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Abstract

The importance of the modulation of pain by emotion is now widely recognised. In particular, stress and anxiety, depending on their nature, duration and intensity, can exert potent, but complex, modulatory influences typified by either a reduction or exacerbation of the pain state. Exposure to either acute or chronic stress can increase pain responding under experimental conditions and exacerbate clinical pain disorders. There is evidence that exposure to chronic or repeated stress can produce maladaptive neurobiological changes in pathways associated with pain processing, resulting in stress-induced hyperalgesia (SIH). Preclinical studies of SIH are essential for our understanding of the mechanisms underpinning stress-related pain syndromes and for the identification of neural pathways and substrates, and the development of novel therapeutic agents for their clinical management. In this review, we describe clinical and pre-clinical models used to study SIH and discuss the neural substrates, neurotransmitters and neuromodulatory systems involved in this phenomenon.

Key words: Pain, Stress, Human, Rodent, Anxiety, comorbidity, Brain

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1 Introduction

The last couple of decades have witnessed an emerging and sustained interest among research scientists and clinicians in understanding the interactions between stress and pain. This interest has been driven by overwhelming clinical and preclinical evidence demonstrating complex and potent effects of stress on pain processing and responding. Critical scrutiny of these data suggests that the nature, duration and intensity of the stressor are key determinants of the effects of stress on pain. Numerous studies have demonstrated that exposure to an acute, robust, intense stress induces a reduction in pain responding, a phenomenon described as stress-induced analgesia (for review see Butler and Finn, 2009).

On the other hand, repeated or chronic exposure to physical or psychological stressors which may be anticipatory/anxiogenic in nature, typically results in the less well understood phenomenon of stress-induced hyperalgesia (SIH) in humans (Gibbons et al. 2012; Kuehl et al. 2010), and rodents (Andre et al. 2005; Bardin et al. 2009; Dina et al. 2011; Khasar et al. 2009; Kuehl et al. 2010; Le Roy et al. 2011; Quintero et al. 2011). Furthermore, it is widely documented that stress exacerbates existing pain associated with chronic pain disorders. Thus, stress is an important etiological factor for chronic pain disorders such as shoulder/neck pain syndrome, complex regional pain syndrome and fibromyalgia (Van Houdenhove and Luyten, 2006; Davis et al. 2011; Nilsen et al. 2007; Grande et al. 2004). These findings lend credence to the concept of SIH as a clinically relevant phenomenon, manifesting in a diverse array of stress-related pain disorders including inflammatory bowel disease, fibromyalgia and complex regional pain syndrome (Aaron and Buchwald, 2003; Egle and van Houdenhove, 2006; Grande et al. 2004; Walker et al. 2012). However, fewer studies have investigated the neurobiological mechanisms underpinning SIH.

A particular area of clinical interest within the context of stress-pain interactions is the comorbidity between chronic pain and affective disorders. The findings from numerous studies indicate a very high prevalence of comorbid chronic pain and psychiatric disorders such as anxiety and depression, (Asmundson and Katz, 2009; Bair et al. 2003). Chronic pain patients are more likely to present with depression (21.7% vs. 10.0%) or anxiety disorders (35.1% vs. 18.1 %) when compared to the general population (McWilliams et al. 2003). The prevalence of clinical anxiety among the population with chronic pain may be as high as 60%, with generalised anxiety disorder being the anxiety disorder that is most prevalent with chronic pain (Fishbain, 1986). The co-occurrence of anxiety and/or depression with chronic pain amplifies the negative effects of each alone, often complicating the treatment and resulting in poor outcome (Asmundson and Katz, 2009; Bair et al. 2003; Lieb et al. 2007). The relationship between altered emotional states and chronic pain disorders is complex and it is difficult to establish whether chronic pain leads to altered emotional states or whether the affective disorder predisposes an individual to the development of a pain disorder. For example, it has been reported that 77% of subjects who met criteria for generalised anxiety disorder comorbid with a pain condition, developed the disorder before the onset of chronic pain (Knaster et al. 2011). Taken together, these findings suggest a reciprocal relationship between chronic pain and affective disorders and support the concept of a self-perpetuating cycle of events that may underpin the chronic nature of such comorbid disorders.

In the present manuscript, we aim to provide a comprehensive review and critical analysis of the current understanding of SIH at pre-clinical and clinical levels. The neuroanatomical alterations associated with SIH, as revealed by both studies in human subjects and animal models will be discussed, as will the role of various neurotransmitter and neuromodulatory systems, including the opioidergic, gamma-aminobutyric acid (GABA)ergic, glutamatergic,

monoaminergic and endocannabinoid systems and the hypothalamo-pituitary-adrenal (HPA) axis.

2 Clinical and pre-clinical models of SIH

2.1 Human models

The high prevalence of comorbid psychiatric and chronic pain states, and the reciprocal relationship which they share, suggests the involvement of common neural substrates and mechanisms in the modulation of pain and emotional states. In addition, the current use of drugs such as pregabalin, amitriptyline and duloxetine for the treatment of both pain and anxiety/depression further illustrates the close associations that exist between pain and affective disorders. Increased understanding of common neural substrates and mechanisms is not only important from a fundamental physiological perspective, but may be of potential therapeutic significance. In pursuit of this, a relatively small number of human models employing either psychological or physiological stressors have been developed for studying SIH.

2.1.1 Psychological stress-based models

In general, psychological stress-based models of SIH usually involve assessment of pain responding during or following exposure to a conditioned or unconditioned aversive stimulus. These paradigms can evoke a state of anticipatory anxiety in the subject which influences the perception of pain upon subsequent challenge with a painful stimulus. Such paradigms have been used to evaluate the effects of stress on pain in healthy individuals as well as those with pre-existing diagnosis of a psychiatric disorder. One such example is an event-related factorial pain paradigm where painful and non-painful heat stimuli were applied to the left volar forearm of subjects while different colour shapes were explicitly used to signal the intensity of the upcoming stimulus (Strigo et al. 2008). Using measures of blood oxygen level-dependent functional magnetic resonance neuroimaging, Strigo and colleagues reported

that unmedicated individuals with a history of major depressive disorder showed increased activity in brain regions associated with pain and emotional modulation, in particular the right amygdala, during anticipation of painful stimuli, compared with healthy controls. The authors conclude that increased emotional reactivity during the anticipation of heat pain may lead to an impaired ability to modulate pain experience in individuals with major depressive disorder (Strigo et al. 2008). It is, however, worth noting that the majority of the subjects in this study were females and while the statistical analysis suggested that there were no gender-related differences between groups, the extent to which differences in natural pain perception between males and females contributed or influenced the outcome of these findings was not clear. In similar experiments relating to anticipation, the threat of electric shock without presentation resulted in decreased pain thresholds to radiant heat (Rhudy and Meagher, 2000). In other experiments, pre-exposure to experimental psychological stress (stress interview and serial subtraction task) was shown to lower pain tolerance in the cold pressor test in children with recurrent abdominal pain (Dufton et al. 2008). Interestingly, the authors of this study did not find any correlation between pain tolerance and physiological reactivity (heart rate) to the stressor, perhaps suggesting that the effects on pain tolerance were related to the psychological, rather than to the physical, components of the stressor. It is, however, important to note that a stimulatory effect of psychological (mental arithmetic performance) stress-induced physiological activity on pain sensitivity has been demonstrated (Caceres and Burns, 1997). The discrepancies between these two studies in relation to the effects of stress-induced physiological activity, must however be viewed within the context of differences in the age of the subjects studied and the stress paradigms employed. More recently, Crettaz and colleagues reported enhanced pain sensitivity to thermal stimuli in female healthy subjects and patients with fibromyalgia following exposure to a psychological stressor based on a standardised psychosocial stress test paradigm (Crettaz et al. 2013). Interestingly enhanced

pain sensitivity in response to pressure pain stimuli was only observed in fibromyalgia patients, but not healthy subjects in this study. The authors conclude that different underlying mechanisms may account for the response to stressors in healthy subjects and patients suffering from chronic pain. Further studies aimed at identifying the neurochemical substrates involved may be necessary to help elucidate the underlying mechanisms involved. Despite differences in the methods used, the overall findings in these studies unanimously depict the negative impact of psychological stressors on pain thresholds in human subjects.

2.1.2 Physiological stress-based models

In parallel with the psychological stress-based models, a number of physiological stress-based paradigms have been developed. These models employ specific physiological stressors to disrupt or interfere with homeostasis. Examples of these models include metyrapone-induced hypocortisolism (a neuroendocrinological correlate of chronic stress), which has been shown to significantly decrease mechanical pain sensitivity thresholds in healthy volunteers (Kuehl et al. 2010). The authors of this study analysed the effects of metyrapone-induced hypocortisolism on quantitatively assessed basal mechanical pain sensitivity, perceptual wind-up and temporal summation of pain elicited by inter-digital web pinching. Results from this study suggest a significant correlation between experimentally-induced reductions in cortisol levels and enhanced temporal summation of inter-digital web pinching-induced pain and perceptual wind-up. These findings suggest that disturbances in HPA axis may contribute to stress-related pain disorders. In another study, Gibbons et al. (2012) report that the induction of a hypoglycaemic state was associated with alterations in stress hormones, release of cytokines and enhanced pain sensitivity to hot, cold and repeated thermal pain stimuli in human subjects. The likely involvement of multiple signalling systems, as demonstrated in this study, highlights the complexity of the mechanisms

underpinning the effects of stress on pain responding, and importantly, presents an opportunity for the identification of multiple therapeutic targets for treating comorbid stress-related affective and pain disorders.

In summary, most human models used to study SIH involve exposure to psychological or physiological stressors prior to, or during assessment of responsivity to noxious stimuli. Further experimentation with these models may advance our understanding of the mechanisms underpinning SIH in humans. However, the usefulness of these models may be limited by a number of confounding factors such as individual differences in pain perception thresholds and subjectivity in the reporting of pain. In addition to these considerations, ethical requirements to provide full disclosure and assure the subject of their safety could skew the data generated using these models.

2.2 Animal models of SIH

In contrast to the relatively few human models that exist, a relatively large number of preclinical animal models have been employed for the study of SIH. These models mainly involve the repeated or persistent application of a stressor (psychological and/or physical) for several days to weeks, in combination with a test for pain responding (see Table 1). Detailed descriptions of the effect of different stressor paradigms on nociceptive responding are provided in the following sections. In the main, these rodent models offer a greater scope than the human models to study the impact of different types of stressors on pain responding and the underlying neural substrates and neurobiological mechanisms involved.

