CHAPTER SIX

# The Role of the Brain's Endocannabinoid System in Pain and Its Modulation by Stress

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## Abstract

Stress has a complex, bidirectional modulatory influence on pain. Stress may either reduce (stress-induced analgesia) or exacerbate (stress-induced hyperalgesia) pain depending on the nature, duration, and intensity of the stressor. The endogenous cannabinoid (endocannabinoid) system is present throughout the neuroanatomical pathways that mediate and modulate responses to painful stimuli. The specific role of the endocannabinoid system in the brain in pain and the modulation of pain by stress is reviewed herein. We first provide a brief overview of the endocannabinoid system, followed by a review of the evidence that the brain's endocannabinoid system modulates pain. We provide a comprehensive evaluation of the role of the endocannabinoid system supraspinally, and particularly in the rostral ventromedial medulla, periaqueductal gray, amygdala, and prefrontal cortex, in pain, stress-induced analgesia, and stress-induced hyperalgesia. Increased understanding of endocannabinoid-mediated regulation of pain and its modulation by stress will inform the development of novel therapeutic approaches for pain and its comorbidity with stress-related disorders.

## ABBREVIATIONS

**2-AG** 2-arachidonoyl glycerol
**ABA** accessory basal nucleus
**AC** adenylate cyclase
**ACC** anterior cingulate cortex
**AEA** anandamide
**BLA** basolateral nucleus
**CB** cannabinoid
**$CB_1$** cannabinoid type 1
**$CB_2$** cannabinoid type 2
**CCI** chronic constriction injury
**CCK** cholecystokinin
**CeA** central nucleus of the amygdala
**CNS** central nervous system
**CUS** chronic unpredictable stress
**dlPAG** dorsolateral periaqueductal gray
**dmPAG** dorsomedial periaqueductal gray
**dPAG** dorsal periaqueductal gray
**eCB** endocannabinoid
**FAAH** fatty acid amide hydrolase
**FABP** fatty acid-binding protein
**FCA** fear-conditioned analgesia
**GiA** gigantocellular reticular nucleus
**HMBA** 4-hydroxy-3-methoxybenzylamine
**i.c.v.** intracerebroventricular
**IL** infralimbic cortex
**LA** lateral nucleus
**lPAG** lateral periaqueductal gray

**MAGL** monoacylglycerol lipase
**MAPK** mitogen-activated protein kinase
**MeA** medial nucleus
**mGlu** metabotropic glutamate receptors
**mPFC** medial prefrontal cortex
**NAPE** *N*-arachidonoyl phosphatidylethanolamine
**NGF** nerve growth factor
**NSAIDs** nonsteroidal anti-inflammatory drugs
**OEA** *N*-oleoylethanolamide
**OX** orexin
**PAG** periaqueductal gray
**PEA** *N*-palmitoylethanolamide
**PFC** prefrontal cortex
**PLD** phospholipase D
**PPARs** peroxisome proliferator-activated receptors
**PrL** prelimbic cortex
**RVM** rostral ventromedial medulla
**SD** Sprague Dawley
**SIA** stress-induced analgesia
**SIH** stress-induced hyperalgesia
**SNI** spared nerve injury
**SNL** spinal nerve ligation
**THC** $\Delta^9$-tetrahydrocannabinol
**TRPV1** transient receptor potential vanilloid 1
**vlPAG** ventrolateral periaqueductal gray
**VPL** ventral posterolateral nucleus of the thalamus
**WKY** Wistar Kyoto

## 1. INTRODUCTION

Pain can be defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (International Association for the Study of Pain [IASP] Task Force on Taxonomy, 1994). Recent data indicate that approximately 20% of the population suffer from chronic pain, the majority of whom also suffer from some other disability or mood disturbance (Blyth et al., 2001; Demyttenaere et al., 2007; Vos et al., 2012). Chronic pain is usually defined as pain persisting for over 3 months. It may be neuropathic, inflammatory, or idiopathic in nature (Aguggia, 2003). Epidemiological studies of 289 diseases and injuries concluded that chronic pain conditions were among the 10 conditions resulting in the longest number of years lived with disability (Vos et al., 2012). Current pharmacotherapies for pain management lack efficacy

in many patients, with ~40% of patients with chronic pain unsatisfied with their treatment (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006). Furthermore, the annual economic cost of pain in the United States has been estimated at a staggering $560–$625 billion annually, including direct and indirect costs (for review, see Gaskin & Richard, 2012; McCarberg & Billington, 2006; Turk, 2002). Despite the efforts of the research community and the pharmaceutical industry to invest in and develop new drugs to manage pain, chronic pain in particular continues to represent a major unmet clinical need. Thus, further research is needed to understand fully the neurobiological mechanisms of pain, and its modulation, with a view to identifying novel targets and developing new, superior analgesics.

Increasing evidence over the past two decades has demonstrated that the endogenous cannabinoid (endocannabinoid; eCB) system has a regulatory role in pain processing and perception (Woodhams, Sagar, Burston, & Chapman, 2015). This regulatory function is facilitated by the expression of the eCB signaling machinery at neuronal synapses within all components of the pain pathway. Activation of cannabinoid (CB) receptors on presynaptic nerve terminals generally functions to reduce neurotransmission, resulting primarily in antinociception/analgesia. However, depending on physiological and pathological state, the tissue concentration of eCBs and expression levels of eCB-sensitive receptors can vary (Alexander & Kendall, 2007; Woodhams et al., 2015), and with it the regulatory potential of this system on nociceptive processing.

The intensity and severity of perceived pain does not necessarily correlate with the degree of tissue damage, injury, or inflammation occurring. The importance of context and modulation of pain by emotion is now widely recognized. Stress, fear, and anxiety exert important modulatory influences on pain (Asmundson & Katz, 2009; Burke, Finn, & Roche, 2015; Butler & Finn, 2009; Fitzgibbon, Finn, & Roche, 2015; Ford & Finn, 2008; Jennings, Okine, Roche, & Finn, 2014; Okine et al., 2014; Rhudy & Meagher, 2000, 2001; Wiech & Tracey, 2009). Regardless of arousal level, positive emotions generally act to inhibit pain, while negative emotions with low to moderate arousal tend to enhance pain, and negative emotions with high arousal inhibit pain (de Wied & Verbaten, 2001; Dougher, 1979; Meagher, Arnau, & Rhudy, 2001; Rhudy & Meagher, 2000, 2001, 2003a, 2003b). Thus, a complex relationship exists between emotion and pain processing. CB receptors are localized in brain regions involved in the modulation of pain including the rostral ventromedial medulla (RVM), the periaqueductal gray (PAG), amygdala, and prefrontal

cortex (PFC) (Herkenham et al., 1991; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998) with these brain regions also key components of stress, fear, and anxiety circuitry. Stress and fear have been shown to alter levels of eCBs in these brain regions (Hill et al., 2013, 2005; Hohmann et al., 2005; Jennings et al., 2014; Olango, Roche, Ford, Harhen, & Finn, 2012; Patel, Cravatt, & Hillard, 2005; Rademacher et al., 2008) (for review, see Carrier, Patel, & Hillard, 2005; Morena, Patel, Bains, & Hill, 2015). Thus, the eCB system is an important common denominator in pain, stress, and fear and its role in the aforementioned brain regions in pain and the modulation of pain by stress is the main focus of this chapter.

We will consider the role of the supraspinal eCB system in acute and chronic pain, as well as its role in both stress-induced analgesia (SIA) and stress-induced hyperalgesia (SIH). The role of the spinal and peripheral eCB system in pain or stress–pain interactions is beyond the scope of this review but has been reviewed previously by ourselves and others (Butler & Finn, 2009; Finn, 2010; Hohmann & Suplita, 2006; Jennings et al., 2014; Maccarrone et al., 2015; Olango & Finn, 2014; Walker & Hohmann, 2005).

## 2. THE ENDOCANNABINOID SYSTEM

The medicinal properties of the *Cannabis sativa* plant have been known for millennia but it was not until the mid to late nineteenth century that its therapeutic potential was examined scientifically. The discovery of $\Delta^9$-tetrahydrocannabinol (THC), the major psychoactive component of the plant *C. sativa* in the 1960s (Mechoulam & Gaoni, 1967) led to extensive studies that have revealed the mechanisms underlying the physiological and pharmacological effects of the eCB system.

The eCB system as we know it today consists of CB type 1 ($CB_1$) receptors (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and CB type 2 ($CB_2$) receptors (Munro, Thomas, & Abu-Shaar, 1993), their endogenous ligands *N*-arachidonylethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995), and the enzymes responsible for their synthesis and degradation. AEA and 2-AG are the best characterized eCBs; however, there are a number of other endogenous ligands with affinity and activity at $CB_1$ and $CB_2$ receptors including 2-AG ether (noladin ether), virodhamine, *N*-arachidonyl dopamine, and others (for review, see Battista, Di

Tommaso, Bari, & Maccarrone, 2012; Di Marzo, 2008; Di Marzo, Stella, & Zimmer, 2015; Henry, Kerr, Finn, & Roche, 2015; Pertwee, 1997, 2001).

The CB receptors in the adult human brain and spinal cord are distributed in a heterogeneous fashion (Glass, Dragunow, & Faull, 1997). $CB_1$ receptors are the most abundant CB receptor subtype in the CNS (Glass et al., 1997; Herkenham et al., 1991; Pertwee, 1997), with particularly high density in brain regions that are key components of the descending inhibitory/facilitatory pain pathways and the stress/fear/anxiety circuitry. $CB_2$ receptors, although expressed in the CNS (Baek, Zheng, Darlington, & Smith, 2008; Concannon, Okine, Finn, & Dowd, 2015; Onaivi et al., 2006; Van Sickle et al., 2005; Zhang et al., 2014) are mainly distributed in the periphery with particularly high density on cells and tissues of the immune system (Berdyshev, 2000; Munro et al., 1993; Sugiura et al., 1995). $CB_1$ and $CB_2$ receptors are Gi/o protein-coupled receptors negatively coupled to adenylate cyclase (AC) (Howlett, 1985; Howlett, Mukhopadhyay, Shim, & Welsh, 1999) and positively coupled to mitogen-activated protein kinase (MAPK) (Bouaboula et al., 1995). Upon binding to $CB_1$ receptors, eCBs also inhibit N- and P/Q-type voltage-activated $Ca^{2+}$ channels and induce inwardly rectifying $K^+$ currents, resulting in inhibition of neurotransmitter release (Demuth & Molleman, 2006).

