is still reflexive in nature rather than voluntary, unlike biting. As such, the authors recommend using voluntary

measures of chronic pain, which verifies that the pain induction method did in fact induce chronic pain.

Article Contribution:

I included this article because this was the only study I came across that attempted to assess chronic pain in an area of the body aside from a limb. Further, tendon ligation is a different method of inducing pain compared to many studies which use CFA, although it did not appear to actually induce myofascial chronic pain. Overall, this study contributes to the literature by investigating a new area of the body, and also an alternative method of pain-induction.

Citation:

Kurejova, M., Nattenmüller, U., Hildebrandt, U., Selvaraj, D., Stösser, S., & Kuner, R. (2010). An improved behavioural assay demonstrates that ultrasound vocalizations constitute a reliable indicator of chronic cancer pain and neuropathic pain. *Molecular Pain, 6*(18), 1 -7. https://doi.org/10.1186%2F1744-8069-6-18

Article Summary:

This article focused on finding a reliable way to measure ultrasound vocalizations (USVs) as an indication of neuropathic and tumor-induced chronic pain in mice. Previously, it has been difficult to study chronic pain in animal models due to lack of a reliable measurement, and previous use of the USV indications have contained many confounding variables, such as mouse-mouse communication and stress due to restraint. This study induced neuropathic pain by severing two of the three branches of the sciatic nerve (spared nerve injury, SNI), and tumor-related pain by injecting sarcoma (cancerous) cells into the heel, inducing bone metastases. They measured USVs at 37 and 50 kHz, which have been previously shown to correlate with experience of acute pain in mice. The researchers accounted for previously encountered confounds by developing a custom plexiglass chamber with ultrasound detectors, and allowing the mice to acclimatize and freely move within the chamber. The study found that, when confounding variables were sufficiently accounted for, USVs were a reliable indication of both neuropathic and tumor-induced chronic pain in mice. This result was further confirmed by treating the mice with fentanyl or gabapentin (drugs used to treat chronic pain), during which the frequencies of USVs declined significantly. This article therefore suggests that USVs are a

reliable behavioural indication of chronic pain in mice. The paper also indicates that other behaviours such as licking and shaking could be related to “disease-induced hypersensitivity”, but it is unclear if this is referring to the experience of chronic pain. These behaviours are not discussed further, and thus it is important to explore other chronic pain-related behavioural indications in mice.

Article Contribution:

I included this article because it includes evidence that ultrasound vocalizations are observed with chronic pain in mice. A reliable behavioural assay had not been found, so this research advanced the study of mouse chronic pain by creating a procedure for measuring this behavioural indication of chronic pain. This study is contradicted by some previous literature, but this discrepancy is likely related to confounding variables in previous studies. The discovery of a reliable behavioural assay is informative in the study of chronic pain, and the results suggest that assays including other pain-related behaviours may be possible under highly controlled conditions.

Citation:

Shepherd, A. J. & Mohapatra, D. P. (2018). Pharmacological validation of voluntary gait and mechanical sensitivity assays associated with inflammatory and neuropathic pain in mice. *Neuropharmacology, 130,* 18-29. https://doi.org/10.1016/j.neuropharm.2017.11.036

Article Summary:

This study aimed to highlight the field’s overreliance on reflexive pain behaviours and suggest an alternative behavioural assay indicative of chronic pain. This was with the goal of better producing mouse models of neuropathic and inflammatory pain, to apply clinical interventions to humans with chronic pain. Authors used spared nerve injury (SNI) by cutting two of the three branches of the sciatic nerve to induce neuropathic pain, and complete Freund’s adjunct (CFA) injection to the intraplantar surface of the hind paw to induce inflammatory pain. Following injury, gait changes, mechanical and temperature hypersensitivity, and mechanical conflict-avoidance were assessed. To confirm measurement of pain behaviours, analgesics (pain mediating drugs) were given to make sure that behaviours attenuated upon drug administration.