2.2.1 Forced Swim Stress

The forced swim test was originally developed as a model for screening antidepressant-like drugs (Porsolt et al. 1978). Exposure to forced swim stress (FSS) has been shown to induce neurochemical changes in several brain regions including the prefrontal cortex (PFC) and amygdala, key regions involved in the emotionality of pain (Connor et al. 1997). Quintero and colleagues have demonstrated inflammatory (formalin test) and thermal (hot plate test) hyperalgesia in rats exposed to repeated swim-stress (Quintero et al. 2000; Suarez-Roca et al. 2006a; Suarez-Roca et al. 2013**),** confirming FSS as a useful model for studying SIH. Their FSS paradigm typically involves placing rats in a cylinder 30cm in diameter - 50cm in height, filled with water to a height of 20cm, for a period of 10 minutes on the first day, and 20-minute duration on the subsequent two days. This is followed by a behavioural assay for pain responding 24 or 48 hours after the last swim. FSS induced thermal hyperalgesia has been associated with increased inflammatory mediators in the spinal cord. This hyperalgesia was prevented by pre-treatment with the anti-inflammatory agents ketoconazole or minocycline (Suarez-Roca et al. 2013). Although Quintero and colleagues have reported that FSS of this nature results in increased thermal and mechanical nociceptive responding in the hotplate and grip strength tests, studies in our laboratory and others (Imbe et al. 2010) failed to observe an effect of FSS in these tests. Inflammatory hyperalgesia in the formalin test has been observed 48 hours after the last swim exposure (Quintero et al. 2000, Imbe et al. 2010). FSS-induced enhancement of inflammatory pain responding has been shown to be inhibited by lesioning of the rostral ventromedial medulla (RVM) using the neurotoxin ibotenic acid, suggesting a role for the descending inhibitory pain pathway in SIH (Imbe et al. 2010). Another study employing swim stress in cold or warm water over 5, 15 or 30 minutes or repeated daily for two weeks in mice, found that cold swim stress reduced grip strength and tail flick latency (TFL) (Abdelhamid et al. 2013). However, it is possible that forced swimming may have

tired the mice and thus affected grip strength. Discrepancies in the effects of the FSS on pain behaviour may be attributed to slight differences in methodological approaches or source/batch of animals. The strength of the FSS model lies in the fact that it represents an innocuous and sub-chronic stress paradigm. However, inconsistencies in reports of the extent of hyperalgesia produced with respect to thermal and mechanical hyperalgesia, differences in rating of inflammatory pain scores and the relatively limited number of studies using this approach to study SIH mechanisms, mean that further investigation and validation of FSS as a model of SIH is warranted.

2.2.2 Repeated cold stress

The repeated cold stress or "specific alternation rhythm of temperature" (SART) model adapted for studying SIH, involves alternate exposure of the animal to normal room temperature and a low temperature of approximately 4°C (Fujisawa et al. 2008; Omiya et al. 2000). SART induces anxiety- and depression-like behaviour in mice in the elevated plus maze (Hata et al. 2001) and forced swim test (Hata et al. 1999), respectively. In addition, this protocol has been shown to dysregulate the HPA axis and induces alterations in the monaminergic system in various brain regions and in the spinal cord (Jaggi et al. 2011; Kawanishi et al. 1997; Hata et al. 1991). Importantly, SART has been shown to decrease the mechano-nociceptive threshold in the Randall–Selitto test and in the von Frey test for both rats and mice (Fujisawa et al. 2008; Nasu et al. 2010; Ohara et al. 1991; Omiya et al. 2000). Moreover, reducing the room temperature to -3°C, instead of the more commonly employed 4°C, produces a longer lasting mechanical hyperalgesia (Nasu et al. 2010), suggesting a direct correlation between the temperature (stressor) and the level of hyperalgesia. Kawanishi and colleagues observed no increase in C-fibre activity following SART, suggesting that SART-induced hyperalgesia is centrally mediated (Kawanishi et al. 1997). It must be noted,

however, that SART stress can affect multiple physiological systems including immune parameters (Hori et al. 1993) and blood flow changes (Hata et al. 1986) which can also modulate pain responding (Austin and Moalem-Taylor, 2010). Thus, the mechnisms involved in SART-induced pain alterations may extend beyond alterations in the nervous system.

2.2.3 Restraint/ Immobilisation stress

Restraint/immobilisation stress is used widely in order to investigate stress-induced changes in neuronal function (Chiba et al. 2012; Gadek-Michalska et al. 2012). Restraint stress paradigms typically involve placing the animal in a well ventilated tube or cage, which restricts but does not completely prevent movement (Jaggi et al. 2011; Bardin et al. 2009b; da Silva Torres et al. 2003; Gameiro et al. 2005). Assessing the effect of this paradigm on nociceptive responding has revealed that animals exposed to restraint for 1 hour every 5 days over a 5-11 week period demonstrate enhanced mechanical, thermal and inflammatory pain responses (Bardin et al. 2009b; da Silva Torres et al. 2003; Gameiro et al. 2005; Spezia Adachi et al. 2012). In addition to these reports of hyperalgesic effects of restraint following a 40-day restraint protocol, da Silva Torres and colleagues reported that a 12-day restraint protocol resulted in a prolonged hyperalgesic response in the tail-flick test that was present 28 days after the last restraint session (da Silva Torres et al. 2003). Furthermore 2 weeks of restraint stress augmented spinal nerve ligation-induced mechanical allodynia, as measured by von Frey test in mice. Treatment prior to restraint stress with metyrapone, a corticosteroid synthesis inhibitor, prevented the enhancement of mechanical allodynia in spinal nerve ligated mice (Norman et al. 2010). More recently, either a single 2- hour restraint session or 2 hour restraint per day over a 4-day period were shown to induce visceral hyperalgesia in rats as measured by colorectal distension (Eutamene et al. 2010; Ohashi-Doi et al. 2010; Shen et

al. 2010). Thus, while visceral hyperalgesia may be induced following exposure to acute or short-term restraint stress, hyperalgesia to somatic pain may require repeated exposure to restrains stress over a much longer duration.

A variant of the restraint stress paradigm involves complete immobilisation of the animal by restriction of limb and head movements of the rodent representing a more intense form of stressor. The duration of immobilisation sessions varies between studies ranging from 90 minutes to 6 hours at a time (Costa et al. 2005; Kumar et al. 2009), and is generally performed for up to 7-10 days. 2 hour immobilisation for 10 days results in anxiety-like behaviour in mice (Qin et al. 2011). Repeated immobilisation of mice results in a reduction in TFL (Costa et al. 2005) and inflammatory hyperalgesia in the formalin test (Seo et al. 2006). Further studies are required in order to determine if immobilisation is a useful model for studying SIH.

2.2.4 Social defeat stress

This model of stress relies on the animal's innate defensive behaviour to induce anxiety (Marcinkiewcz et al. 2009). The model involves the introduction of a smaller intruder rat (stressed) into the cage of the larger resident rat. The procedure is carried out in two phases: in the first phase, the intruder is placed in a protector cage whilst allowing for visual, auditory and olfactory, but not physical contact with the resident rat. In phase II, the protective cage is removed, to facilitate interaction between the intruder and resident animal. During this second phase, 3–4 confrontations of 10 seconds duration are permitted, during which the intruding animal is dominated by the resident rat. This process is most commonly repeated over a three- to four-day period. Assessing nociceptive responding following this type of stress paradigm has revealed enhanced inflammatory and mechanical nociceptive behaviours

in Sprague-Dawley rats assessed using the formalin test and von Frey tests, respectively (Rivat et al. 2010; Andre et al. 2005). Furthermore, in Long Evans rats, thermal sensitivity was increased following social defeat stress in the thermal preference and thermal escape tests (Marcinkiewcz et al. 2009). The potential risk of injury and pain to either animal through physical confrontation associated with this model makes it a little less attractive as a model of SIH. Nonetheless, the broad spectrum of effects on inflammatory, thermal and mechanical hyperalgesia over a relativiely short time-frame mean that the social defeat paradigm is a useful model of SIH.

2.2.5 Water avoidance stress

The water avoidance stress (WAS) paradigm is a well-characterised model for human irritable bowel syndrome (IBS), recapitulating various features associated with the disease (Bradesi et al. 2005; Chen et al. 2011; Hong et al. 2009; Myers et al. 2007; Wang et al. 2013) 2007). In the WAS model, the healthy rodent is placed on a platform (8cm length X 8cm width X 10cm height) mounted in a plexiglass tank (45cm length_X 25cm width_X 25cm height: for rats) which is filled with water up to 1cm below the height of the platform. The rodent is left on the platform for 1hour every day for 10 consecutive days (Bradesi et al. 2006; Chen et al. 2011; Larauche et al. 2010). Acute (1hour) WAS has been demonstrated to produces transient visceral hyperalgesia (Bradesi et al. 2006; Chen et al. 2011; Larauche et al. 2010). In comparison, persistent visceral hyperalgesia and enhanced mechanical nociceptive behaviour in the von Frey test has been demonstrated following chronic 10 day WAS (Chen et al. 2011). The effects of acute WAS appear to be specific for visceral nociception and chronic exposure to 10 days of WAS is required to modulate mechanical nociception.

2.2.6 Chronic Mild Stress

Chronic mild stress (CMS) has been proposed as a clinically relevant model of depression (Willner, 1997; Willner, 2005). Typically, the paradigm involves exposing the animal to unpredictable stressors of varying type and duration over a 5-9 week period. This regimen prevents habituation of the animal to the stressor. The CMS paradigm is thought to be relevant to the range, diversity and chronic nature of life stressors that humans experience (Willner, 1997). CMS induces anhedonia, assessed using the sucrose preference test, which is amenable to treatment with tricyclic antidepressants (Willner et al. 1987) or selective serotonin receptor uptake inhibitors (SSRIs) (Mutlu et al. 2012). Recently, the effect of CMS on nociceptive responding has been employed to model SIH. Shi and colleagues found that rats exposed to unpredictable CMS rats displayed increased inflammatory pain behaviours as assessed in the formalin test. Treatment with the SSRI fluoxetine, reversed CMS-induced increases in inflammatory pain behaviours (Shi et al. 2010a; Shi et al. 2010b). However in comparison they also found that rats exposed to CMS had an increased threshold to evoked thermal and mechanical stimuli in the hot plate and von Frey tests respectively, under both normal and complete Freund's adjuvant (CFA)-induced chronic pain conditions (Shi et al. 2010b), suggesting that this model has divergent effects on spontaneous and evoked pain responding. Examination of the effect of CMS on neuropathic pain-related behaviour revealed that rats exposed to CMS after spinal nerve ligation (SNL) again exhibited increased thermal and mechanical pain thresholds in comparison to the non-stressed SNL animals, as measured by hot plate and von Frey tests. It should be noted that the mechanical allodynia induced by SNL was restored to control levels over the six weeks of CMS (Shi et al. 2010a). Therefore although this model is relevant to the chronic nature of human stressors, it is associated with analgesic response to evoked pain and hyperalgesic responding was only observed in the formalin test.