The biosynthetic pathways for AEA are not fully characterized but the best described mechanism involves the formation of AEA from the precursor *N*-arachidonoyl phosphatidylethanolamine (NAPE), due to the hydrolytic activity of the phospholipase D enzyme known as NAPE-PLD (Bisogno, Ligresti, & Di Marzo, 2005). 2-AG is synthesized almost exclusively by phospholipase C (PLC) hydrolysis producing 1,2-diacylglycerol which is then converted to 2-AG by diacylglycerol lipases (DAGL) (Di Marzo, 2008; Howlett & Mukhopadhyay, 2000). For a more complete discussion of the biosynthetic routes for AEA and 2-AG, please refer to chapter "The endocannabinoid signaling system in the CNS: A primer" by Hillard. AEA is primarily degraded to arachidonic acid and ethanolamine by the enzyme fatty acid amide hydrolase (FAAH), located in the endoplasmic reticulum of the postsynaptic neuron (Cravatt et al., 1996; Giang & Cravatt, 1997) (for review, see Otrubova, Ezzili, & Boger, 2011). FAAH also catabolizes additional *N*-acylethanolamines including *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA) which themselves do not have appreciable activity at $CB_1$ or $CB_2$ receptors but which can elevate levels of AEA through substrate competition at FAAH (Di Marzo et al., 1994; Sugiura et al., 1995). In contrast, 2-AG is primarily

metabolized to arachidonic acid and glycerol by the enzyme monoacylglycerol lipase (MAGL) (Ueda, Tsuboi, Uyama, & Ohnishi, 2011), with other enzymes including FAAH, ABHD6, and ABHD12 accounting for a modest degree of 2-AG catabolism (Blankman, Simon, & Cravatt, 2007; Goparaju, Ueda, Yamaguchi, & Yamamoto, 1998). FAAH is primarily a postsynaptic enzyme, whereas MAGL is presynaptic (Egertova, Cravatt, & Elphick, 2003; Gulyas et al., 2004; Tsou, Nogueron, et al., 1998) (for review, see Blankman & Cravatt, 2013; Di Marzo, 2008; Lichtman, Blankman, & Cravatt, 2010).

The mechanisms underlying eCB biosynthesis, signaling, and degradation are relatively well understood although controversy remains surrounding the mechanisms by which eCBs are transported across cell membranes. It has been proposed that due to their lipophilic nature, eCBs are readily transported via a simple diffusion mechanism (Glaser et al., 2003; Kaczocha, Hermann, Glaser, Bojesen, & Deutsch, 2006) while others suggest the existence of a protein-facilitated transport process (Beltramo & Piomelli, 2000; Hillard, Edgemond, Jarrahian, & Campbell, 1997). Most recently, a FAAH-like anandamide transporter has been described as the main mediator for AEA transport (Fu et al., 2012). Furthermore, fatty acid-binding proteins (FABPs) are small cytoplasmic lipid transport proteins (Furuhashi & Hotamisligil, 2008) located both peripherally (De Leon et al., 1996) and in the CNS (Yamamoto et al., 2009). FABP5 and FABP7 are capable of binding eCBs and regulating their signaling and catabolism by FAAH (Cravatt et al., 2001; Kaczocha, Glaser, & Deutsch, 2009; Kaczocha, Vivieca, Sun, Glaser, & Deutsch, 2012).

In addition to the two classical CB receptors ($CB_1$ and $CB_2$), several lines of evidence suggest that eCBs act at numerous other non-$CB_1$/non-$CB_2$ including the transient receptor potential vanilloid 1 (TRPV1), members of the nuclear receptor family of peroxisome proliferator-activated receptors (PPARs), and the G-protein-coupled receptors GPR55 and GPR119 (Alexander & Kendall, 2007; Brown, 2007; O'Sullivan, 2007).

## 3. THE ENDOCANNABINOID SYSTEM IN THE BRAIN REGULATES PAIN

Considerable effort has been invested in investigating the brain regions involved in mediating the antinociceptive effects of eCBs and CB receptor agonists. Later sections will discuss in more detail the role of the eCB system in individual brain regions in pain and its modulation by stress. Presented

in this section is an overview of the studies that have identified a role of the eCB system, supraspinally, in the modulation of pain (and summarized in Fig. 1).

Strong evidence of a role for the supraspinal eCB system in the modulation of pain was provided by Hohmann, Tsou, and Walker (1999). Here, systemic administration of the CB receptor agonist WIN55,212-2 resulted in an antinociceptive effect in the tail flick test in rats. Transection of the spinal cord and thus blockade of descending pain processes inhibited the CB-induced suppression of noxious heat-evoked activity in the tail flick test, thus indicating that WIN55,212-2 acted supraspinally to mediate its antinociceptive efficacy. This study paved the way for the investigation of the role of supraspinal sites in CB-induced antinociception.

Antinociceptive activity of CB receptor agonists had been demonstrated in the mouse and rat tail flick tests following intracerebroventricular (i.c.v.)

| Brain region | Receptor event | Functional consequence |
|---|---|---|
| ACC | $CB_1$ receptor activation | Decreased pain-related behavior/nociception |
| rACC | $CB_1$ receptor inhibition | Increased pain-related behavior/nociception |
| IL/PrL | $CB_1$ receptor activation coupled with TRPV1 inhibition | Decreased pain-related behavior/nociception |
| ACC | PPARα inhibition | Decreased pain-related behavior/nociception |
| BLA | $CB_1$ receptor inhibition | Decreased pain-related behavior/nociception |
| CeA | $CB_1$ receptor inhibition | Decreased pain-related behavior/nociception |
| CeA/BLA | $CB_1$ receptor activation | Decreased pain-related behavior/nociception |
| PAG | $CB_1$ receptor activation | Decreased pain-related behavior/nociception |
| PAG | TRPV1 activation, opposite at higher dose | Decreased pain-related behavior/nociception, opposite at higher dose |
| PAG | $CB_1$ receptor activation coupled with TRPV1 inhibition | Increased pain-related behavior/nociception |
| RVM | $CB_1$ receptor activation | Decreased pain-related behavior/nociception |
| GiA | $CB_1$ receptor activation | Decreased pain-related behavior/nociception |

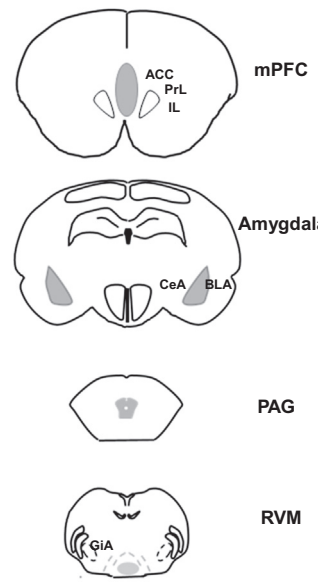


**Figure 1** A synthesis of the literature reviewed herein on the role of the supraspinal endocannabinoid system in discrete brain regions in pain. mPFC, medial prefrontal cortex; ACC, anterior cingulate cortex; PrL, prelimbic cortex; IL, infralimbic cortex; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; PAG, periaqueductal gray; RVM, rostral ventromedial medulla; GiA, gigantocellular reticular nucleus; TRPV1, transient receptor potential vanilloid 1; PPARs, peroxisome proliferator-activated receptors.

administration. Specifically, i.c.v. injection of the CB agonist WIN55, 212-2, $\Delta^9$-THC, and CP-55,940 produced antinociception in the rat tail flick test (Lichtman, Cook, & Martin, 1996; Martin, Lai, Patrick, Tsou, & Walker, 1993). In spinally transected rats, i.c.v. administration of the $CB_1$ receptor antagonist/inverse agonist rimonabant completely blocked the antinociceptive effects of $\Delta^9$-THC and CP-55,940 in the rat tail flick test, indicating that these effects are mediated through $CB_1$ receptors in the brain. The antagonist failed to block the effects of morphine, indicating its selectivity for CB receptors (Lichtman & Martin, 1997). However, when administered via the same route, i.c.v. administration of $\Delta^9$-THC enhances the antinociceptive potency of morphine (Welch, Thomas, & Patrick, 1995), suggesting a synergistic interaction between the opioid and CB systems. Similar to Martin et al. (1993), i.c.v. injection of WIN55,212-2 and THC produced dose-related antinociceptive effects in the mouse tail flick test (Raffa, Stone, & Hipp, 1999). In addition, i.c.v. administration of WIN55,212-2 induces antinociception in the mouse tail flick and paw withdrawal test (Fang et al., 2012). It was also shown that rimonabant has greater efficacy in the mouse tail flick test at a supraspinal rather than spinal level when blocking the action of THC, HU210, CP-55,940, and AEA (Welch, Huffman, & Lowe, 1998). Thus, taken together, supraspinal $CB_1$ receptors are important in modulating pain processes. Although i.c.v. administration of pharmacological agents is a useful means of investigating the contribution of the brain in general, alternative approaches are required to study the role of the eCB system in specific brain regions in pain and its modulation by stress. These approaches and the results obtained are discussed later in the review for each of the key brain regions that comprise the descending pain pathways (RVM, PAG, amygdala, and PFC). Additionally, less characterized mechanisms and targets will also be discussed toward the end of the review. Lines of evidence implicating the supraspinal eCB system in pain, stress, and their interaction will be considered briefly now.