Some gait indices changed in response to pain, but they did not reverse upon analgesic administration, suggesting that these changes may be related biomechanical changes in the animal rather than pain-induced behaviours. To verify analgesic efficacy, drugs were administered prior to validated mechanical and temperature hypersensitivity assays, and pain-related behaviours attenuated as expected. A custom mechanical conflict avoidance test was proposed as a new measure of chronic pain. To avoid an aversive bright light, mice were made to cross a raised probe-covered plate between themselves and a darkened region. They found that SNI and CFA animals took significantly longer to cross the probed plate than controls (therefore staying in the brightly lit region longer), suggesting voluntary pain-associated place avoidance. This confirms previous research suggesting that behavioural measures more accurately measure chronic pain, compared to reflexive measures which simply demonstrate hypersensitivity to aversive stimuli following injury.

Article Contribution:

I included this article because it proposes a new way of measuring a chronic pain-related behaivour in mice. Although place and stimulus avoidance have been previously used to measure chronic pain, this study highlights limitations in previous methods and proposed a new, highly controlled way to measure avoidance of aversive stimuli. As such, it differs from the previous literature by demonstrating voluntary measures can be used to measure chronic pain under highly controlled conditions and contributes to the field by suggesting new ways we can measure a voluntary pain-related behaviour.

Demonstrating Proximate and/or Ultimate Influences

Citation:

Lister, K. C., Bouchard, S. M., Markova, T., Aternali, A., Denecli, P., Pimentel, S. D., Majeed, M., Austin, J., Williams, A., & Mogil, J. S. (2020). Chronic pain produces hypervigilance to predator odor in mice. *Current Biology, 30*, 866-867. https://doi.org/10.1016/j.cub.2020.06.025

Article Summary:

This article presented the hypothesis recently proposed by other pain researchers that chronic pain represents an adaptive function in squid and crustaceans. The current authors indicated that acute (short-lived) pain has been previously noted as adaptive in that it promotes escape from the aversive stimulus and prevents against further tissue damage. However, the adaptive significance of chronic pain has been historically rejected, and it has been seen as an oversensitive response to injury after the injury has already healed. In response to recent research suggesting that the experience of chronic pain reduced predation risk in squid, this study aimed to examine this phenomenon in mice.

Experimental mice underwent spared nerve injury (SNI) which involves severing two of the three branches of the sciatic nerve to produce neuropathic pain, or pain due to nerve damage. Mice were then introduced to an octagonal O-maze by which they could access a food reward through a “long route” or a “short route”. The short route was infused with the odor of fox urine to see if SNI mice would avoid the short route (and thus a potential predator) more often than control mice. As suspected, mice with chronic pain chose the long route significantly more often than control mice, suggesting chronic pain confers an adaptive response to predation risk in the form of hypervigilance. The mechanism of this adaptive significance is suggested to be that chronic pain continually reminds the animal of its risk of predation, leading it to take additional measures in the avoidance of danger (hypervigilance).

Article Contribution:

I included this article because I think it strongly demonstrates an ultimate influence on chronic-pain related behaviours, because predator avoidance based on increased predation risk has been influenced over millennia through natural selection. It adds to the field by exploring voluntary behaviour in a unique model which allows us to extrapolate to mice in the wild, as opposed to simply laboratory mice. Additionally, it focused on the behaviour of vigilance, which is measured in few other studies because of its complexity and that it is laborious to measure.

Citation:

Millecamps, M., Shi, X. Q., Piltonen, M., Echeverry, S., Diatchenko, L., Zhang, J., & Stone, L. S. (2020). The geriatric pain experience in mice: Intact cutaneous thresholds but altered

responses to tonic and chronic pain. *Neurobiology of Aging*, *89*, 1-11. https://doi.org/10.1016/j.neurobiolaging.2019.12.018

Article Summary:

This study sought to investigate differences in behavioural response to pain in young versus geriatric mice, which has been historically under investigated in pain research. The authors hypothesized that older animals have an altered experience of pain compared to young, injured animals, suggesting an important influence of age on the experience of chronic pain.