2.2.7 Maternal Separation/deprivation/early life stress

Maternal separation (MS) involves neonatal separation on post-natal days (PND) 2-14, for 3 hours a day, and is associated with visceral hyperalgesia in rats when measured using colorectal distension (Chung et al. 2007a; Wouters et al. 2012; O'Mahony et al. 2010). This model of stress-induced visceral hyperalgesia has also been shown to be associated with increased activation (Zhang et al. 2009b) and plasticity (Chung et al. 2007a; Chung et al. 2007b) of neurons in the dorsal root ganglia (DRG) in the lumbosacral section of the spinal cord. Furthermore, MS rats subjected to colorectal distension displayed significant increases in regional blood flow in the cerebellum and in particular, the periaquaductal gray (PAG), which is a key component of the descending inhibitory pain pathway (Wouters et al. 2012). In addition, MS rats exposed to 1 hour of water avoidance in adult life displayed a further augmentation in visceral sensitivity (van den Wijngaard et al. 2012; Wouters et al. 2012).

In comparison to maternal separation, maternal deprivation (MD) involves a single prolonged separation of 24 hours before PND 14. Recent data have demonstrated that MD of male and females rat pups at PND 9 resulted in thermal and mechanical allodyina as tested by hot plate test and von Frey respectively, of MD females in adulthood compared to non-MD counterparts (Burke et al. 2013). Furthermore, following spinal nerve ligation, a model of peripheral nerve injury and neuropathic pain, MD female, but not male, rats exhibit exacerbated mechanical and cold allodynia of the ipsilateral hind paw post-SNL (Burke et al. 2013). This study is interesting in two aspects, not only does it further demonstrate that early life stress can result in enhanced nociceptive and neuropathic pain responding in adult life but it also illustrates sexually dimorphic effects of early-life stress on pain responding.

An early life stress model which does not separate the pups with their mother is the neonatal limited bedding stress model. Mother rats and their pups are placed in cages fitted with a

stainless steel mesh bottom on post-natal day 2 to 9. Adult rats exposed to neonatal limited bedding as pups display mechanical hyperalgesia as measured by a digital force transducer (Alvarez et al. 2013; Green et al. 2011). This mechanical hyperalgesia is exacerbated by sound stress in adult rats (Alvarez et al. 2013). This further supports the evidence that early-life stress can affect stress-coping mechanisms and pain responding in adult life.

2.2.8 Noise, Vibration, Rotation, Air and Whisker pad stimulation stress and PTSD model

Noise stress typically involves exposing rodents to 105 dB tone over several different frequencies ranging from 11 to 19 kHz, each lasting for 5 to 10 seconds within a 30 minute period, over 2 to 4 days (Khasar et al. 2009; Khasar et al. 2005). This type of stress has been shown to enhance both inflammatory and mechanical hyperalgesia in rats, as assessed with bradykinnin-induced nociceptive test and algesimeter respectively (Strausbaugh et al. 2003; Green et al. 2011). Increased plasma corticosterone levels have been reported following noise stress (Babb et al. 2013; Khasar et al. 2009) and noise stress-induced mechanical hyperalgesia has been reported to be prevented by adrenal medullectomy (Khasar et al. 2009), and restored upon intraperitoneal administration of stress-related levels of adrenaline (Khasar et al. 2009). While this paradigm appears to model several features seen in other SIH models, the underlying mechanisms, specifically changes in the sympatho-adrenal axis observed following this stressor, mean that it has been proposed as a model of fibromyalgia.

Vibration has been proposed as an acute mild stressor and the effects of vibration stress on thermal nociceptive responding have been investigated. This paradigm involves placing a rat in a restraining tube to which it has been habituated previously. Following assessment of baseline TFL, the restraining tube with the rat inside is then vibrated at 4Hz for 5 minutes,

after which time TFL is measured intermittently over a 25 minute period. This vibration stress protocol has been shown to induce short-term thermal hyperalgesia in male rats and female rats in late diestrus (Devall et al. 2009; Devall et al. 2011), an effect more persistent in female rats.

Rotational stress, whereby rats are placed in cages which are then rotated at 45rpm for 10 minutes daily over 2 weeks, has been shown to produce increased formalin-evoked nociceptive behaviour in mice. In particular, the second phase of formalin-evoked nociceptive behaviour is augmented in mice exposed to this regime (Boccalon et al. 2006). Further studies may be warranted to determine the validity of this stressor in modelling SIH.

Air stress employs a continuous stream of air at room temperature directed at the face of rat restrained in a tube for a period of 30 minutes and has been shown to reduce response thresholds to mechanical stimulation with von Frey filaments (Wagner et al. 2013). In this study, Wagner and colleagues also demonstrated roles for both the dorsomedial hypothalamus and the RVM in this form of SIH since inactivation of these brain regions blocked SIH. The authors suggest that anatomical and functional connections between the dorsomedial hypothalamus and the RVM play key roles in stress-induced facilitation of pain (Wagner et al. 2013).

Whisker pad stimulation involves placing a rat in a chamber, larger than a restraint cage, with holes at the front. In this model, a von Frey filament is then inserted through the holes into the chamber and is directed towards the whisker pad to act as a stressor. This is repeated 10 times over 15 minutes throughout each 1 hour stress session. The 1 hour sessions are repeated every 5 days for 2 weeks. Control rats were placed in the chamber but without whisker pad

stimulation. Stressed rats developed mechanical hypersensitivity of the hind paw in the von Frey test, as well as aggressive behaviour. Microinjection of dermorphin-saporin into the RVM, a specific toxin targeting neurons expressing μ-opioid receptors, prevented the development of hind paw mechanical hypersensitivity, but did not affect the aggressive behaviours (Reynolds et al. 2011). As von Frey filaments and a similar arena were used for both induction of stress and nociceptive testing, there is a possibility that rats developed contextual learning which could then affect behaviour in the von Frey test. This is noted by the authors and they suggest a need for additional examination of contextual learning in this model. To our knowledge, this is the only published study to-date on whisker pad stimulation SIH so further studies are required to validate this model.

In research aimed at modelling links between post-traumatic stress disorder (PTSD) and pain, exposure to single prolonged stress, an established animal model of PTSD, induced visceral hypersensitivity to colorectal distension (He et al. 2013), in addition to thermal hyperalgesia and mechanical allodyina as measured by paw withdrawal latencies to a heat stimulus and von Frey test respectively (Zhang, 2012). Interestingly this enhanced nociception is not evident until day 7 post stress exposure and lasted until day 28. Furthermore, there was a statistically significant correlation between mechanical allodynia and anxiety-like behaviour, as measured in the elevated plus maze, at day 9. However anxiety-like behaviour in stressed rats dissipated by day 14 (Zhang, 2012). These results suggest that a single intense stressful episode is able to induce long-term maladaptive changes in pain processing long after the stressful episode occurs (He et al. 2013) and that the stress-induced exacerbation of pain responding is not dependent on ongoing anxiety-related behaviour.

3 Neural substrates of SIH

Multiple brain regions are engaged in a complex manner to mediate the experience of pain. Following exposure to a noxious stimulus, nociceptive information is transmitted via the ascending pain pathway to the somatosensory cortex, facilitating perception of pain. Subsequently, descending facilitatory or inhibitory pathways may be activated to potentiate or inhibit nociceptive transmission, respectively (Millan, 2002). Nociceptive transmission can be influenced by stress, through stress-induced alterations within the different spinal and supraspinal components of the pain pathways, including, but not limited to, the cortex, amygdala, PAG, RVM and spinal cord dorsal horn. Such alterations may impair the body's ability to suppress pain, resulting in enhanced pain perception. In this regard, a key to unravelling the mechanisms underlying the interactions between stress and pain is the identification of neuroanatomical substrates which are critically involved in modulating stress and pain responses. Presented below is the current data highlighting a role for these neural substrates in the interaction between stress and pain and in particular SIH.

3.1 Cortex

The cortex is a highly organised structure and a site of convergence of both ascending and descending pain pathways. Reorganisation of the primary somatosensory cortex (S1) has been found in chronic pain conditions with or without nerve damage (Vartiainen et al. 2009; Wrigley et al. 2009).

Evidence from neuroimaging studies also suggests that chronic pain and stress-related psychiatric disorders are associated with structural and functional reorganisation of cortical structures (Burgmer et al. 2009; Flor et al. 2001; Karl et al. 2001; Pleger et al. 2006; Hayes et al. 2012; Asami et al. 2008; Qiu et al. 2013). There is evidence for the involvement of S1 in the modulation of the sensory aspects of pain perception (Bushnell et al. 1999). Increased

gray matter thickness has been found in patients with a chronic pain disorder as measured by MRI (Desouza et al. 2013). Changes in gray matter of S1 cortex may be due to non-neuronal mechanisms i.e. increased vasculature, however, they may also be due to neuronal mechanisms such that consistent nociceptive/sensory input produces activity dependent plasticity. It is possible that certain people may be more susceptible to developing a pain condition due to pre-existing alterations in their cortex including the S1. In fact healthy individuals with lower pain thresholds exhibit more frequent and more robust pain-induced activation of the S1 cortex in addition to the anterior cingulate cortex (ACC) and PFC than individuals with higher thresholds (Coghill et al. 2003). Sub-cortical structures such as the ACC and insular cortex are thought to play a key role in the affective component of pain processing (Basbaum et al. 2009; Xie et al. 2009). Healthy volunteers experienced increased pain intensity to electrical stimulation in the presence of a negative context, which was associated with increased pain-related activation in the ACC (Senkowski et al. 2011, Yoshino et al. 2012; Yoshino et al. 2010). An imaging study in IBS patients exposed to a psychological stressor revealed that increased pain sensitivity upon colorectal distension was associated with pronounced activation of several brain regions including the insula, mid-cingulate and ventrolateral PFC, compared with healthy study participants (Elsenbruch et al. 2010) (Fig.1). In addition enhanced visceral sensitivity in rats exposed to WAS is associated with increased colorectal distension evoked activation in the insular cortex, but reduced activation in the prelimbic area of PFC (Wang et al. 2013)

Animal studies have demonstrated that continuous electrical stimulation of the ACC increases C-fibre activation to noxious thermal stimulation in the Hargreaves test, suggesting a role for the ACC in descending facilitation of pain (Zhang et al. 2005). Furthermore, lesioning of the ACC prevented formalin-induced conditioned place avoidance (Gao et al.