## 4. THE MODULATION OF PAIN BY STRESS: ROLE FOR THE BRAIN'S ENDOCANNABINOID SYSTEM

Both painful (Alexander & Kendall, 2007; Kwilasz, Abdullah, Poklis, Lichtman, & Negus, 2014; Walker, Huang, Strangman, Tsou, & Sanudo-Pena, 1999; Woodhams et al., 2015) and aversive (stress/fear) (Hill et al., 2013, 2005; Hohmann et al., 2005; Jennings et al., 2014; Olango et al.,

2012; Patel et al., 2005; Rademacher et al., 2008) stimuli have been shown to alter eCB levels and expression of key components of the eCB system in supraspinal regions (for review, see Morena et al., 2015). As highlighted earlier, emotion and stress can profoundly impact on nociceptive processing, with chronic stress paradigms shown to enhance pain perception under a variety of experimental conditions. Chronic unpredictable stress (CUS), a widely used model for inducing anxiety and depressive-like behavior in mice, has been shown to enhance thermal (hot plate test) and mechanical (von Frey) hyperalgesia. It has also been shown to induce long lasting widespread hyperalgesia following intramuscular injection of nerve growth factor (NGF) (Lomazzo et al., 2015). The FAAH and MAGL inhibitors URB597 and JZL184 attenuated the CUS-induced anxiety-related behavior in the light–dark box and thermal hyperalgesia in the hot plate test. URB597 significantly reduced the widespread hyperalgesia induced by combining CUS and NGF in this study, while JZL184 had no significant effect. Both drugs enhanced the levels of AEA and 2-AG, respectively, in the midbrain and cingulate cortex (Lomazzo et al., 2015). These data highlight the strong potential for pharmacological inventions aimed at increasing eCB levels supraspinally in both anxiety- and pain-related disorders.

I.c.v. administration of rimonabant increases levels of the stress hormones, adrenocorticotropic hormone, and corticosterone, in rats, suggesting a role for supraspinal $CB_1$ receptors in the neuroendocrine response to stress (Manzanares, Corchero, & Fuentes, 1999). $CB_1(-/-)$ knockout mice develop normal mechanical hypersensitivity but more pronounced anxiety-related behavior following partial sciatic nerve ligation, indicating a potential role for the EC system in chronic comorbid pain/anxiety disorders (Racz, Nent, Erxlebe, & Zimmer, 2015). Indeed, the acquisition, expression, and extinction of fear-related behavior have all been shown to involve eCB signaling (see Chhatwal & Ressler, 2007). Our group has shown an interaction between the eCB and opioid systems in fear-conditioned analgesia (FCA). FCA was modeled by assessing formalin-evoked nociceptive behavior in an arena previously paired with footshock. Systemic administration of the FAAH inhibitor URB597 enhanced FCA in rats, an effect blocked by the $CB_1$ and $CB_2$ receptor antagonists rimonabant and SR144528, respectively (Butler, Rea, Lang, Gavin, & Finn, 2008). These findings corroborated and extended our earlier work demonstrating that $CB_1$ receptors play a key role in mediating FCA (Finn et al., 2004). The use of transgenic mice lacking components of the eCB system further implicates a role for eCBs in SIA (Valverde, Ledent,

Beslot, Parmentier, & Roques, 2000) and studies that have investigated the role of the EC system in specific brain regions in FCA/SIA will be discussed in detail below.

It has been shown that the neuropeptide cholecystokinin (CCK) plays a role in pain sensitivity via its regulation of opioid tone in the CNS. A recent study has demonstrated an interaction between CCK and ECs in the regulation of SIA (Kurrikoff, Inno, Matsui, & Vasar, 2008). Intraperitoneal rimonabant prevented SIA, in the tail flick test, in response to footshock in wild-type mice. SIA was present in CCK type 2 receptor-deficient mice regardless of rimonabant treatment while naloxone weakened SIA in both wild-type and CCK type 2 receptor-deficient mice. The $CCK_2$ receptor gene, along with genes implicated in eCB-mediated neurotransmission, were upregulated in the mesolimbic area of the brain. $CCK_2$ receptors may therefore modulate the action of eCBs. This study demonstrates a clear involvement of the $CCK_2$ receptor in eCB-mediated SIA.

See Table 1 for a summary of studies (excluding those focused on the RVM, PAG, amygdala, and PFC) investigating the role of the brain's eCB system in pain and its modulation by stress. Tables 2–5, Fig. 2, and the sections that follow below then deal with the role of the eCB system within the RVM (Table 2), PAG (Table 3), amygdala (Table 4), and PFC (Table 5) in pain and its modulation by stress.

## 5. THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE ROSTRAL VENTROMEDIAL MEDULLA IN PAIN, STRESS-INDUCED ANALGESIA, AND STRESS-INDUCED HYPERALGESIA

### 5.1 Pain

The RVM is made up of the nucleus raphe magnus, the nucleus gigantocellularis pars alpha (GiA), and the adjacent reticular formation; and is a major component of the descending inhibitory pain pathway (Meng, Manning, Martin, & Fields, 1998). $CB_1$ receptors have been shown to be expressed in the RVM using receptor autoradiography and immunohistochemistry (Glass et al., 1997; Herkenham et al., 1991; Herzberg, Eliav, Bennett, & Kopin, 1997; Mailleux, Parmentier, & Vanderhaeghen, 1992; Thomas, Wei, & Martin, 1992; Tsou, Brown, et al., 1998). The RVM contains ON and OFF cells which are involved in descending facilitation and inhibition of nociception, respectively (Vanegas, Barbaro, & Fields, 1984), and it projects to the dorsal horn of the spinal cord and the trigeminal

**Table 1** Summary of Studies Investigating the Role of the Brain's Endocannabinoid System in Pain and Its Modulation by Stress (Excluding Studies on RVM, PAG, Amygdala, and PFC Which Are Summarized in Tables 2–5)

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| $CB_1/CB_2$ agonist | WIN55,212-2 | i.v. | Rat | Spinal transection; tail flick | Inhibits noxious activity; blocked by spinal transection | Hohmann et al. (1999) |
| $CB_1/CB_2$ agonist | THC; CP-55,940 | i.c.v. | Rat | Spinal transection; tail flick | Antinociception; blocked by rimonabant | Lichtman and Martin (1991, 1997) |
| $CB_1/CB_2$ agonist | THC | i.c.v. | Mouse | Tail flick | Enhances the antinociceptive effects of morphine | Welch et al. (1995) |
| $CB_1/CB_2$ agonist | WIN55,212-2; CP-55,940 | i.c.v. | Rat | Tail flick | Antinociception | Martin et al. (1993) |
| $CB_1/CB_2$ agonist | WIN55,212-2; THC | i.c.v. | Mouse | Tail flick | Dose-related antinociception | Raffa et al. (1999) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | i.v. | Rats | Pressure stimulus to hindpaw; electrophysiological recording of nociceptive neurons in thalamus | Decrease in nociceptive transmission in the thalamus | Martin, Hohmann, and Walker (1996) |

| | | | | | | |
|---|---|---|---|---|---|---|
| $CB_1/CB_2$ agonist | WIN55,212-2 | Microinjection; GiA, thalamus, noradrenergic A5 region | Rats | Tail flick | Antinociception when administered to each region | Martin et al. (1999) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | i.c.v. | Mice | Tail flick; paw withdrawal in the lamp-foot-flick assay | Antinociception | Fang et al. (2012) |
| $CB_1$antagonist/ inverse agonist | Rimonabant | i.c.v.; i.t.; intraperitoneal | Mouse | Tail flick | Rimonabant exhibits greater efficacy supraspinally rather than spinally | Welch et al. (1998) |
| $CB_2$ agonist | JWH-133 | Intra-VPL | Rats | Spinal nerve ligation | Reduced noxious activity in SNL rats; blocked by SR144528 ($CB_2$ antagonist) | Jhaveri et al. (2008) |
| FAAH inhibitor | FAAH (−/−); URB597 PF3945 | | Mice | Nitroglycerin-induced migraine-like pain | FAAH (−/−), URB597, and PF3945 reduce nociceptive behavior; blocked by rimonabant | Nozaki, Markert, and Zimmer (2015) |
| | FAAH (−/−) | | Mice | Tail immersion, hot plate, formalin tests, CCI and carrageenan | FAAH (−/−) increased response latency in tail immersion and hot plate | Lichtman, Shelton, |

*Continued*

**Table 1** Summary of Studies Investigating the Role of the Brain's Endocannabinoid System in Pain and Its Modulation by Stress (Excluding Studies on RVM, PAG, Amygdala, and PFC Which Are Summarized in Tables 2–5)—cont'd

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| | | | | | test; reduced formalin-evoked nociceptive behavior | Advani, and Cravatt (2004) |
| FAAH inhibitor; MAGL inhibitor | URB597; JZL184 | i.p. | Mice | CUS; NGF hyperalgesia; tail flick; hot plate | Anti-hyperalgesic in carrageenan model | Lomazzo et al. (2015) |
| TRPV1 antagonist | A-784168 | i.c.v. | Rats | Model of osteoarthritis (sodium monoiodoacetate); complete Freund's adjuvant (chronic inflammatory pain) | Effects in CUS mice—enhanced levels of AEA and 2-AG; decreased thermal hyperalgesia; URB597 decreased NGF hyperalgesia | Cui et al. (2006) |
| TRPV1 antagonist | Capsazepine | i.c.v. | Mice | Formalin | Decreased weight bearing; decreased chronic inflammatory thermal hyperalgesia | Santos and Calixto (1997) |
| | GPR55(−/−) | | Mice | Inflammatory mechanical hyperalgesia (von Frey; complete Freund's adjuvant) | Attenuation of nociceptive behavior | Castane et al. (2006) and Staton et al. (2008) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | GPR55(−/−) | | Mice | Nerve ligation; mechanical hyperalgesia | Hyperalgesia absent in GPR55(−/−) | Staton et al. (2008) |
| PPARγ agonist | Rosiglitazone; 15d-PGJ(2) | i.c.v. | Rats | Plantar carrageenan model of inflammatory pain | Mechanical hyperalgesia absent following nerve ligation in GPR55(−/−) | Morgenweck et al. (2010) |
| | | | | SIA—footshock; tail flick | Anti-inflammatory and antihyperalgesia effects | Kurrikoff et al. (2008) |
| $CB_1$ antagonist/ inverse agonist; opioid antagonist | Rimonabant; naloxone; $CCK_2$ knockout mice | i.p. | Mice | | Rimonabant prevented SIA, an effect not seen in $CCK_2$ knockout mice; naloxone weakened SIA in wild-type and $CCK_2$ knockout mice | |
| FAAH inhibitor | URB597 | i.p. | Rats | FCA—conditioned fear (footshock) and formalin test | URB597 enhances FCA; attenuated by rimonabant, SR144528 and naloxone | Butler et al. (2008) |
| FAAH inhibitor | URB597 | intra-ventral hippocampus | Rats | FCA–conditioned fear (footshock) and formalin test | URB597 enhanced FCA, an effect blocked by rimonabant. | Ford et al. (2011) |

$CB_{1/2}$, Cannabinoid receptor type 1/2; i.v., intravenous; i.c.v., intracerebroventricular; GiA, nucleus reticularis gigantocellularis pars alpha; i.t., intra-thecal; intra-VPL, ventral posterolateral nucleus; FAAH, fatty acid amide hydrolase; SNL, spinal nerve ligation; (−/−), knock out; CCI, chronic constriction injury; MAGL, monoacylglycerol lipase; PPARα, peroxisome proliferator-activated receptor alpha; TRPV1, transient receptor potential cation channel subfamily V member 1; CUS, chronic unpredictable stress; NGF, nerve growth factor; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; GPR55, G protein-coupled receptor 55; CCK2, cholecystokinin 2; SIA, stress-induced analgesia; FCA, fear-conditioned analgesia.