Experimental young and geriatric mice underwent partial sciatic nerve ligation (PSNL) where part of the sciatic nerve is cut at thigh level to produce neuropathic pain. They were then exposed to a variety of aversive temperature and mechanical stimuli and tonic painful stimuli (acetone and capsaicin exposure). Behavioural pain-related responses were measured in two different place avoidance tests which investigate aversion to light, and either mechanical pain or heat. It was found that older mice had typical but prolonged behavioural reactions to both the acetone (cold) and capsaicin (hot) tonic pain stimuli when compared to younger mice. Conversely, geriatric mice appear to lack avoidance behaviours after PSNL, behaving significantly more like their control counterparts than younger PSNL mice did in response to acetone application. The authors suggest that there is a cognitive impairment of the frontal cortex in older animals, leading them to demonstrate hyperreactivity to tonic stimuli, but hyporeactivity to acetone after PSNL. In other words, it does not seem to be a lack of sensory perception driving this behavioural response, but a lack of stimulus avoidance due to cognitive deficit in old age. Overall, the authors suggest that there are age-related difference in behavioural patterns to chronic pain, which should be taken into account in future chronic pain studies in mice, and other animals.

Article Contribution:

I included this article because it investigated a less-frequently researched variable in mouse chronic pain. Due to the high variability in results in mouse chronic pain studies, and the debate over reflexive versus voluntary behaviours, this is one of few studies that investigated age as an extremely important influencer of the experience of pain. It also indicates an influence on chronic pain-related behaviours that is somewhere between proximate and ultimate, lasting the

animal's lifetime. Simultaneously, age could be considered a proximate influence, as it is the aging of individual cells in the prefrontal cortex that is directly affecting behaviour.

Citation:

Piardi, L. N., Pagliusi, M., Bonet, I. J. M., Brandão, A. F., Magalhães, S. F., Zanelatto, F. B., Tambeli, C. H., Parada, C. A., & Sartori, C. R. (2020). Social stress as a trigger for depressive-like behavior and persistent hyperalgesia in mice: Study of the comorbidity between depression and chronic pain. *Journal of Affective Disorders, 274*, 759-767. https://doi.org/10.1016/j.jad.2020.05.144

Article Summary:

This study investigated the reciprocal relationship between social defeat stress (SDS, depressive symptoms related to negative social interactions) and chronic pain based on the observation that people often experience both chronic pain and major depressive disorders in clinical studies. As such, the researchers aimed to investigate chronic pain-related hypersensitivity to an electrical stimulus using various protocols to induce mild or chronic pain, and mild or chronic SDS in mice.

A mild seven-day protocol of prostaglandin E2 (PGE_2) injection to the intraplantar surface of the hind paw was used to induce predisposition to chronic pain, but was known not to be sufficient to induce chronic pain alone. PGE_2 was used because it is known to be produced in response to injury and therefore promotes inflammation, and thus when injected causes inflammation and pain. On the other hand, a persistent 14-day protocol of PGE_2 injection was used to induce persistent hyperalgesia (chronic pain). Mice were also exposed to a social defeat stress (SDS) due to prolonged exposure to a particularly aggressive mouse of another species, or subthreshold social defeat stress (SSDS) protocol in order to examine the relatedness between social avoidance behaviour and the experience of chronic pain.

Mice that underwent the SDS protocol and subsequently underwent the mild PGE_2 protocol were found to show symptoms of chronic hyperalgesia (lasting at least two weeks), despite the mild PGE_2 protocol being unable to induce this same effect alone. Similarly, mice that underwent the persistent hyperalgesia protocol and subsequently underwent the SSDS protocol were found to be significantly more social avoidant, although the SSDS protocol is

unable to induce social avoidance alone. These results demonstrate that social defeat stress and hypersensitivity are complexly interrelated and can predispose mice to either disorder reciprocally.

Article Contribution:

I chose to include this article because this was the only study I came across which used the PGE_2 injection protocol to induce chronic pain, and because socialization is an important influence on the subjective experience of chronic pain which seems under-researched in this area. It contributes to the literature in that it indicates a reciprocal relationship between social stress and chronic pain, rather than only measuring a one way relationship. Finally, socialization is an influence of chronic pain that is relatively proximate temporally, but simultaneously influenced through natural selection.