2004; Johansen et al. 2001), which may suggest a decrease in aversion and indicate that the ACC plays a role in the negative affective component of pain. In a rat model of neuropathic pain employing SNL, periodic MRI scans reveals widespread changes in cortical structure (Seminowicz et al. 2009). Increased mechanical and cold allodynia, as measured by acetone and von Frey tests, respectively, were associated with decreased volume in the ACC and insula cortices. Anxiety-like behaviour in the elevated plus maze and open field developed in SNL rats 5 weeks post surgery and this was associated with a reduction in PFC volume (Seminowicz et al. 2009). The extent to which other sub-cortical structures are involved in SIH has yet to be fully characterised. Yet there is strong clinical and pre-clinical data indicating that abnormal activation of the ACC in stress-related disorders and/or during stress/anxiety states may increase nociception and augment the affective aspects of the pain experience (Fig.1).

3.2 Amygdala

The amygdala plays a key role in the modulation of anxiety and fear, two emotionally distinct states which generally result in opposite effects on nociceptive responding (Butler and Finn; 2009, Rhudy and Meagher, 2000). Reduced μ-opioid receptor availability in the amygdala has also been seen in a positron emission tomography study in healthy volunteers during sustained pain (Zubieta et al. 2001) (Fig. 1). Mouse models of neuropathic (SNL) and chronic inflammatory (CFA) pain have been shown to exhibit increased anxiety-like behaviour, an effect accompanied by reduced binding of μ- and δ- opioid receptor binding in the amygdala, while κ-opioid receptor agonist binding in the amygdala was affected only in the CFA model (Narita et al. 2006).

Implantation of micropellets loaded with corticosterone into the central nucleus of the amygdala (CeA) resulted in increased anxiety-related behaviour (as assessed with the elevated plus maze) and also increased somatic (von Frey test) and visceral (colonic distension) pain behaviour (Myers et al. 2007; Myers and Greenwood-Van Meerveld, 2007; Myers and Greenwood-Van Meerveld, 2010). Conversely, micropellets loaded with either the glucocorticoid receptor antagonist mifepristone, or the mineralocorticoid receptor antagonist spironolactone, prevented the development of visceral hyperalgesia in the WAS model when injected into the CeA (Myers and Greenwood-Van Meerveld, 2012). The wistar-kyoto (WKY) stress-hyperresponsive inbred rat strain expresses a higher level of corticotropin-releasing factor receptor 1 (CRF_1) receptors in the CeA compared to other strains (Bravo et al. 2011), which may account, at least in part, for the visceral hyperalgesia observed in this stress-sensitive strain. In the WKY rat, intra-CeA implantation of micropellets loaded with the CRF_1 receptor antagonist, CP 376395, and not the glucocorticoid and mineralocorticoid receptor antagonists,,mifepristone or spironolactone, abolished the enhanced colonic sensitivity (Johnson et al. 2012). These results highlight differences in SIH models, particularly when examining the influence of genotype.

3.3 Periaqueductal grey

Given the key role of the PAG in the descending inhibitory pain pathway and aversive responding, it is not surprising that this structure has been proposed as a possible substrate mediating SIH. Lesions to the ventrolateral PAG attenuate nociception but do not affect the emotional behaviour associated with pain, including anxiety-related behaviour (Mendes-Gomes et al. 2011). Early life stress in the form of MS predisposes to the development of visceral hypersensitivity to colorectal distension in adulthood (Wouters et al. 2012). MS rats displayed increased PAG activation in response to colorectal distension both pre- and post-

WAS. MS rats exposed to WAS also displayed increased activation of the S1 compared with pre-stress activity, suggesting that stress in the adult MS rat results in enhanced sensory input to visceral noxious stimulation. Therefore, the increased response to colorectal distension in MS rats post-WAS compared with the pre-stress response may be due to enhanced pain perception rather than effects on endogenous pain inhibition, as WAS did not further affect PAG activity to colorectal distension. However, it should be noted that no non-MS rats were included in this study, making it difficult to fully interpret the data in the context of MS-induced alterations.

Further evidence for a role of the PAG in SIH comes from research demonstrating that vibration stress-induced reduction in TFL was associated with reduced expression of c-Fos, a correlate of neuronal activity, in the dorsolateral, lateral, and ventrolateral columns of the PAG (Devall et al. 2011). The identity of these neurons is unknown; however it is possible that the reduction in neuronal activity could result from decreased activation of inhibitory interneurones controlling activity in descending inhibitory pathways. Further studies, employing dual immunohistochemistry and or electrophysiological recordings from the PAG would be required to clarify this. Chronic restraint SIH is associated with a decrease in the expression of glial fibrillary acidic protein (GFAP), an astrocyte biomarker, and excitatory amino acid transporter 2 in the PAG (Imbe et al. 2012) (Fig.1). However, SNL mice also exposed to restraint stress had an increase in GFAP mRNA expression in the PAG, and also an increase in brain-derived neurotrophic factor and interleukin (IL)-1 mRNA expression (Norman et al. 2010). Thus, the directionality of changes in expression of GFAP in the PAG depends on the type of SIH model employed. Overall restraint stress-induced changes in PAG neurobiology could alter the activity of the descending inhibitory pain pathway to facilitate mechanical hyperalgesia.

3.4 Rostral ventromedial medulla

The RVM consists of OFF and ON cells (Vanegas et al. 1984) which are involved in descending inhibition and facilitation of pain, respectively. Neutral cells represent a third subset, the exact function of which is as yet unknown. The RVM has descending projections to the dorsal horn of the spinal cord and to the trigeminal nucleus (Aicher et al. 2012) and exerts bidirectional control over nociception. Either analgesia or hyperalgesia can result, depending on the receptors and neuronal subtypes activated within the RVM (Heinricher et al. 2009).

Chronic restraint-induced thermal hyperalgesia in rats has been shown to be associated with significantly higher numbers of phospho-ERK-immunoreactive neurons, upregulation of tryptophan hydroxylase in the RVM (Imbe et al. 2004) and a decrease in GFAP (Imbe et al. 2013) (Fig.1). Forced swim SIH in the formalin test is attenuated after ibotenic acid-induced lesion of the RVM, while air stress-induced mechanical hyperalgesia is attenuated by lidocaine-mediated inactivation of the RVM, suggesting a descending facilitatory role of the RVM during SIH (Imbe et al. 2010). More specifically, social defeat-induced anxiety (elevated plus maze) and mechanical hyperalgesia (von Frey and Randall–Selitto tests) (Rivat et al. 2010) were attenuated by direct injection of a cholecystokinin $(CCK)_2$ receptor antagonist into the RVM (Rivat et al. 2010). However, in the air stress-induced thermal hyperalgesia model in rats, direct injection of the CCK_2 receptor antagonist YM022 into the RVM had no effect. Selective ablation of neurons expressing μ-opioid receptors in the RVM with dermorphin-saporin prevented the development of whisker pad stimulation stress–induced mechanical hyperalgesia (Reynolds et al. 2011). Thus, μ-opioid receptor-expressing neurons in the RVM appear to be involved in the development of SIH (Fig.1).

Recently, work from our laboratory has demonstrated that pharmacological blockade of the cannabinoid receptor 1 (CB_1) potentiates hyperalgesia to formalin injection in stress-hyperresponsive WKY rats, while inhibition of the endocannabinoid catabolising enzyme, fatty acid amide hydrolase (FAAH), attenuates this hyperalgesia (Rea et al. 2013). Moreover, we reported blunted formalin-evoked mobilisation of endocannabinoids in the RVM of WKY rats, compared with SD controls and intra-RVM administration of the FAAH inhibitor URB597 reduced formalin-evoked nociceptive behaviour in WKY rats (Rea et al. 2013). Adult Wistar rats which had previously been exposed to MD, display enhanced visceral hypersensitivity to colorectal distension which is associated with increased expression of tyrosine kinase receptor B but not its ligand, brain-derived neurotrophic factor, in the RVM (Chung et al. 2009). Thus, mounting evidence supports a role for the RVM in SIH and it is clear that it plays a critical role in determining pro-nociceptive versus anti-nociceptive tone during or following exposure to stress.

3.5 Spinal Cord

When pain becomes persistent, it is in part due to alterations in spinal circuitry which lead to the exacerbation and maintenance of the pain condition (Basbaum, 1999). Less is known about the effect of stress at the level of the spinal cord and how it can alter nociception. It has been shown that stress can alter the balance between inhibitory and excitatory transmission in the spinal cord. Increased formalin-evoked nociceptive behaviour in rats exposed to FSS (3 days) was associated with increased c-Fos expression in the dorsal horn of the spinal cord and increased levels of glutamate and decreased levels of GABA in the lumbar spinal cord (Quintero et al. 2011; Quintero et al. 2003). Prior administration of the anxiolytic diazepam inhibited the hyperalgesic response and prevented changes in spinal glutamate levels

(Quintero et al. 2011), indicating a possible role for spinal glutamate transmission in mediating this form of SIH. In another model of SIH, socially defeated rats that displayed mechanical hyperalgesia had a transient increase in cyclooxygenase-2 and inducible nitric oxide synthase in the dorsal horn of the spinal cord. Chronic intrathecal treatment with aspirin attenuated this hyperalgesia (Rivat et al. 2010). Together, these findings suggest that exposure to psychological stress modulates nociceptive processing at the level of the spinal cord (Fig.1). Whether this modulation is achieved via direct effects of stress on the cord circuitry, or via top-down modulation arising from perception and supraspinal effects of stress, remains to be determined. However, the latter would seem to us to be the most likely explanation.

4 Neurotransmitters and neuromodulatory systems involved in SIH

Neurotransmitters, neuropeptides and other neuromodulators play a key role in both stress and pain processing within the CNS. As a consequence, pathophysiological alterations or adaptations in the levels of these neurochemicals could influence the outcome of stress-pain interactions. This section will discuss the evidence for involvement of key neurotransmitter and neuroendocrine systems (opioid, glutamate, GABA CCK, monoamines, endocannabinoid, HPA axis and the sympathetic adrenomedullary system) in SIH. Full consideration and discussion of the role of these systems in pain and stress *per se* is beyond the scope of this review and we will focus on their involvement in stress-pain interactions.