**Table 2** Summary of Studies Investigating the Role of the Endocannabinoid System in the RVM in Pain and Its Modulation by Stress

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| $CB_1/CB_2$ agonists | WIN55,212-2; HU210 | Intra-RVM | Rat | Tail flick | Nociceptive behavior suppression | Martin, Tsou, and Walker (1998) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intra-RVM | Rat | Tail flick | Increased tail flick latency; inhibition of ON-cell activity; increase in OFF-cell activity; effects blocked by rimonabant | Meng and Johansen (2004) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intra-GiA | Rat | Tail flick | Increased antinociception; blocked by rimonabant | Monhemius, Azami, Green, and Roberts (2001) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intra-GiA | Rat | Partial nerve ligation; formalin test | Decrease in formalin-evoked nociceptive behavior following nerve ligation; reversed by rimonabant | Monhemius et al. (2001) |
| $CB_1$ antagonist/ inverse agonist; dual FAAH/ TRPV1 inhibitor | Rimonabant; AA-5-HT | Intra-RVM; | Rats | SIA—Footshock, Formalin | Suppression of SIA by rimonabant; enhancement of SIA by AA-5-HT | Suplita, Farthing, Gutierrez, and Hohmann (2005) |

| | | | | | | |
|---|---|---|---|---|---|---|
| $CB_1$ antagonist/ inverse agonist; FAAH inhibitor | AM251; URB597 | Intraperitoneal (AM251 and URB597); intra-RVM AM251 | Wistar Kyoto and Sprague Dawley rats | Formalin | Systemic AM251 potentiates hyperalgesia in WKY, URB597 attenuates hyperalgesia in WKY associated with impaired pain-related mobilization of ECs in RVM of WKY rats as seen from intra-RVM AM251 | Rea et al. (2014) |

$CB_{1/2}$, cannabinoid receptor type 1/2; RVM, rostral ventromedial medulla; GiA, nucleus reticularis gigantocellularis pars alpha; SIA, stress-induced analgesia; WKY, Wistar Kyoto; TRPV1, transient receptor potential cation channel subfamily V member 1; FAAH, fatty acid amide hydrolase.

**Table 3** Summary of Studies Investigating the Role of the Endocannabinoid System in the PAG in Pain and Its Modulation by Stress

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| $CB_1/CB_2$ agonist | HU-210 | vlPAG; systemic | Rat | Hot plate test | HU210 enhanced antinociceptive effect of morphine and morphine enhanced the antinociceptive effect of HU210 | Wilson-Poe, Pocius, Herschbach, and Morgan (2013) |
| $CB_1/CB_2$ agonist | HU210 | Intra-dPAG | Rat | Formalin | Reduced formalin-evoked nociceptive behavior; blocked following rimonabant administration | Finn et al. (2003) |
| $CB_1/CB_2$ agonist | CP-55,940 | Intra-vlPAG; intra-dPAG; | Rat | Tail flick | Intra-vlPAG microinjection produced antinociception; intra-dPAG had no effect | Lichtman et al. (1996) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intra-dPAG | Rats | Tail flick | Increased tail flick latency | Martin, Patrick, Coffin, Tsou, and Walker (1995) |
| $CB_1$ antagonist/inverse agonist | AM251 | Intra-PAG; intra-RVM | Rat | Metazinol-induced antinociception in a carrageenan model of inflammation | Reverses metazinol-induced analgesia | Escobar et al. (2012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| TRPV1 agonist | Capsaicin (low dose); capsaicin (high dose) | Intra-dlPAG | Rat | Plantar test | Low dose—antinociception; high dose—blocked antinociception | Palazzo et al. (2002) |
| TRPV1 agonist; TRPV1 antagonist | Capsaicin; capsazepine | Intra-dlPAG | Rat | Tail flick | Capsaicin—hyperalgesia followed by antinociception; capsazepine—blocked hyperalgesic effect of capsaicin | McGaraughty et al. (2003) |
| FAAH inhibitor | URB597 | Intra-vlPAG | Rat | Plantar test | Low dose—hyperalgesia—coadministration with AM251 converted hyperalgesia to analgesia—coadministration with capsazepine and AM251 abolished any effect of URB597; high dose—antinociception; intermediate dose—biphasic response – blocked by AM251, hyperalgesic following coadministration of URB597 with capsazepine | Maione et al. (2006) |

*Continued*

**Table 3** Summary of Studies Investigating the Role of the Endocannabinoid System in the PAG in Pain and Its Modulation by Stress—cont'd

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| Dual FAAH/TRPV1 inhibitor; $CB_1$ antagonist/inverse agonist; TRPV1 antagonist; FAAH inhibitor | AA-5-HT; AM251; I-RTX; URB597 | Intra-PAG | Rat | Tail flick | AA-5-HT-induced antinociception—blocked by AM251 and I-RTX; URB597 and I-RTX-induced analgesia | de Novellis et al. (2008) |
| $CB_1$ antagonist/inverse agonist; MAGL inhibitor | Rimonabant; URB602 | Intra-dlPAG | Rat | SIA—footshock and tail flick | Rimonabant-attenuation of SIA; URB602—enhances SIA | Hohmann et al. (2005) |
| $CB_1$ antagonist/inverse agonist; dual FAAH/TRPV1 inhibitor | Rimonabant; AA-5-HT | Intra-dlPAG | Rat | SIA—footshock and tail flick | Rimonabant-suppression of SIA; AA-5-HT—enhances SIA | Suplita et al. (2005) |
| $CB_1$ antagonist/inverse agonist | Rimonabant | Intra-dlPAG | Rat | FCA—formalin and footshock | Rimonabant attenuated FCA | Olango et al. (2012) |

$CB_{1/2}$, cannabinoid receptor type 1/2; TRPV1, transient receptor potential cation channel subfamily V member 1; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; PAG, periaqueductal gray; vlPAG, ventrolateral periaqueductal gray; dPAG, dorsal periaqueductal gray; RVM, rostral ventromedial medulla; dlPAG, dorsolateral periaqueductal gray; SIA, stress-induced analgesia; FCA, fear-conditioned analgesia.

**Table 4** Summary of Studies Investigating the Role of the Endocannabinoid System in the Amygdala in Pain and Its Modulation by Stress

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intra-BLA; intra-CeA | Rat | Tail flick | Increased tail flick latency | Martin et al. (1999) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intra-BLA | Rat | Formalin; tail flick | Increased tail flick latency; decreased formalin-evoked nociceptive behavior—attenuated via AM251 | Hasanein, Parviz, Keshavarz, and Javanmardi (2007) |
| $CB_1/CB_2$ agonist | WIN55,212-2; | Intra-CeA, intra-BLA | Rats | Tail flick | Antinociception | Manning, Martin, and Meng (2003) |
| $CB_1/CB_2$ agonist | WIN55,212-2 | Intramuscularly | Rhesus monkey | Warm-water tail-withdrawal assay | Dose-dependent antinociception; attenuated via bilateral amygdala lesions | Manning, Merin, Meng, and Amaral (2001) |
| $CB_1$ antagonist/ inverse agonist | Rimonabant | Intra-BLA | Rat | SIA—tail flick, footshock | Suppression of SIA | Connell, Bolton, Olsen, Piomelli, and Hohmann (2006) |
| $CB_1$ antagonist/ inverse agonist | Rimonabant | Intra-BLA | Rat | FCA—formalin, footshock | Reduced formalin-evoked nociceptive behavior; no effect on FCA | Roche, O'Connor, Diskin, and Finn (2007) and Roche et al. (2010) |

*Continued*

**Table 4** Summary of Studies Investigating the Role of the Endocannabinoid System in the Amygdala in Pain and Its Modulation by Stress—cont'd

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| FAAH inhibitor | URB597 | Intraperitoneal | Rat | FCA—formalin, footshock | URB597 enhances FCA; weakened by rimonabant and SR144528; FCA associated with enhanced phospho-ERK in the amygdala | Butler et al. (2008) |
| $CB_1$ antagonist/inverse agonist | AM251 | Intraperitoneal; intra-BLA; intra-CeA | Rat | FCA—formalin, footshock | AM251 prevents expression of FCA following intraperitoneal and intra-BLA but not intra-CeA injection | Rea et al. (2013) |

$CB_1$, cannabinoid receptor type 1; FAAH, fatty acid amide hydrolase; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; SIA, stress-induced analgesia; FCA, fear-conditioned analgesia.