4.1 Opioids

Drugs that target the endogenous opioid system are among the most commonly prescribed analgesics (Trescot et al. 2008) and endogenous opioids play an important role in the modulation of both inflammatory and neuropathic pain (Przewlocki and Przewlocka, 2001; Bushlin et al. 2010). The opioid system is composed of 3 receptor subtypes; μ (mu)-, δ

(delta)- and κ(kappa)- opioid receptors; the endogenous ligands for these receptors; β-endorphin, enkephalin and dynorphins, respectively, and the enzymes which metabolise and synthesis these ligands. Both μ- and δ-opioid receptors are expressed at key peripheral, spinal and supraspinal sites involved in pain processing (DuPen et al. 2007). Increasing evidence has implicated a role for the endogenous opioid system in the modulation of stress and emotion (Ribeiro et al. 2005). As such, it is not surprising that the opioid system has been implicated in SIH. Recent evidence has demonstrated that selective ablation of μ-opioid receptors in the RVM prevents the induction of mechanical hyperalgesia in a rat model of SIH using whisker pad stimulation as a stressor (Reynolds et al. 2011). In addition, the pre-stress administration of naloxone or naloxonazine, μ-opioid receptor antagonists, prevents the development of hyperalgesia in swim stressed rats (Le Roy et al. 2011; Suarez-Roca et al. 2006b).

In comparison, administration of nor-binaltorphimine, a selective κ-opioid receptor antagonist, or naltrindole, a selective δ-opioid receptor antagonist, did not affect the development of hyperalgesia in rats exposed to FSS (Suarez-Roca et al. 2006a), suggesting that μ-opioid receptors are involved in mediating SIH. These findings suggest that exposure to non-noxious stressful events may be associated with the sustained release of endogenous opioids, resulting in the desensitisation of the $\mu\text{-}_1$ opioid receptor (Le Roy et al. 2011; Suarez-Roca et al. 2006b). μ-opioid receptor antagonists, administered prior to stressful events, may prevent this desensitisation, thereby restoring endogenous analgesic tone and attenuating SIH.

4.2 Glutamate and GABA

Glutamate is the main excitatory neurotransmitter in the brain and spinal cord and acts at NMDA, AMPA/kainate or metabotropic glutamate receptors. Microdialysis and post-mortem analysis of cold or forced swim-stressed rats exposed to a noxious stimulus revealed higher levels of glutamate release in the dorsal horn of the lumbar region of the spinal cord (Okano et al**.** 1997; Raftery et al. 2011). Spinal administration of the NMDA receptor antagonist ketamine prevented and abolished hyperalgesia in rats exposed to FSS (Suarez-Roca et al. 2006b). Furthermore, intrathecal administration of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid to cold-stressed rats prevented hyperalgesia without affecting mechanical pain thresholds in control rats (Okano et al. 1995b; Okano et al. 1995a). The WAS model of visceral SIH, was associated with decreased spinal expression of the glial glutamate transporter GLT1, the astrocytic marker GFAP, and the glutamate conversion enzyme glutamine synthetase, whereas expression of the glial glutamate transporter, GLAST, was upregulated (Bradesi et al. 2011). Moreover, visceral hyperalgesia was blocked by pharmacological inhibition of spinal NMDARs and by the glial modulating agent propentofylline (Bradesi et al. 2011). Overall, these findings suggest that the NMDA receptor, at least at the level of the spinal cord, is important in maintaining the hyperalgesic state induced by chronic stress exposure. However, it must be noted that long-term use of drugs targeting the NMDA receptor is also associated with alterations in brain function in both mice and humans (Narendran et al. 2005, Sun et al. 2011), and therefore may be problematic for treating chronic comorbid disorders clinically.

GABA is the main inhibitory neurotransmitter in the CNS, acting on $GABA_A$ (ligand-gated ion channel receptor) and $GABA_B$ (G-protein coupled receptor) receptors (Bowery and Smart, 2006). Decreased levels of GABA in the dorsal horn of the spinal cord have been

reported in a variety of animal models of SIH (Eaton et al. 1999; Narita et al. 2011; Suarez-Roca et al. 2008; Ibuki et al. 1997). GABA release in the spinal cord, as measured by *in vivo* microdialysis, has been reported to be reduced in swim-stressed rats upon exposure to a noxious inflammatory stimulus when compared to non-stressed control rats (Quintero et al. 2011; Suarez-Roca et al. 2008), suggesting that swim stress results in altered GABAergic neurotransmission in the spinal cord and consequent exacerbation of pain responding. Systemic administration of the $GABA_A$ receptor positive allosteric modulator diazepam, 1 hour prior to each stress session, did not affect GABA release but reduced pain scores and overexpression of c-Fos. Flumazenil, an antagonist of the benzodiazepine binding site, reversed these effects (Suarez-Roca et al. 2008). These findings indicate that GABAergic neurotransmission in the spinal cord is important in the development and to some extent the maintenance of swim stress-induced hyperalgesia.

4.3 Cholecystokinin

Cholecystokinin (CCK) is a neuropeptide widely distributed in the brain. The sulphated octapeptide, CCK-8S, is the predominant CCK isoform found in the CNS. There are two CCK receptor subtypes, CCK_1 (CCK_A) and CCK_2 (CCK_B), both of which are G-protein coupled receptors which and expressed in the CNS, but the CCK_2 receptor is particularly highly expressed in key areas involved in pain and fear/emotional processing (Bowers et al. 2012; Kurrikoff et al. 2004; Chen et al. 2010; Li et al. 2013). CCK induces panic attacks in healthy human subjects (Bradwejn and Koszycki, 1994) and in rats (Zanoveli et al. 2004). Deletion of the CCK_2 receptor in mice reduces hyperalgesia in a mouse model of neuropathic pain (Kurrikoff et al. 2004). Moreover, deletion of CCK_2 also leads to the up-regulation of μ- and δ-opioid receptors with an increase in levels of circulating endogenous opioids (Pommier et al. 2002). Hawranko and Smith (1999) showed that repeated exposure to a noxious thermal

stimulus (as a stressor) reduced the antinociceptive efficacy of morphine in the TFL and that intrathecal administration of the CCK_2 receptor antagonist, L-365,260, restored morphine's efficacy (Hawranko and Smith, 1999).

Moreover, direct injection of a CCK_2 receptor agonist into the PAG causes anxiety-like behaviour in rats (Bertoglio and Zangrossi, 2005; Zanoveli et al. 2004). CCK also has pro-nociceptive effects and is implicated in chronic pain conditions through activation of descending facilitation (Lovick, 2008). CCK-8S attenuates $GABA_A$ receptor-mediated inhibitory postsynaptic currents in the dorsal root ganglia (Ma et al. 2006) and PAG (Mitchell et al. 2011). As previously mentioned, a decrease in spinal GABAergic transmission or activity is seen in animal models of SIH.

A number of studies examining pro-nociceptive properties of CCK have focused on the RVM, with direct injections into the RVM producing increased nociception (Friedrich and Gebhart, 2003; Heinricher and Neubert, 2004). However, the PAG does appear to play a central role also, and its projections to the RVM may mediate pro-nociceptive effects of CCK (Lovick, 2008). In addition, projections from the dorsomedial hypothalamus (DMH) to the RVM have also been suggested to play a role in SIH. Using reterograde tracing and immunohistochemistry, it has been demonstrated that CCK-expressing neurons in the DMH are a significant supraspinal source of CCK in the RVM. However, not all neurons projecting from the DMH to the RVM express CCK (Wagner et al. 2013). Direct injection into the RVM with a CCK_2 receptor antagonist in a SIH model using social defeat, prevented anxiety-like behaviour as assessed in the open field and elevated plus maze, and transient mechanical allodynia in the von Frey and the Randall–Selitto test compared to vehicle-treated socially defeated rats. The RVM is not the only brain region in which CCK receptors

have been suggested to modulate SIH. In a SIH model of social defeat, rats had increased CCK-like material in frontal cortex microdialysates. Pre-treatment with a CCK-B receptor antagonist, CI-998, prevented stress-induced increases in formalin-evoked nociceptive behaviour (Andre et al. 2005). In summary, the CCK system, given its important role in modulating pain and aversion *per se*, also appears to be involved in SIH; however further studies are required to determine the exact mechanisms underpinning the involvement of CCK in SIH.

4.4 Monoamines

Monoamines, and in particular noradrenaline (NA) and 5-hydroxytryptamine (5-HT; serotonin), are widely recognised as important in nociceptive transmission and descending inhibition, and in mediation and modulation of the stress response (Millan, 2002). In addition to treating depression, tricyclic antidepressants which elevate synaptic levels of monoamines are commonly used to treat neuropathic pain, although the mechanisms involved have not yet been fully elucidated. Thus, it is not surprising that monoamines have been implicated in SIH. Several pharmacological studies have implicated 5-HT, NA or both of these neurotransmitters in various forms of SIH. Exposure to 6 days of cold stress is associated with reductions in levels of both 5-HT and its metabolite 5-hydroxyindoleacetic acid in the rat hypothalamus, thalamus, midbrain, pons and medulla oblongata and spinal cord (Hata et al. 1991). Repeated cold SIH in mice was suppressed by the systemic administration of 5-Hydroxytryptophan, a precursor of 5-HT, and by L-DOPA, a precursor of catecholamines (Ohara et al. 1991). Administration of fluoxetine, an SSRI, has been shown to attenuate chronic restraint stress-induced enhancement of formalin-induced nociceptive behaviour (SIH) (Gameiro et al. 2006) and unpredictable chronic mild stress-induced thermal and inflammatory hyperalgesia (Shi et al. 2010a). In addition, forced swim SIH was inhibited by

acute pre-treatment with tryptophan, a precursor of 5-HT, and chronic pre-treatment with clomipramine and fluoxetine (Quintero et al. 2000).

Clonidine, an alpha-2-adrenoceptor agonist and inhibitor of the synaptic release of NA, was shown to prevent vibration-induced mechanical hyperalgesia in rats (Jorum, 1988); indicating that enhanced noradrenergic system activity is involved in mediating this form of SIH. In addition, pre-treatment with milnacipran, a dual 5-HT/NA uptake inhibitor, reversed repeated forced-swim stress-induced muscle hyperalgesia without altering nociceptive behaviour in non-stressed animals, suggesting that enhanced central NA and/or 5-HT can reverse SIH without affecting normal nociception (Suarez-Roca et al. 2006a).

When examining enhanced visceral sensitivity in the WKY rat, systemic and intracerebroventricular administration of a 5-HT_{2B} antagonist reduced pain behaviours during colorectal distension (O'Mahony et al. 2010) and there is also evidence that 5-HT_{2B} receptors may mediate restraint stress-induced visceral hypersensitivity in mice (Ohashi-Doi et al. 2010). The serotonin system can affect gastrointestinal motility (Coates et al. 2006), however, the exact mechanisms as to how a 5-HT_{2B} receptor agonist works centrally is yet to be determined. Overall, treatments targeting monoamines are already used to treat both depression and pain disorders and monoamines clearly play a key role in SIH.