**Table 5** Summary of Studies Investigating the Role of the Endocannabinoid System in the PFC in Pain and Its Modulation by Stress

| Class of Pharmacological Compound | Pharmacological or Genetic Intervention | Route of Administration | Species | Model | Effect | References |
|---|---|---|---|---|---|---|
| Dual FAAH/TRPV1 inhibitor | AA-5-HT | Intra-PrL; intra-IL | Rats | Noxious mechanical stimuli (Von Frey); SNI rats | Reduced mechanical allodynia in rats following SNI | Giordano et al. (2012) |
| Dual FAAH/TRPV1 inhibitor; FAAH inhibitor; TRPV1 antagonist | AA-5-HT; URB597; I-RTX | Intra-PrL; intra-IL | Rats | SNI rats | All drugs produced antinociception; AA-5-HT produced antinociception more efficiently than URB597 or I-RTX | de Novellis et al. (2011) |
| PPARα antagonist; PPARα agonist | GW6471; GW7647 | Intra-mPFC | Rats | Formalin | GW6471, but not GW7647, delayed onset of the second phase of formalin-evoked nociceptive behavior | Okine et al. (2014) |
| $CB_1$ antagonist/ inverse agonist | AM251 | Intra-PrL | Rat | FCA—tail flick, footshock | Attenuated FCA | Freitas, Salgado-Rohner, Hallak, Crippa, and Coimbra (2013) |

$CB_1$, Cannabinoid receptor type 1; TRPV1, transient receptor potential cation channel subfamily V member 1; FAAH, fatty acid amide hydrolase; PPARα, peroxisome proliferator-activated receptor alpha; SNI, spared nerve injury; mPFC, medial prefrontal cortex; IL, infralimbic cortex; PrL, prelimbic cortex; FCA, fear-conditioned analgesia.

| Brain region | Receptor event | Functional consequence |
|---|---|---|
| BLA | $CB_1$ receptor inhibition | Decreased SIA |
| Amygdala | eCB dysfunction in WKY rats | Decreased SIH |
| dlPAG | $CB_1$ receptor inhibition | Decreased SIA |
| dlPAG | $CB_1$ receptor activation | Increased SIA |
| dlPAG | $CB_1$ receptor activation coupled with TRPV1 inhibition | Increased SIA |
| RVM | $CB_1$ receptor inhibition | Decreased SIA |
| RVM | $CB_1$ receptor activation coupled with TRPV1 inhibition | Increased SIA |
| RVM | eCB dysfunction in WKY rats | Decreased SIH |

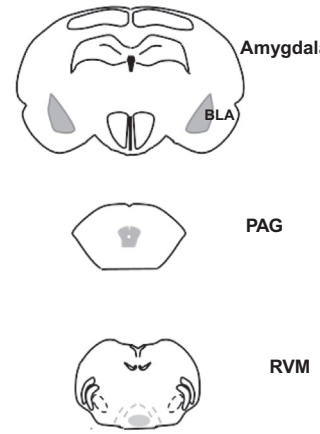


**Figure 2** A synthesis of the literature reviewed herein on the role of the supraspinal endocannabinoid system in discrete brain regions in stress-induced analgesia (SIA) and stress-induced hyperalgesia (SIH). eCB, endocannabinoid; BLA, basolateral amygdala; PAG, periaqueductal gray; dlPAG, dorsolateral PAG; RVM, rostral ventromedial medulla; TRPV1, transient receptor potential vanilloid 1; WKY, Wistar Kyoto rat.

nucleus to exert bidirectional control over nociception (Aicher, Hermes, Whittier, & Hegarty, 2012; Basbaum & Fields, 1984).

The RVM shares connections with the PAG, forming the PAG-RVM pathway (Basbaum & Fields, 1984). CBs activate descending analgesia via this pathway through a process of "GABA disinhibition." According to the GABA disinhibition hypothesis of analgesia, $CB_1$ receptor-mediated inhibition of GABAergic interneurons in the PAG and RVM results in disinhibition of projection neurons within the descending inhibitory pain pathway, resulting in analgesia (Basbaum & Fields, 1984; Lau & Vaughan, 2014; Szabo & Schlicker, 2005).

Microinjection of the CB receptor agonists WIN55,212-2 and HU210 into the RVM suppressed nociceptive behaviors in the tail flick test, an effect attenuated by coadministration with the $CB_1$ receptor antagonist rimonabant (Martin et al., 1998). Nociceptive behavior remained unchanged upon CB receptor agonist injection outside of the RVM. CBs induce antinociception by modulating neuronal activity in the RVM and inactivation of the RVM prevents CB-induced analgesia (Meng et al., 1998). As previously mentioned, ON cells in the RVM increase firing in response to painful stimuli, whereas OFF cells decrease firing, facilitating,

and inhibiting pain, respectively. Intra-RVM microinjection of WIN55,212-2 increases tail flick latencies while inhibiting ON-cell activity and increasing OFF-cell activity, thus decreasing nociception. Coinfusion with rimonabant blocked these effects, indicating a role for the eCB-$CB_1$ receptor system in the RVM in nociception (Meng & Johansen, 2004).

Microinjection of WIN55,212-2 into the GiA resulted in behavioral analgesia in the rat tail flick test, an effect blocked by coadministration of rimonabant (Monhemius et al., 2001). In the same study, animals with partial sciatic nerve ligation were given intra-GiA WIN55,212-2 and rimonabant and intraplantar formalin, contralaterally to the site of nerve ligation. Formalin-evoked nociceptive behavior was significantly reduced in partial nerve ligated rats, an effect reversed by microinjection of rimonabant into the GiA. This study demonstrated a role for the $CB_1$ receptor in GiA-mediated antinociception and modulation of nociceptive transmission in both acute pain and chronic neuropathic pain (Monhemius et al., 2001).

## 5.2 SIA

To our knowledge, only one study to date has investigated the role of the eCB system in the RVM in the modulation of pain by acute stress (SIA). Intra-RVM administration of rimonabant attenuated SIA in a rat model that combined footshock and a tail flick test. The FAAH inhibitor and TRPV1 antagonist AA-5-HT, administered systemically or intra-RVM, enhanced SIA in rats in a $CB_1$ receptor-dependent manner (Suplita et al., 2005). This study provides evidence for an important role of $CB_1$ receptors in the RVM in mediating and modulating SIA.

## 5.3 SIH

While there is evidence for a role of the RVM in SIH (for review, see Jennings et al., 2014), few studies have specifically investigated the role of the eCB system in the RVM in SIH. Genetic background plays a key role in determining the effect of stress on pain. The Wistar Kyoto (WKY) rat displays increased sensitivity to noxious stimuli and exhibits a depressive/anxiety-like phenotype and hypersensitivity to stress, compared with other rat strains including Sprague Dawley (SD) rats (Burke et al., 2010; O'Mahony et al., 2010). We have recently reported an impairment in pain-related mobilization of the eCBs AEA and 2-AG, along with their synthesizing enzymes, NAPE-PLD and DAGL, respectively, in the RVM of WKY rats compared with SD rats, following intraplantar injection of

formalin (Rea et al., 2014). Systemic administration of AM251 potentiated while systemic administration of the FAAH inhibitor URB597 attenuated hyperalgesia to formalin injection in WKY rats, but not SD rats, an effect mediated by $CB_1$ receptors in the RVM. These data suggest eCB dysfunction in the RVM underlies the hypersensitivity to noxious stimuli in WKY rat model of negative affective state (Rea et al., 2014).

See Table 2 for a summary of studies investigating the role of the eCB system in the RVM in pain and its modulation by stress.

## 6. THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE PERIAQUADUCTAL GRAY IN PAIN, STRESS-INDUCED ANALGESIA, AND STRESS-INDUCED HYPERALGESIA

### 6.1 Pain

The periaquaductal gray (PAG) is a midbrain/brainstem structure that can be divided into four columns along its rostro-caudal axis: dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (lPAG), and ventrolateral (vlPAG) columns (Bandler & Keay, 1996). Exposure to an aversive stimulus activates the descending inhibitory pain pathway, of which the PAG is a key component. The PAG, via the RVM, modulates nociceptive transmission at the level of the spinal cord (Fields, Heinricher, & Mason, 1991). The PAG possesses a larger density of CB receptors than other brainstem structures (Herkenham et al., 1991). CBs act in the PAG to inhibit GABAergic and glutamatergic synaptic transmission and to produce analgesia by a disinhibitory mechanism (Vaughan, Connor, Bagley, & Christie, 2000).

$CB_1$ receptor-mediated antinociception and increased levels of AEA were reported following electrical stimulation of the dorsal PAG (dPAG) and lPAG (Walker et al., 1999). These authors also showed that subcutaneous injection of formalin elicited a pain response in rats and substantially increased AEA levels in the PAG, measured by *in vivo* microdialysis. Increased levels of the eCBs, AEA, and 2-AG were also seen in the PAG and RVM of rats 7 days post chronic constriction injury (CCI) of the sciatic nerve, when hyperalgesia and mechanical allodynia were observed to be maximal (Petrosino et al., 2007).

Intra-vlPAG administration of morphine in rats enhanced the antinociceptive effect of the $CB_1$ receptor agonist HU-210 in the hot plate test (Wilson-Poe et al., 2013). Likewise, intra-vlPAG and systemic administration of HU-210 enhanced the antinociceptive effect of morphine (Wilson,

Maher, & Morgan, 2008). This study provides evidence for a dual role of morphine and CBs in pain and antinociception. Formalin-evoked nociceptive behavior was reduced following microinjection of HU210 into the dorsal PAG (dPAG) of rats, an effect blocked by coadministration with the $CB_1$ receptor antagonist rimonabant (Finn et al., 2003). Microinjection of CP-55,940 into the vlPAG, but not the posterior dlPAG or the anterior vlPAG, areas produces antinociception in the rat tail flick test (Lichtman et al., 1996). WIN55,212-2 increased tail flick latencies following microinjection into the rat dlPAG (Martin et al., 1995). Microinjection of WIN55,212-2 into the PAG increased the latency of the nociceptive response in the plantar test in rats, an effect blocked by coadministration with rimonabant. MPEP, a metabotropic glutamate receptor mGlu5 antagonist, also completely blocked the antinociceptive effect of WIN55,212-2 (Palazzo et al., 2001), indicating a $CB_1$-glutamatergic interaction in the PAG in mediating CB-induced analgesia.

Studies investigating the analgesic effect of the nonsteroidal anti-inflammatory drugs (NSAIDs) in supraspinal structures indicate a role for eCBs and $CB_1$ receptors in the PAG and RVM. Inflammation-induced hyperalgesia can be attenuated by microinjection of the NSAID metazinol into the PAG (Vazquez, Escobar, Ramirez, & Vanegas, 2007). Injection of the $CB_1$ receptor antagonist AM251 into the PAG or RVM reverses metazinol-induced analgesia, suggesting a role for the eCB system in these brain regions in NSAID-induced analgesia (Escobar et al., 2012).