4.5 Endocannabinoids

The endogenous cannabinoid (endocannabinoid) system is composed of two G-protein coupled cannabinoid (CB) receptors, CB_1 and CB_2, the endocannabinoids, the best characterised of which are anandamide (AEA) and 2-arachidonylglycerol (2-AG), the enzymes responsible for the synthesis and metabolism of these ligands. The presence of endocannabinoids and their receptor targets in key loci associated with pain, both in the

periphery and in the CNS, supports the reported role of the endocannabinoid system in pain modulation (Guindon and Hohmann, 2009). Direct injection of cannabinoid receptor agonists into the RVM, amygdala, thalamus or PAG results in anti-nociception (Finn et al. 2003; Martin et al. 1999; Meng et al. 1998). Moreover, exposure to noxious stimuli results in increased levels of AEA in the PAG, suggesting that pain triggers endocannabinoid release to suppress pain (Walker et al. 1999). Stress-induced dysfunction in the endocannabinoid system can lead to altered responses to stress (Hu et al. 2011; Wamsteeker et al. 2010). Increasing levels of AEA and 2-AG by preventing their degradation either through genetic or pharmacological manipulation reduces anxiety-like behaviour in rodents (Moreira et al. 2008, Sciolino et al. 2011).

Mounting evidence supports a role for the endocannabinoid system in SIH. Down-regulation of CB_1 receptor expression in the dorsal root ganglion has been reported in a rat model of WAS-induced enhancement of visceral sensitivity (Hong et al. 2009; Hong et al. 2011). Treatment with mifepristone, a glucocorticoid receptor antagonist, prevented these stress-induced changes in expression of the CB_1 receptor in this model (Fig. 1) (Hong et al. 2011). In addition, 10 days of subcutaneous corticosterone administration also resulted in down-regulation of CB_1 receptor expression in a manner similar to that induced by WAS, thus demonstrating the regulatory role of the HPA axis on the endocannabinoid system (Hong et al. 2009). Partial restraint stress-induced visceral hyperalgesia has been reported to be attenuated following sub-chronic (3 day) intraperitoneal injection of a CB_1 receptor agonist, ACEA (Shen et al. 2010). Conversely, sub-chronic administration of the CB_1 receptor antagonist SR141716A, exacerbated visceral hyperalgesia (Shen et al. 2010).

Dysfunction in the endocannabinoid system has been reported in the WKY rat strain (Vinod et al. 2012), which, as previously mentioned, displays anxiety-like behaviour and enhanced pain responding (Burke et al. 2010; O'Mahony et al. 2010). Findings revealed higher levels of the enzyme fatty acid amide hydrolase (FAAH) and lower levels of its substrate AEA in the cortex and hippocampus (Vinod et al. 2012). In addition, we have recently reported blunted pain-related mobilisation AEA and 2-AG and transcription of their synthesising enzymes, NAPE-PLD and DAGLα, in the RVM of WKY rats after intraplantar formalin injection, compared with SD rats (Rea et al. 2013). Moreover, systemic administration of the CB_1 receptor antagonist/inverse agonist AM251 potentiated the hyperalgesic response of WKY rats to formalin injection while systemic or intra-RVM administration of the FAAH inhibitor URB597 attenuated it (Rea et al. 2013). These results suggest that dysfunction in the endocannabinoid system may underlie the heightened nociceptive responding (thermal, mechanical, inflammatory, visceral) in stress-hyperresponsive WKY rats (Burke et al. 2010; Johnson et al. 2012)

4.6 The HPA axis

The hypothalamo-pituitary-adrenal (HPA) axis plays a major role in the physiological response to stress (Herman and Cullinan, 1997) and pain (Bomholt et al. 2004; Ulrich-Lai et al. 2006) and alterations within the HPA axis may also contribute to SIH. Acute stress or pain causes the release of CRF from the hypothalamus, which stimulates the pituitary gland to secrete adrenocorticotropic hormone, which in turn induces the secretion of cortisol (humans) or corticosterone (rodents) from the adrenal cortex. Evidence from clinical studies shows that chronically stressed and pain patients exhibit alterations in HPA axis activity and responsivity (Aloisi et al. 2011; Chang et al. 2009; Tanriverdi et al. 2007). For example, patients with IBS had lower baseline plasma levels of CRF but had increased or exaggerated levels of CRF

upon stress exposure, which was also associated with enhanced visceral pain from colorectal distension (Posserud et al. 2004). Further evidence suggests that the CRF receptor antagonist, alpha-helical CRF, significantly reduces the abdominal pain evoked by electrical stimulation in IBS patients (Sagami et al. 2004). CRF receptors, in particular the CRF_1 receptor subtype, have been shown to play an important role in animal models of stress-induced visceral hyperalgesia (Tache et al. 2004). Systemic administration of a CRF_1 receptor antagonist to rats significantly inhibited WAS-induced visceral hyperalgesia (Million et al. 2003; Schwetz et al. 2004). In contrast, systemic administration of a CRF_1 receptor agonist resulted in visceral hyperalgesia in rats, an effect apparently mediated through peripheral mechanisms as intracerebroventricular injection of a CRF_1 antagonist did not prevent this hyperalgesia (Larauche et al. 2009). It has also been reported that CRF_1 receptors are involved in stress-induced visceral hyperalgesia in a rat model of neonatal stress (Schwetz et al. 2005). Moreover, there is evidence for involvement of CRF_2 receptors in stress-induced musculoskeletal hyperalgesia in mice exposed to FSS (Abdelhamid et al. 2013).

Earlier studies by Vidal and colleagues demonstrated a role for the pituitary gland, a key component of the HPA axis, in the development of SIH. Hypophysectomy potentiated inescapable holding-induced hyperalgesia, but attenuated novelty-induced hyperalgesia (Vidal et al. 1982). However, dexamethasone, which is known to block the stress-induced release of adrenocorticotropic hormone and endorphin from the anterior lobe of the pituitary did not affect novelty-induced hyperalgesia but enhanced hyperalgesia induced by holding (Vidal et al. 1982). These findings led the authors to conclude that hypophyseal factors, not affected by dexamethasone and originating from the pituitary, may participate in novelty-induced hyperalgesia, whereas analgesic mediators originating in the anterior pituitary (e.g. opioids) appear to counteract the holding-induced hyperalgesia.

Recent studies have demonstrated that chronic subcutaneous administration of corticosterone to rats produces visceral hyperalgesia, as measured by colorectal distension, which is comparable to that seen following water avoidance (Hong et al. 2011). In addition, chronic water avoidance induces changes in transient receptor potential receptor $TRPV_1$ and CB_1 receptor expression in the dorsal root of the lumbar region of the spinal cord, effects that are prevented by treatment with a glucocorticoid receptor antagonist (Hong et al. 2009; Hong et al. 2011). These findings are also consistent with a previous report demonstrating that adrenalectomy abolishes FSS-induced hyperalgesia in rats (Fereidoni et al. 2007). Moreover, the implantation of corticosterone micropellets in the central amygdala of rats results in increased anxiety-related behaviour and visceral and mechanical hyperalgesia (Greenwood-Van Meerveld et al. 2001; Johnson et al. 2010; Myers and Greenwood-Van Meerveld, 2010; Tran and Greenwood-Van Meerveld, 2012), indicating that the action of glucocorticoids in the amygdala may be involved in regulating visceral sensitivity.

4.7 The sympathetic adrenomedullary and peripheral nervous systems

A contribution of peripheral components of stress response axes, in particular the adrenergic system, and other key mediators of pain processing in the periphery such as the dorsal root ganglia (DRG), to SIH, has been demonstrated. For example, noise- and footshock-induced thermal hyperalgesia in the hot plate test in rats and wild-type mice pre-treated systemically with α_2-adrenoceptor antagonists, or in α_{2A}- adrenoceptor knockout mice, was attenuated by sympathectomy or systemic administration of an α_1-adrenoceptor antagonist (Donello *et al.*, 2011). The authors interpret their findings as suggesting a role for sympathetic postganglionic

nerves in enhancing pain sensation, possibly via a peripheral α_1-adrenoceptor mediated mechanism. It is, however, worthwhile noting that these findings are at variance with previous reports suggesting no involvement of the adrenergic system in SIH based on a rat model of acute restraint stress (Oyadeyi *et al.*, 2005). While these discrepancies warrant further investigation on the role of the adrenergic system in SIH, a key consideration in the interpretation of these data is the choice of the experimental paradigms employed to examine/model SIH. In this regard, it is possible that differences in the nature of stressors and pain models used in both studies account for the different outcomes.

Excitation of DRG neurones by stress hormones represents another potential mechanism underlying SIH. Studies in cultured DRG neurones suggest that stress hormone signalling to colonic DRG neurons may play a role in sustained hyperexcitability of nociceptors (Ochoa-Cortes *et al.*, 2014). In this study, overnight exposure of mouse DRG neurons to adrenaline and corticosterone together induced hyperexcitability in mouse DRG neurones which was blocked by antagonists of the β_2-adrenoreceptor and glucocorticoid receptor, either individually or together. Studies in whole-animal systems showed differential effects of antagonist treatment on visceromotor reflexes to colorectal balloon distension in rats exposed to the WAS model of SIH, in that separate administration of β_2-adrenoreceptor and glucocorticoid receptor antagonists reduced visceromotor reflexes, but paradoxically had the opposite effect when given together. The authors suggest that these differences may reflect different peripheral and central actions on sensory signalling (Ochoa-Cortes *et al.*, 2014). Other studies have also presented evidence for interactions between stress hormones and primary afferent fibres, in particular C-fibers (Chen *et al.*, 2005). These authors showed that intradermal administration of adrenaline resulted in excitation of a group of C-fibers (single unit electrophysiological recordings) and a decrease in the mechanical activation threshold in a non-overlapping group. Interestingly, fibers that were not excited or did not demonstrate a

decrease in threshold demonstrated a significant increase in response to sustained suprathreshold mechanical stimuli. However, the extent to which these interactions contribute to the development of SIH warrants further investigation. Moreover, stress-induced alterations in other molecules including cytokines and neuropeptides may also modulate the activity of primary afferent sensory neurones and could contribute to SIH.