TRPV1, a target of AEA, is expressed in the PAG (Palazzo, Rossi, & Maione, 2008) and a role for TRPV1 in pain modulation in the PAG has also been demonstrated. Intra-dlPAG injection of the TRPV1 agonist capsaicin increased the latency of nociceptive responses in the rat plantar test (Palazzo et al., 2002). A higher dose administered to the same region produced opposite effects, decreasing the latency of nociceptive responses and inducing hyperalgesia followed by analgesia (McGaraughty et al., 2003). Similar to Palazzo et al. (2002), intra-vlPAG administration of capsaicin also increased the latency of nociceptive responses in the hot-plate responses in rats (Liao, Lee, Ho, & Chiou, 2011) (for review, see Starowicz, Nigam, & Di Marzo, 2007). Thus, TRPV1 agonism in the PAG elicits antinociceptive effects in several pain models. For a recent review, see Madasu, Roche, and Finn (2015).

Intra-vlPAG injection of the FAAH inhibitor URB597 produced a robust hyperalgesic response at low doses, an analgesic response at high doses, and a biphasic effect on nociception at intermediate doses, in the

rat plantar test (Maione et al., 2006). AEA and 2-AG levels were increased in a dose-dependent manner following URB597 administration into the vlPAG. Coadministration of a low dose of URB597 with the $CB_1$ receptor antagonist AM251 converted the hyperalgesic effect to an analgesic one, while coadministration of URB597 with both the TRPV1 antagonist capsazepine and AM251 abolished all effects. In comparison, the early hyperalgesic effect of the intermediate dose of URB597 was blocked by AM251, while the later URB597-induced analgesic effect became hyperalgesic following TRPV1 antagonism. $CB_1$ receptor-dependent analgesia was seen at the highest dose of intra-vlPAG URB597 administration (Maione et al., 2006). The URB597-induced antinociceptive effects (TRPV1-mediated) and pronociceptive effects ($CB_1$ receptor mediated) were associated with enhanced or reduced RVM OFF cell activity, respectively, suggesting URB597-induced alteration in the activity of excitatory PAG output neurons. This study indicates a role for both $CB_1$ and TRPV1 receptors in the eCB-mediated control of the descending pain pathway.

Diabetes is frequently associated with neuropathy, with many patients suffering from hyperalgesia or allodynia. A role for TRPV1 and $CB_1$ receptors in the PAG has been proposed in diabetic thermal hyperalgesia (Mohammadi-Farani, Sahebgharani, Sepehrizadeh, Jaberi, & Ghazi-Khansari, 2010). Intra-vlPAG administration of capsaicin and WIN produced antinociception in the hot plate test of nondiabetic mice (Mohammadi-Farani et al., 2010). In contrast, the antinociceptive effects of intra-vlPAG capsaicin and WIN were reduced in hyperalgesic diabetic mice, an effect associated with $CB_1$ upregulation and TRPV1 downregulation in the vlPAG (Mohammadi-Farani et al., 2010). Taken together, the data demonstrate that diabetic neuropathy is associated with altered eCB signaling in the PAG, effects which may underlie the associated hyperalgesia and allodynia.

Systemic administration of the FAAH inhibitor and TRPV1 antagonist AA-5-HT produced antinociceptive effects in both rats and mice treated with formalin and in rats with CCI of the sciatic nerve (Maione et al., 2007), effects associated with increased levels of AEA in both the PAG and RVM. These antinociceptive effects were blocked by both $CB_1$ receptor and TRPV1 antagonists. Intra-vlPAG injection of AA-5-HT increased eCB levels and induced a pronociceptive effect at low doses and an antinociceptive effect at higher doses in the rat tail flick test (de Novellis et al., 2008). These effects were blocked by antagonism of vlPAG $CB_1$ receptors (AM251) or TRPV1 (I-RTX). Furthermore, administration of

the FAAH inhibitor URB597 with the TRPV1 antagonist I-RTX into the vlPAG also induced antinociceptive effects in the rat tail flick test and inhibited RVM ON- and OFF-cell activity (de Novellis et al., 2008), thus indicating that the antinociceptive effects of FAAH substrates in the vlPAG may be mediated by $CB_1$ receptors. In the formalin test of inflammatory pain, intra-PAG AA-5-HT prevented the changes in the ON and OFF cell firing activity induced by intraplantar injection of formalin. Since $CB_1$ and TRPV1 antagonists blocked the effects of AA-5-HT (de Novellis et al., 2008), it suggests that these two eCB receptors in the PAG may be responsible for AA-5-HT-induced analgesia. Furthermore, intra-PAG administration of the GPR55 agonist LPI reduced the nociceptive threshold in the rat hot plate test, an effect blocked upon pretreatment with the GPR55 antagonist ML-193 (Deliu et al., 2015). This study suggests that altering GPR55 activity in the PAG may affect nociceptive behaviour. Taken together these studies suggest that $CB_1$ receptors, TRPV1 and GPR55 in the PAG all play important roles in modulating pain behavior.

Orexin (OX) A and B are peptides and endogenous agonists for the OX1 and OX2 receptors which are localized in the lateral and perifornical area of the hypothalamus (de Lecea et al., 1998; Sakurai et al., 1998; Tsujino & Sakurai, 2009). They exert antinociceptive effects (Chiou et al., 2010) including following direct administration into the PAG (Azhdari Zarmehri et al., 2012). Orexin A decreases GABA release in an eCB-dependant manner in the vlPAG. Activation of OX receptors in the vlPAG leads to antinociception, measured electrophysiologically in brain slices. Intra-vlPAG microinjection of orexin-A reduced hot-plate nociceptive responses in rats in a manner blocked AM 251 (Ho et al., 2011).

## 6.2 SIA

A number of studies have demonstrated an important role for the eCB system in the PAG in SIA/FCA. Intra-dPAG administration of the $CB_1$ receptor antagonist rimonabant attenuated SIA, observed as an increase in the tail flick latency following exposure of rats to footshock stress (Hohmann et al., 2005). The same dose of this drug administered i.c.v., intra-vlPAG, and intra-lPAG had no effect on SIA in this study, highlighting a particular role of $CB_1$ receptors in the dlPAG in mediating SIA. Increased levels of 2-AG were seen in the dlPAG directly after footshock stress, implicating this eCB in the dlPAG in SIA. Moreover, inhibition of the 2-AG-degrading enzyme MAGL in the dlPAG using URB602 enhanced SIA (Hohmann et al., 2005).

A subsequent study by the same group confirmed the $CB_1$ receptor-dependant attenuation of SIA following intra-dlPAG administration of rimonabant and the $CB_1$-dependant enhancement of SIA following AA-5-HT administration (Suplita et al., 2005). These studies provide evidence that the PAG is a key neural substrate for eCB-mediated SIA. Another follow-up study from this group showed that mGlu5 receptor activation mobilizes 2-AG in the dlPAG to produce SIA in rats (Gregg et al., 2012). Thus, unconditioned SIA mediated by $CB_1$ receptor stimulation in the PAG is under the control of glutamatergic neurotransmission via mGlu5 receptors.

Our group has reported a role for the eCB system in the PAG in a model of SIA associated with conditioned learned fear (FCA) (Olango et al., 2012). FCA in these studies was measured as the reduction of formalin-evoked nociceptive behavior upon re-exposure of rats to a conditioning arena previously paired with footshock. Systemic administration of the FAAH inhibitor URB597 enhanced FCA, an effect associated with reduced phospho-ERK1/2 expression in the PAG (Butler et al., 2008). FCA was attenuated by intra-dlPAG administration of rimonabant (Olango et al., 2012), confirming a role for $CB_1$ receptors in the dlPAG in mediating both conditioned and unconditioned forms of SIA.

### 6.3 SIH

While there is evidence for a role of the PAG in SIH (for review, see Jennings et al., 2014), there is currently a paucity of studies addressing the role of the eCB system in the PAG in SIH and this is an area that warrants investigation.

See Table 3 for a summary of studies investigating the role of the eCB system in the PAG in pain and its modulation by stress.

## 7. THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE AMYGDALA IN PAIN, STRESS-INDUCED ANALGESIA, AND STRESS-INDUCED HYPERALGESIA

### 7.1 Pain

The amygdala is a key region of the limbic system located in the medial temporal lobe. It contains a number of different nuclei including the lateral nucleus (LA), basolateral nucleus (BLA), the central nucleus (CeA), accessory basal nucleus (ABA), and the medial nucleus (MeA). The amygdala plays a key role in the interaction between pain and emotion. The CeA, in

particular, is involved in the emotional-affective component of persistent pain (Neugebauer, Galhardo, Maione, & Mackey, 2009; Neugebauer, Li, Bird, & Han, 2004), while the BLA may be involved in the modulation of acute or tonic nociceptive processing (Oliveira & Prado, 1998). The amygdala is a key region of the ascending and descending pain pathways and shares connections with other key regions including the PFC and PAG. Pain-related changes have been identified in the amygdala in animals and humans using PET and fMRI neuroimaging studies (Neugebauer et al., 2004).

All components of the eCB system are expressed in the amygdala, although $CB_1$ receptors are expressed in highest density in the BLA (Herkenham et al., 1991; Tsou, Brown, et al., 1998). The amygdala contributes to the antinociceptive effects produced by systemically administered CBs. WIN55,212-2 produces dose-dependent antinociceptive effects in rats characterized as increased tail flick latencies (Manning et al., 2003). Intra-CeA, but not intra-BLA, administration of muscimol, significantly attenuated these antinociceptive effects of systemically administered WIN55, 212-2. Moreover, unilateral CeA inactivation via muscimol reduced the suppression of formalin-evoked c-Fos expression by WIN55,212-2 in the superficial dorsal horn of the spinal cord but not in the deeper "nociceptive" laminae (Manning et al., 2003). Another study from the same group found that the amygdala also plays a role in antinociception in non-human primates (Manning et al., 2001). WIN55,212-2 produced dose-dependent analgesia in rhesus monkeys. Bilateral lesions to the amygdala of the monkeys significantly reduced CB-induced analgesia. Both of these lesion studies indicate that the eCB system in the amygdala, in particular the CeA, can mediate antinociceptive effects.

Tail flick latencies have been shown to be increased upon microinjection of WIN55,212-2 into the CeA and BLA in rats (Hasanein et al., 2007; Martin et al., 1999). Furthermore, intra-BLA administration of WIN55,212-2 has also been shown to reduce formalin-evoked nociceptive behavior in rats, an effect attenuated by intra-BLA administration of the $CB_1$ receptor antagonist AM251 (Hasanein et al., 2007). Interestingly, intra-BLA administration of rimonabant has also been shown to attenuate formalin-evoked nociceptive behavior and associated increases in c-Fos immunoreactivity in the hippocampus and RVM in rats (Roche et al., 2007, 2010), although intra-BLA administration of a different $CB_1$ receptor antagonist, AM251, did not exert a similar effect (Rea et al., 2013).