Khasar and colleagues (2008) reported that prolonged enhancement of bradykinin-induced hyperalgesia by unpredictable sound stress in rats requires a contribution from both the sympathoadrenal and the HPA axes (Khasar et al. 2008). The specific contributions of the sympathoadrenal axis to bradykinin-induced hyperalgesia was demonstrated by the absence of cutaneous and muscle hyperalgesia in unpredictable sound-stressed rats subjected to adrenal medullectomy (Khasar et al. 2009). SIH was restored following the administration of adrenaline at concentrations similar to those reached during stress, thus demonstrating the significant contribution of the sympathoadrenomedullary system in the induction, as well as the maintenance, of SIH. In another study, dams and neonatal rats submitted to a restriction of nesting material (neonatal limited bedding) for 1 week exhibited mild muscle hyperalgesia which was inhibited following intrathecal treatment with antisense directed against the pro-inflammatory cytokine interleukin-6 receptor subunit gp130, but not antisense against tumor necrosis factor receptor type 1 (TNFR1) (Alvarez et al. 2013). Muscle hyperalgesia in rats exposed to neonatal limited bedding was markedly aggravated by mild sound stress in adulthood. Adrenal medullectomy prevented the aggravation of muscle hyperalgesia by sound stress, but did not modify hyperalgesia in rats exposed to neonatal limited bedding. Moreover, the hyperalgesic effects of sound stress were restored by sustained administration of adrenaline to rats exposed to neonatal limited bedding. Furthermore, treatment with antisense against either gp130 or TNFR1 inhibited sound stress-induced enhancement of

hyperalgesia. Taken together, these findings suggest that a possible mechanism of SIH is the release of pro-inflammatory cytokines during exposure to early life stressors which primes the organism for enhanced pain responding in adulthood and is facilitated by the stress-induced release of adrenaline from the adrenal medulla.

4.8 Future Considerations

A full understanding of the specific mechanisms mediating SIH is complicated by the involvement of multiple central and peripheral substrates and neurotransmitter systems. Research to date has tended to focus on the role of a particular brain region or a particular neurotransmitter in isolation and there is a paucity of information on how the multiplicity of substrates and neurotransmitter systems interact with one another to mediate and modulate SIH. Future studies should focus on cross-talk between different neurotransmitter and receptor systems and adopt a neural networks approach to better understand SIH.

There is no doubt that the numerous animal models that have been developed have facilitated an increased understanding of the neurobiology of SIH. However, it is, perhaps, worth considering which of these animal models most appropriately models SIH in humans. This, of course, is a very complex consideration and it is very difficult to single out any one model as being the one that most closely models the human condition when the human condition itself is not yet fully understood. One could argue that the models of SIH that are based on social defeat, chronic mild stress and early life stress including maternal separation/deprivation are more relevant in the context of stressors that humans may be exposed to. Clearly these models utilise stressors that humans are more likely to be exposed to, compared with stressors such as whisker pad stimulation, restraint or forced swimming,

for example. However, while these former models may have greater translational value, particularly if coupled with a chronic pain model, their reproducibility across laboratories can be more difficult than models that use a more robust, acute/sub-acute, homotypic stressor. In addition, it seems likely that different models/stressors engage different brain regions and neurobiological substrates, thereby contributing to our understanding of the role of those regions and substrates in SIH. In this respect, it will be important that studies of SIH continue to adopt an integrative, whole-systems neurobiological approach whereby behavioural data are integrated with neurochemical, physiological, genetic and pharmacological data to enable strong conclusions with respect to underlying mechanisms. Moreover, recent years have seen significant interest in the role of epigenetics in stress and pain and future studies should investigate the potential involvement of epigenetic mechanisms in SIH.

Another important consideration is that the majority of SIH studies have used male rodents only, and do not address the influence of sex and sex hormones on SIH. The clinical manifestation of the influence of sex is evident from the gender-biased representation of some stress-related pain disorders such as IBS and fibromyalgia which have a higher incidence in females thank in males. Addressing this imbalance in animal studies of SIH will be important for a full understanding of SIH in the context of sex differences and their potential therapeutic implications for stress-related pain disorders.

Finally, there is a need for additional studies investigating SIH over the life-course, and the influence of aging on SIH, both in animal models and in humans. Human studies that utilise imaging modalities such as fMRI to further elucidate the neural circuitry of SIH would be

particularly useful and could inform the design of animal studies aimed at further elucidating neurochemical and molecular mechanisms in a back-translational approach.

4.9 Concluding Remarks

Our understanding of the physiological, biochemical and molecular mechanisms mediating and modulating both pain and stress has become much clearer in recent years. However, the full extent of stress-pain interactions and the detailed aetiology of anxiety/depression and pain comorbidity has yet to be determined. It is abundantly clear that stress has a significant impact on multiple systems in the body, including the pain pathways and particularly the descending inhibitory pain pathway. Animal models of SIH have facilitated a greater mechanistic insight into SIH, and with that, novel pharmacological targets may be identified. It must be noted that different models and strains used to study SIH have found some conflicting observations. Choosing the best translational SIH models needs to be confirmed by comparison with human studies. However, the precise mechanisms involved in SIH are yet to be uncovered, including the role of neural substrates, neurotransmitters and neuroendocrine alterations in SIH. It appears that there are two aspects of SIH, induction and maintenance. Although a particular neurotransmitter system may be involved in the induction of SIH through down-regulation or desensitisation of receptors, it is also important to understand what mechanisms maintain enhanced nociception.

Our ever-increasing understanding of overlap and interactions that exist between the neural substrates and neurochemical mechanisms that regulate pain and stress means that it may be possible to develop new drugs which can treat both pain and co-occurring anxiety/depression. Attractive drug targets include the GABAergic, monoaminergic, CCK and endocannabinoid systems and the HPA axis. Many glutamate receptor antagonists have been linked to altered

brain function following chronic administration (Narendran et al. 2005; Sun et al. 2011) thus making them less appealing targets. Although opioids play an important role in the SIH, they appear to be more important in the development of SIH through repeated stress-induced opioid release linked to desensitisation of the opioid receptors. While current clinical and pre-clinical models are facilitating identification of important physiological, biochemical and molecular mechanisms involved in SIH, it is clear that both pain and stress are processed through multiple brain pathways involving several excitatory and inhibitory systems and that multiple complex alterations likely give rise to SIH. However given the high incidence of comorbidity between pain and stress related psychiatric disorders, and the need to develop improved treatments, a full understanding of the neurobiological mechanisms underpinning SIH is very important.

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Conflict of interest

None

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Figure Captions.

Fig 1. Summary of some of the key sites and neurobiological mechanisms thought to mediate stress-induced hyperalgesia (SIH). Abbreviations: ACC (anterior cingulate cortex), PAG (periaqueductal grey), RVM (rostral ventromedial medulla), DRG (dorsal root ganglia), pERK (phosphorylated extracellular signal regulated kinase) , 5-HT (5-hydroxytryptamine), NA (noradrenaline), GABA (gamma-aminobutyric acid), CRF-R (corticotrophin releasing factor receptor subtype 1), EAAT2 (excitatory amino acid transporter 2), CCK (cholecystokinin), TRPV1 (transient receptor potential cation channel subfamily V member 1), GFAP (glial fibrillary acidic protein).

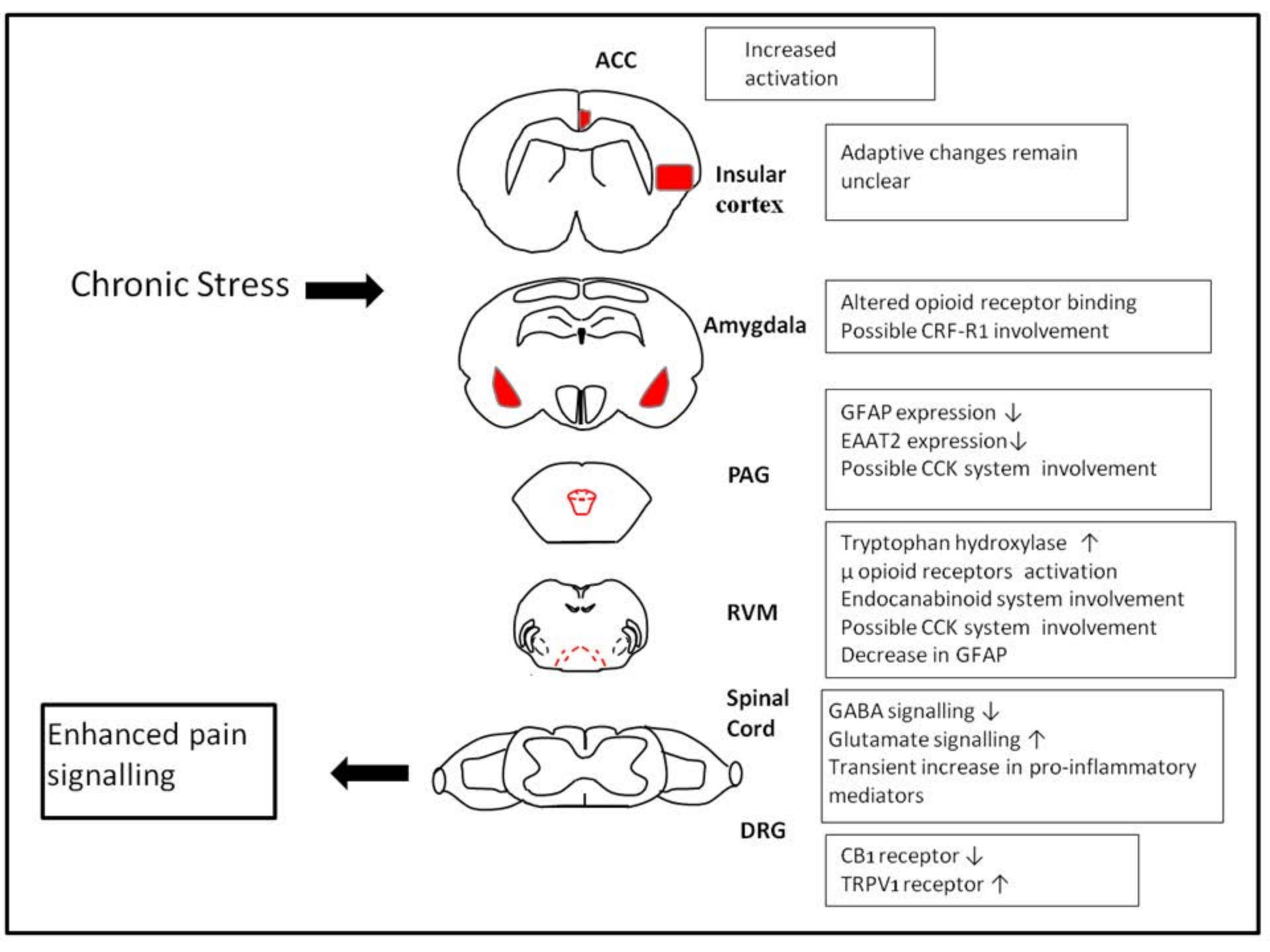
ACC
Increased activation
Insular cortex
Adaptive changes remain unclear
Chronic Stress
Amygdala
Altered opioid receptor binding
Possible CRF-R1 involvement
PAG
GFAP expression ↓
EAAT2 expression ↓
Possible CCK system involvement
RVM
Tryptophan hydroxylase ↑
μ opioid receptors activation
Endocanabinoid system involvement
Possible CCK system involvement
Decrease in GFAP
Spinal Cord
GABA signalling ↓
Glutamate signalling ↑
Transient increase in pro-inflammatory mediators
DRG
CB1 receptor ↓
TRPV1 receptor ↑
Enhanced pain signalling