Using fMRI, it has been shown that the amygdala may play a role in the modulation of pain perception by $\Delta^9$-THC in humans (Lee et al., 2013).

Cutaneous ongoing pain and hyperalgesia induced by capsaicin were monitored in healthy cannabis-naive volunteers. $\Delta^9$-THC reduced "painfulness" but not the intensity of pain and hyperalgesia, an effect positively correlated with amygdala activity. A $\Delta^9$-THC-related reduction in sensory-limbic functional activity was also seen between the amygdala and primary sensorimotor areas (Lee et al., 2013).

While the evidence points to a clear role for the eCB system in the amygdala in antinociception, there is a paucity of studies investigating its impact on the emotional aspect of pain. As a region with a clear role for the interaction between pain and emotion, it is necessary to further investigate this area and the role of the eCB system therein.

## 7.2 SIA

The amygdala plays a role in both unconditioned and conditioned SIA (Helmstetter, 1992; Helmstetter & Bellgowan, 1993; Helmstetter, Bellgowan, & Poore, 1995; Werka, 1994, 1997; Werka & Marek, 1990). Intra-BLA microinjection of rimonabant has been shown to suppress unconditioned SIA in rats exposed to footshock stress and then tested in the tail flick test, whereas intra-CeA microinjection had no effect on this form of SIA (Connell et al., 2006). Intra-BLA administration of FAAH and MAGL inhibitors, however, had no effect on SIA (Connell et al., 2006), suggesting that $CB_1$ receptors in the BLA, but not CeA, mediate SIA, although the role of the individual eCBs requires further investigation. Roche et al. (2007, 2010) reported no effect of unilateral or bilateral intra-BLA administration of rimonabant on FCA in rats (Roche et al., 2010, 2007). However, a subsequent study showed that the expression of FCA in rats was reduced following systemic or intra-BLA, but not intra-CeA, administration of a different $CB_1$ receptor antagonist, AM251 (Rea et al., 2013).

URB597 enhances the expression of FCA when administered via the intraperitoneal route, an effect blocked by $CB_1$, $CB_2$, or μ-opioid receptor antagonists (Butler et al., 2008). Interestingly, FCA in this study was associated with increased expression of phospho-ERK2 in the amygdaloid complex. In contrast, the URB597-induced enhancement of FCA was associated with reduced phospho-ERK1 and phospho-ERK2 expression in the amygdala. This dichotomy is not consistent with a causal role of ERK signaling in FCA (Butler et al., 2008).

$CB_1$ receptors are expressed on GABAergic and glutamatergic neurons in the BLA (Herkenham et al., 1991; Katona et al., 2001). Expression of FCA in rats was reduced following systemic or intra-BLA, but not intra-

CeA, administration of the $CB_1$ receptor antagonist AM251 (Rea et al., 2013), an effect attenuated by intra-BLA administration of both the $GABA_A$ receptor antagonist, bicuculline, and the mGlu5 receptor antagonist, MPEP, suggesting that $CB_1$ receptors in the BLA facilitate the expression of FCA, through a mechanism which is likely to involve the modulation of GABAergic and glutamatergic signaling. FCA was associated with increased levels of AEA in the left BLA (side contralateral to intraplantar formalin injection). Fear-conditioned, formalin-treated rats displayed increased levels of 2-AG and PEA in the left and right BLA, respectively (Rea et al., 2013).

It is clear, therefore, that the eCB system in the amygdala, and specifically the BLA, plays an important role in mediating both unconditioned and conditioned SIA with likely interactions with GABAergic and glutamatergic signaling.

### 7.3 SIH

A recent study from our group investigated the effects of repeated exposure to forced swim stress on formalin-evoked nociceptive behavior in rats in stress normo-responsive (SD) and stress hyper-responsive (WKY) rat strains. Formalin-evoked nociceptive behavior was increased in SD rats following 10 days of forced swim stress (Jennings, Okine, Olango, Roche, & Finn, 2015). AEA levels were reduced in the contralateral amygdala (relative to formalin injection) of SD rats but not WKY rats. There were also strain differences in components of the eCB system within the amygdala. For example, decreased levels of AEA and 2-AG were observed in the ipsilateral amygdala of SD, but not WKY, rats. Lower levels of $CB_1$ receptor mRNA were seen in the ipsilateral, but not contralateral, amygdala of WKY rats. These data indicate a role for the eCB system in the amygdala in SIH as well as implicating it in the strain differences seen in WKY and SD rats (Jennings et al., 2015). Additional studies are warranted to fully understand the role of the eCB system in the amygdala in SIH.

See Table 4 for a summary of studies investigating the role of eCB system in the amygdala in pain and its modulation by stress.

## 8. THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE PREFRONTAL CORTEX IN PAIN, STRESS-INDUCED ANALGESIA, AND STRESS-INDUCED HYPERALGESIA

### 8.1 Pain

The PFC is involved in both the top-down descending modulation of pain and also in the affective dimension of the pain experience. The medial PFC

(mPFC) is comprised of the prelimbic cortex (PrL), infralimbic cortex (IL), and anterior cingulate cortex (ACC). Imaging studies have shown that the PFC is consistently activated by noxious stimuli (Casey, Minoshima, Morrow, & Koeppe, 1996; Davis, Taylor, Crawley, Wood, & Mikulis, 1997; Derbyshire et al., 1997; May et al., 1998; Millan, 1999; Neal, Pearson, & Powell, 1990; Svensson, Minoshima, Beydoun, Morrow, & Casey, 1997). $CB_1$ receptors are expressed in the PFC (Herkenham et al., 1991; Sim-Selley, Vogt, Vogt, & Childers, 2002; Tsou, Brown, et al., 1998). This, along with its projections to the PAG and amygdala (Diorio, Viau, & Meaney, 1993; Little & Carter, 2013; Marchand & Hagino, 1983), suggests a role for the EC system in the PFC in pain.

$CB_1$ receptors in the rodent mPFC are expressed on GABAergic interneurons (Marsicano & Lutz, 1999; Wedzony & Chocyk, 2009). $CB_1$ receptors on presynaptic axon terminals face pyramidal neurons with postsynaptic mGluR5 (Lafourcade et al., 2007). A rat arthritis pain model, induced via intra-articular injections of kaolin and carrageenan through the patellar ligament, shows hyperactivity in amygdala output neurons and abnormal inhibition of mPFC pyramidal neurons (Ji et al., 2010). Another study investigated the effect of mGluR5 and $CB_1$ receptor activation on the activity of the mPFC cells in rats in the previously described arthritis pain model (Ji & Neugebauer, 2014). Coactivation of mGluR5 and $CB_1$ receptors increased mPFC activity and inhibited pain-related neuronal activity in the CeA in the arthritis pain model. Thus, there appears to be an inverse link between activation of mPFC neurons and amygdala output and a role for the eCB system in this top-down cortical control (Ji & Neugebauer, 2014). Further evidence for a role of the eCB system in the PFC in arthritic conditions comes from work demonstrating that osteoarthritis pain is associated with increased 2-AG levels in the PFC of mice in the monosodium iodoacetate model of arthritis (La Porta et al., 2015).

$CB_1$ receptor activity is decreased in the rostral ACC 10 days post CCI in mice, compared with sham controls (Hoot et al., 2010). $CB_1$ receptor levels in the rostral ACC of CCI and sham rats remained unchanged and there were no significant differences in the levels of 2-AG or AEA in the ACC between CCI and sham-operated mice. The ACC is associated with the affective component of pain (Kulkarni et al., 2005; Kuo, Chiou, Liang, & Yen, 2009; LaBuda & Fuchs, 2005; Treede, Kenshalo, Gracely, & Jones, 1999). It is possible therefore that reduced $CB_1$ receptor activity in the ACC is associated with the negative affective component of neuropathic pain.

TRPV1 expression is increased, in glutamatergic neurons, in the mPFC (namely the PrL and IL) following spared nerve injury (SNI) (Giordano et al., 2012). Intra-PL/IL administration of AA-5-HT reduced mechanical allodynia in rats following SNI to a greater extent than that seen with a FAAH inhibitor or TRPV1 antagonist alone (Giordano et al., 2012). SNI-induced neuropathic pain is also associated with increased levels of endovanilloids and eCBs in the mPFC. Intra-PrL/IL injection of AA-5-HT produced antinociceptive effects more efficiently (de Novellis et al., 2011). These studies suggest that both the eCB and endovanilloid systems in the mPFC may play a role in neuropathic pain. Therapies which target both of these systems may prove useful in the treatment of chronic neuropathic pain.

We have studied the role of PPARα in the mPFC in formalin-evoked nociceptive behavior in rats. The PPARα antagonist GW6471 delayed the onset of the second phase of formalin-evoked nociceptive behavior. This reduction in nociceptive behavior was associated with a reduction in the levels of *N*-palmitoylethanolamide and *N*-oleoylethanolamide (PPARα ligands) in the mPFC (Okine et al., 2014). Together these data suggest a facilitatory role for PPARα in the mPFC in formalin-evoked nociceptive behavior.

## 8.2 SIA

Lesion studies have indicated a role for the PFC in acquisition, consolidation, and extinction of conditioned fear in rodents (Sierra-Mercado, Corcoran, Lebron-Milad, & Quirk, 2006). This region has also been shown to project to other regions important in fear neurocircuitry, including the previously discussed amygdala and PAG (LeDoux, 2000). $CB_1$ receptors in the PrL cortex are involved in the amplification of panic-like aversive reactions and SIA. Thus, microinjection of bicuculline into the dorsomedial and ventromedial hypothalamus-induced aversive panic-like behavior and SIA, an effect attenuated by microinjection of AM251 into the PrL (Freitas et al., 2013). This work suggests that $CB_1$ receptor signaling in the PrL may facilitate or augment SIA induced by stimulation of the hypothalamus. Further investigation of the roles of the eCB system in the PrL, IL, and ACC in SIA and FCA is warranted.

## 8.3 SIH

To our knowledge, there have been no published studies to date investigating the role of the eCB system in the PFC in SIH.

See Table 5 for a summary of studies investigating the role of the eCB system in the PFC in pain and its modulation by stress.

## 9. LESS CHARACTERIZED SUPRASPINAL ENDOCANNABINOID MECHANISMS IN PAIN MODULATION

Systemic administration of the CB receptor agonist WIN55,212-2 dose-dependently inhibited stimulus-evoked activity, in the form of graded pressure stimuli to the paw, of nociceptive neurons in the ventroposterolateral thalamus (VPL) of anesthetized rats (Martin et al., 1996). Further evidence for a role of $CB_1$ receptors in the thalamus in mediating and modulating nociceptive responding was observed following microinjection of WIN55,212-2 into the thalamus which resulted in antinociceptive effects in the tail flick test in rats (Martin et al., 1999). Similar effects were observed following microinjection into the alpha part of the gigantocellular reticular nucleus (GiA) and the noradrenergic A5 region. Furthermore, intralocus coeruleus microinjection of the hypothalamic peptide orexin-A decreased formalin-evoked nociceptive behavior in rats (Mohammad-Pour Kargar, Azizi, Mirnajafi-Zadeh, Reza Mani, & Semnanian, 2015), an effect reversed following pretreatment with either the OX1 receptor antagonist SB-334867 or the $CB_1$ receptor antagonist AM251. Intra-locus coeruleus microinjection of SB-334867 and AM251 alone induced hyperalgesia (Mohammad-Pour Kargar et al., 2015). The results from this study suggest a new mechanism by which orexin-A modulates nociceptive information in the locus coeruleus via interaction with $CB_1$ receptors.

There is now increasing evidence supporting the role of $CB_2$ receptors in the supraspinal modulation of pain (for review, see Guindon & Hohmann, 2008). For example, microinjection of the $CB_2$ receptor agonist JWH-133 into the ventral posterolateral nucleus of the thalamus (VPL) has been shown to reduce noxious activity, recorded with a multichannel electrode array in VPL neurons, in a rat model of neuropathic pain (spinal nerve ligation; SNL) (Jhaveri et al., 2008). No significant differences in the levels of eCBs in the thalamus of SNL rats compared to sham rats were observed (Jhaveri et al., 2008). The results from this study suggest that $CB_2$ receptors in the thalamus may contribute to the modulation of neuropathic pain responses.

The eCB system has also been proposed to play a role migraine-related pain (for review, see Greco, Gasperi, Maccarrone, & Tassorelli, 2010;

Russo, 2004; Smith & Wagner, 2014). FAAH-deficient mice (FAAH (−/−)) express less nitroglycerin-induced migraine-like pain, with similar effects observed following pharmacological inhibition of FAAH inhibitors using URB597 and PF3945. Administration of the $CB_1$ receptor antagonist rimonabant blocked these antinociceptive effects in this migraine model, demonstrating a key role for $CB_1$ receptors in mediating the effects of the FAAH substrates (i.e., AEA) (Nozaki et al., 2015). Similarly, several other studies have demonstrated that genetic and/or pharmacological inhibition of FAAH is associated with increased AEA levels in the brain, and associated with antinociceptive effects in several pain models (Kwilasz et al., 2014; Lichtman, Shelton, et al., 2004). For example, URB597 (intraperitoneal), a selective FAAH inhibitor, produced antinociception in the form of $CB_1$ dependant decreases in acid-stimulated stretching in a lactic acid model of pain, an effect associated with increased AEA levels in the brain (Kwilasz et al., 2014). Increased FAAH activity and an increased density of CB-binding sites have also been found in the hypothalamus in animal models of migraine (nitroglycerin-induced hyperalgesia) (Greco, Gasperi, Sandrini, et al., 2010). It is clear that elevation of brain eCB levels produces robust modulatory effects in mouse models of pain antinociception (Cravatt et al., 2001; Holt, Comelli, Costa, & Fowler, 2005; Jayamanne et al., 2006; Lichtman, Leung, et al., 2004; Lichtman, Shelton, et al., 2004), suggesting supraspinal $CB_1$ receptor-dependant antinociception.

PET imaging with a $CB_1$ receptor radioligand demonstrated that patients with in functional dyspepsia have higher $CB_1$ receptor availability in the hypothalamus and ACC (Ly et al., 2015). It is possible that eCB dysfunction and abnormal brain activity in these areas may be related to the pain felt in patients with functional dyspepsia; however, further work is warranted (Ly et al., 2015).

As previously mentioned, eCBs act on other non-$CB_1$/non-$CB_2$ receptors, such as the ligand-gated ion channel, TRPV1. TRPV1 on primary afferent neurons plays a key role in the sensation of pain and thermal hyperalgesia (Caterina et al., 2000). However, increasing evidence suggests a role for TRPV1 in pain modulation in supraspinal regions (Madasu et al., 2015). Central administration of the TRPV1 antagonist A-784168 induced potent analgesia in the rat sodium monoiodoacetate model of osteoarthritic pain and reduced thermal hyperalgesia and mechanical allodynia in the complete Freund's adjuvant model of inflammatory pain (Cui et al., 2006). Moreover, i.c.v. administration of TRPV1 antagonists reduced nociceptive behavior in the rat formalin test (Santos & Calixto, 1997). Following spinal

cord injury, $CB_1$ and TRPV1 receptors interact and play a role in the plastic changes that occur in the rat brain (Knerlich-Lukoschus et al., 2011). In the same study, 7 days following spinal cord lesion, $CB_1$ receptor immunoreactivity was increased in the thalamus and hippocampus and downregulated in the ACC, amygdala and PAG, brain regions related to pain, emotion, learning, and memory in rats. Double labeling studies revealed that TRPV1 was coexpressed with $CB_1$ (Knerlich-Lukoschus et al., 2011). Thus, alterations in $CB_1$-TRPV1 expression/activity may underlie the emotional-affective and somatosensory pain responses following spinal cord lesion.

Paracetamol (acetaminophen) is a well-recognized and potent analgesic drug (Toms, McQuay, Derry, & Moore, 2008) and a number of recent studies have demonstrated that paracetamol is metabolized to the TRPV1 agonist and AEA transport blocker AM404, which contributes to the antinociceptive activity of paracetamol (Hogestatt et al., 2005; Mallet et al., 2010; Zygmunt, Chuang, Movahed, Julius, & Hogestatt, 2000). The breakdown of paracetamol to AM404 occurs in the brain and is dependent on FAAH (Hogestatt et al., 2005). The deacetylated paracetamol metabolite 4-aminophenol and 4-hydroxy-3-methoxybenzylamine (HMBA) produces antinociception in a variety of rodent (both mice and rats) pain tests (Barriere et al., 2013) and is metabolized in the brain to form AM404 plus HPODA or arvanil plus olvanil. The antinociceptive effects of arvanil were dependent on FAAH, TRPV1, and $CB_1$ receptors (Barriere et al., 2013). FAAH-dependent generation of TRPV1-active analgesic drug metabolites may be useful in the production of novel pain therapeutics (Barriere et al., 2013).

GPR55, a putative novel CB receptor, has recently been shown to be involved in the development of hyperalgesia in models of inflammatory and neuropathic pain. Inflammatory mechanical hyperalgesia was absent in GPR55(−/−) knockout mice (Castane et al., 2006; Staton et al., 2008). Furthermore, following partial sciatic nerve ligation, GPR55 (−/−) mice failed to express mechanical hyperalgesia (Staton et al., 2008). Together, these results suggest a pro-nociceptive role for GPR55. However, as discussed below, only one study to date has investigated the role of this novel target supraspinally in the modulation of pain or stress (Deliu et al., 2015).

The PPARs are also targets for eCBs and may play a role in eCB-induced analgesia. PPARγ agonists produced anti-inflammatory and antihyperalgesic effects in carrageenan-treated rats, effects which were supraspinally mediated (Morgenweck et al., 2010). Similarly, i.c.v. administration of PPARα

ligands produced anti-inflammatory and antihyperalgesic effects in mice and rats in the carrageenan model of inflammation (D'Agostino et al., 2007, 2009; Taylor, Kriedt, Nagalingam, Dadia, & Badr, 2005). Thus, central PPARs play an important role in inflammatory nociceptive processing and responding.

See Table 1 for a synthesis of the studies described above.

## 10. CONCLUDING REMARKS

This review has provided a detailed overview of the role of the supraspinal eCB system in pain, SIA, and SIH. Work in animal models has provided clear evidence that activation of supraspinal CB receptors (particularly $CB_1$) or elevation of supraspinal eCB levels can induce antinociception. Although our understanding of the physiological, biochemical, and molecular mechanisms mediating these processes has become much clearer in recent years, there is still a need for further work to provide full details on the neurobiological mechanism underlying these effects.

We have highlighted a few areas where further investigation is warranted. In particular, studies investigating the role of the supraspinal eCB system in SIA and SIH are still relatively sparse, and particularly for SIH. Further studies in this area would enhance our understanding of pain-stress interactions. Pain is processed via many interconnected receptor-mediated pathways utilizing different neurotransmitter systems including GABA, glutamate, monoamines, opioids, and the eCB system, among others. In order to fully understand the role of the supraspinal eCB system in pain, and its modulation by stress, it is imperative that we look at the interactions between the supraspinal eCB system and other neurotransmitter systems. To date there has been a relative paucity of studies investigating these interactions.

The majority of studies discussed in this review and the bulk of our understanding on this topic has come from laboratory animal studies. While there are many clinical trials investigating the effects of CBs in pain and psychiatric disorders (International Clinical Trials Registry Platform), to our knowledge there are no studies that specifically target the supraspinal eCB system to evaluate its effect on pain or comorbid pain and stress disorders, likely owing to the technical and ethical challenges that would be involved. It will be necessary to develop therapeutic approaches relevant to the clinical setting without overt side effects and these may include sub-region specific targeting of $CB_1$ receptors, elevation on eCB levels rather than potent $CB_1$

receptor agonism and targeting of $CB_2$ receptors or other non-$CB_1$ receptor targets of relevance within the eCB system—all of which have been reviewed in this manuscript. Given the high incidence of pain disorders and their comorbidity with stress-related disorders, there is an urgent need to fully understand the neurobiological mechanisms underpinning supraspinal modulation of pain, SIA, and SIH and develop new, more effective treatments with more favorable adverse side effect profiles.

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