Table 1. Summary of commonly used animal models of stress-induced hyperalgesia

Species, sex and Strain	Frequency/ Duration	Pain Test	Pain behaviour observations	Reference
Forced Swim-Stress				
Male Sprague-Dawley rats	Day 1: 10mins Day 2-3: 20mins	Formalin test and hot plate test	Inflammatory and thermal hyperalgesia	(Quintero et al. 2011; Quintero et al. 2003; Quintero et al. 2000; Suarez-Roca et al. 2008) (Quintero et al. 2000; Suarez-Roca et al. 2006a; Suarez-Roca et al. 2006b)
		Carrageenan intra-muscular injection followed by grip strength	Mechanical hyperalgesia as determined by reduced grip strength	(Suarez-Roca et al. 2006a)
Male Swiss albino mice	Two swim stress sessions 6mins duration, 8hrs apart	Hot plate	Thermal hyperalgesia	(Suaudeau and Costentin, 2000)
Male Wistar rats	5 minute sessions daily for 5 days	Tail flick test	Thermal hyperalgesia	(Fereidoni et al. 2007)
Adult female Swiss Webster mice	15 daily swims	Tail flick test and grip strength	Thermal and mechanical hyperalgesia	(Abdelhamid et al. 2013)
Male albino mice	6 minute sessions daily for 15 days	Tail immersion test	Thermal hyperalgesia	(Dhir and Kulkarni, 2008)

Repeated Cold Stress/SART				
Male 4-week old ddY mice	Over 7 days: Alternating 24˚C/ 4˚C every hour for 7 hours; 4˚C for final 17 hours	Randall-Selitto apparatus	Mechanical hyperalgesia (days 5-7)	(Ohara et al. 1991)
Male Sprague-Dawley rats	Over 5 days: Alternating 24˚C/ 4˚C or -3˚ every 30 mins for 7 ½ hours; 4˚C/-3˚ for final 16 ½ hours	Randall–Selitto test and the von Frey hair test	Mechanical hyperalgesia (greater in -3˚C group than 4˚C group)	(Nasu et al. 2010)
Male Wistar Rats	Over 5 days: Alternating 24˚C/-3˚ every hour for 4 hours; -3˚ for remaining 20 hours	Randall–Selitto test	Mechanical hyperalgesia	(Fujisawa et al. 2008)
Male Wistar rats	Over 5 days: Alternating 24˚C/ -3˚ every 2 hours for 6 hours; -3˚ for remaining 18 hours	Footshock on one of two floors	Decreased escape latency	(Kawanishi et al. 1997)
Restraint Stress				
Male and female (mixed estrous	Daily 1 hr restraint for 40 days	Tail flick test	Thermal hyperalgesia in males, no effect in females	(Gamaro et al. 1998)

phases) Wistar rats				
Male Wistar rats	Acute: 15mins, 30mins or 1hr restraint Subchronic: 1hr restraint for 3 days Chronic: 1h daily, 5 days per week for 40 days	Formalin injection into the temporomandibular joint (TMJ)	Increased inflammatory pain in chronic restraint stress rats	(Gameiro et al. 2006a)
Male Sprague-Dawley rats	Daily 1hr restraint for 4 days over for 5 weeks	von Frey, Randall-Selitto, Tail immersion test, Acetone-induced cold allodynia, Formalin test	Inflammatory, thermal and mechanical hyperalgesia	(Bardin et al. 2009)
Adult male Wistar rats	1hr daily restraints for 5 days per week over 8 weeks	Tail flick test [a] Formalin injection into TMJ [b]	Thermal and inflammatory hyperalgesia	(da Silva Torres et al. 2003[a];Gameiro et al. 2005[b])
Male Sprague-Dawley rats	6hr restraint once or over 1, 2 or 3 weeks	Tail flick test	Thermal hyperalgesia following 2 and 3 week restraint	(Imbe et al. 2004)
Male Sprague[a] Dawley Rats Male and female Wistar rats[b]	Acute restraint for 2hrs in restraint cage	Colorectal distension	Visceral hyperalgesia	(Ohashi-Doi et al. 2010[a], Eutamene et al. 2010[b])

Male Sprague Dawley rats	2hr restraint stress 4 days	Colorectal distension	Visceral hyperalgesia	(Shen et al. 2010)
Male Wistar rats	1hr restraint 5 days a week for 11 weeks	von Frey Test and hot plate	Mechanical allodynia and thermal hyperalgesia	(Spezia Adachi et al. 2012)
Immobilisation Stress				
Adult male Sprague Dawley rats	90 mins daily for 7 days	Tail-Flick test	Thermal hyperalgesia	(Costa et al. 2005)
Male ICR mice	1hr daily for 5 days	Formalin test	Inflammatory hyperalgesia	(Seo et al. 2006)
Social Defeat				
Male Sprague-Dawley Rats (Long Evans rats as intruder)	Four daily intruder sessions divided into two periods (see above)	von Frey, Randall–Selitto test and formalin test	Mechanical and inflammatory hyperalgesia	(Rivat et al. 2010)
Male Long Evans Rats	Resident rats were vasectomised prior to testing. Five daily intruder sessions divided into two periods (see above)	Formalin test [a], thermal preference and thermal escape tests[b]	Inflammatory and thermal hyperalgesia	(Andre et al. 2005b)[a] (Marcinkiewcz et al. 2009) [b]

Water Avoidance				
Male Wistar Rats	1hr per day for 10 consecutive days	Colorectal distension	Visceral hyperalgesia	(Bradesi et al. 2006; Bradesi et al. 2007; Bradesi et al. 2009; Bradesi et al. 2005; Larauche et al. 2008;Wang et al. 2013)
Male Sprague-Dawley rats	1hr per day for 10 consecutive days	von Frey test [a] , colorectal distension [b]	Mechanical and visceral hyperalgesia	(Chen et al. 2011[a] ; Green et al. 2011[b]
Adult male C57Bl/6 mice	1hr per day for 10 consecutive days	Colorectal distension	Visceral hyperalgesia	(Hong et al. 2009; Larauche et al. 2010)
Male Sprague-Dawley Rats	Over Three days: Tones played over four frequencies over 30 minute time period	Randall Selitto test [a,] Colorectal distension [b]	Mechanical and Visceral hyperalgesia	(Khasar et al. 2009a; Khasar et al. 2005[a] Green et al. 2011[b])
Chronic Mild Stress				
Male Wistar Rats	Unpredictable Chronic stress for 6 weeks;	von Frey and hot plate in normal and complete Freund's adjuvant chronic pain rat model and formalin test	Increased mechanical and thermal thresholds and inflammatory hyperalgesia	(Shi et al. 2010a)
Male Wistar Rats	Unpredictable Chronic stress for 6 weeks;	Hot Plate and von Frey tests in naive and SNL rats	Increased thermal and inflammatory pain thresholds for both normal and SNL rats	(Shi et al. 2010b)

Rotation Stress				
Male CBA/J mice	Rotational movement in spinning cages at 45rpm for 10mins every hour daily for 2 weeks	Formalin test	Inflammatory hyperalgesia	(Boccalon et al. 2006)
Maternal Separation /Deprivation/ Early life stress				
Wistar male rats [a] Sprague Dawley [b]male rats	Pups separated from mother for 180 minutes from days 2-14	Colorectal distension	Visceral hyperalgesia	(Chung et al. 2007a; Chung et al. 2007b [a] (Chung et al. 2007a; Chung et al. 2007b; Zhang et al. 2009a; Zhang et al. 2009b; Zhang et al. 2008 [b])
Long-Evans rats	Pups separated from mother for 180 minutes from days 2-14	Colorectal distension	Visceral hyperalgesia	(van den Wijngaard et al. 2012; Wouters et al. 2012;Welting et al. 2005)
Wistar male and female rats	24 hours MD on PND 9	Hot Plate, von Frey, acetone test and prior to and after spinal nerve ligation	Thermal hypoalgesia, mechanical allodynia in females	(Burke et al. 2013)
Sprague Dawley rats	Mother and pups are placed in cages fitted with a stainless steel mesh bottom on post-natal day 2 to 9.	Digital force transducer	Mechanical hyperalgesia	(Alvarez et al. 2013; Green et al. 2011)

Noise Stress				
Male Sprague Dawley rats	105 dB tone of mixed frequencies, ranging from 11 to 19 kHz over 30 minutes over 3 to 4 days	Paw-withdrawal threshold	Enhanced inflammatory pain	(Khasar et al. 2009; Khasar et al. 2005)
Vibration Stress				
Male and female Wistar rats	4 Hz applied to restraint tube for 5 min	Tail flick test	Hyperalgesia developed at 2-10 minutes after stress in male rats. Thermal hyperalgesia and female responding was oestrus dependent	(Devall et al. 2009; Devall and Lovick, 2010)
Whisker pad stimulation				
Male Sprague–Dawley rats	Light tactile whisker pad stimulation: 10 applications/ session, 4 sessions/h in 1 day, sessions on days 1–5 and 8–12	von Frey test	Mechanical hyperalgesia	(Reynolds et al. 2011)
Air stress				
Male Sprague–Dawley rats	Continuous stream of air at room temperature was directed at the face for 30 min	von Frey test	Mechanical hyperalgesia	(Wagner et al. 2013)
PTSD model				
Male Sprague–Dawley rats	2 hr restraint, 20 mins swim followed by 15 min rest, inhalation of an ether until unconscious	von Frey test and paw withdrawal to heat stimulus	Mechanical allodyina and thermal hyperalgesia	(Zhang et al. 2012)

Male Sprague–Dawley rats	2 hr restraint, 20 mins swim followed by 15 min rest, inhalation of an ether until unconscious then footshock when conscious	Colorectal distension	Visceral hyperalgesia	(He et al. 2013)