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Neurosteroids and Neuropathic Pain Management: basic evidence and therapeutic perspectives

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ABSTRACT (150 words)

Complex mechanisms involved in **neuropathic pain** that represents a major health concern make its management complicated. Because neurosteroids are bioactive steroids endogenously synthesized in the nervous system, including in pain pathways, they appear relevant to develop effective treatments against **neuropathic pain**. Neurosteroids act in paracrine or autocrine manner through genomic mechanisms and/or via membrane receptors **of neurotransmitters** that pivotally modulate pain sensation. Basic studies which uncovered a direct link between neuropathic pain symptoms and endogenous neurosteroid production/regulation, paved the way for the investigations of neurosteroid therapeutic potential against pathological pain. Concordantly, antinociceptive properties of synthetic neurosteroids were evidenced in humans and animals. Neurosteroids promote peripheral analgesia mediated by T-type calcium and **gamma-aminobutyric acid** type A channels, counteract chemotherapy-induced neuropathic pain and ameliorate neuropathic symptoms of injured spinal cord animals by stimulating anti-inflammatory, remyelinating and neuroprotective processes. Together, these data open interesting perspectives for neurosteroid-based strategies to manage/alleviate efficiently **neuropathic pain**.

Keywords: Chronic pain; Neuroactive steroid; neurosteroid; neuroprotection; nociception; peripheral neuropathy

Highlights

Bioactive neurosteroids are endogenously synthesized in pain neural pathways

Neurosteroids interact with membrane receptors of neurotransmitters controlling pain

Neurosteroidogenic pathway is differentially regulated in neuropathic pain condition

In vivo blockade of neurosteroidogenesis in spinal circuit modifies pain thresholds

Neurosteroid-treatment suppresses pain symptoms in several models of neuropathic pain

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References

1. Introduction

Neurosteroids are bioactive steroids endogenously synthesized in neurons and/or glial cells (for reviews, Baulieu et al., 1999; Mensah-Nyagan et al., 1999). The chemical structure of neurosteroids is not necessarily different from that of hormonal steroids but the main criterion required before considering an endogenous steroid as a neurosteroid is its production in the central (CNS) or peripheral (PNS) nervous systems independently from the activity of endocrine glands such as the adrenals and gonads. In addition to the genomic action generally used by all steroidal hormones, neurosteroids modulate the nervous system activity in a paracrine or autocrine manner by acting through various membrane receptors including gamma-aminobutyric acid type A (GABAA), N-methyl-D-aspartate (NMDA), Purinergic 2X (P2X) and sigma receptors as well as voltage-gated calcium channels or VGCCs (Balthazart et al., 2018; Baulieu et al., 1999; Belelli and Lambert, 2005; Gunn et al., 2015; Joksimovic et al., 2018a; Maurice et al., 2006; Mellon and Griffin, 2002; Mensah-Nyagan et al., 1999; Olsen, 2018; Rudolph et al., 2016; Tuem and Atey, 2017). So far, the non-genomic interactions of neurosteroids with the GABAA membrane receptor are the most well documented and neurosteroid binding sites have been identified in the receptor's transmembrane domains mediating the potentiating and direct activation effects of neurosteroids (Belelli and Lambert, 2005; Hosie et al., 2006; Majewska, 1992). In particular, it has been demonstrated that the interactions of neurosteroids with a cavity formed by the α -subunit transmembrane domains allosterically potentiate GABA responses but neurosteroids can also directly initiate GABAA receptor activation from the interfacial residues between α and β subunits and this activation may be increased by steroid binding to the allosteric potentiation site. Thus, significant receptor activation by neurosteroids relies on occupancy of both the activation and potentiation sites (Hosie et al., 2006).

Two main categories of steroids can be distinguished in the neurosteroid family (Pattē-Mensah and Mensah-Nyagan, 2008): (i) the non-exclusive neurosteroids such as pregnenolone (PREG), progesterone (PROG) or dehydroepiandrosterone (DHEA) are steroidal hormones that can also be synthesized by neurons or glial cells; (ii) semi-exclusive neurosteroids such as tetrahydroprogesterone ($3\alpha,5\alpha$ -THP) also called allopregnanolone are mainly synthesized in the nervous system even if substantial amounts can be produced in endocrine glands. The existence of exclusive neurosteroids such as epiallopregnanolone (which may only be synthesized in nerve cells) has been suggested but the arguments supporting this idea are rare (Higashi et al., 2007). The occurrence of neurosteroidogenesis or neurosteroid production in a neural structure requires the presence of active steroidogenic enzymes such as cytochrome P450_{side-chain-cleavage} (P450_{scc}), cytochrome P450_{c17} (P450_{c17}), 3α -hydroxysteroid oxidoreductase (3α -HSOR), 3β -hydroxysteroid dehydrogenase (3β -HSD) and 5α -reductase (5α -R) (Baulieu et al., 1999; Compagnone and Mellon, 2000; Mensah-Nyagan et al., 1996a, 1996b). The process of neurosteroidogenesis is well conserved through the vertebrate phylum (Baulieu et al., 1999; Le Goascogne et al., 1987; Mellon and Griffin, 2002; Mensah-Nyagan et al., 1999), suggesting that neurosteroid formation might be crucial for life. This idea raises a great hope for the development of neurosteroid-based therapies to treat effectively complex disorders of the nervous system.

Among chronic disorders of the nervous system that involve complex mechanisms is neuropathic pain which represents a major health concern and the reason for medical consultation in many diseases. The International Association for the Study of Pain defined neuropathic pain as “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system” (Treede et al., 2008). The etiology of neuropathic pain includes several disorders such as diabetic polyneuropathies, postherpetic neuralgia, trigeminal neuralgia, painful radiculopathies, central post-stroke pain, spinal cord injury pain, traumatic,

postsurgical and chemotherapy-induced neuropathies (Colloca et al., 2017; Cruccu and Truini, 2017; Finnerup et al., 2016; Jensen et al., 2011). About 10% of the general population is affected by neuropathic pain that is refractory to current analgesic drugs. This percentage is likely to increase because of the global ageing population and the augmentation of diabetic patients as well as the number of cancer survivors after chemotherapy (Colloca et al., 2017; Cruccu and Truini, 2017; Hershman et al., 2014).

Neuropathic pain is characterized by a diversity of symptoms including burning sensation, sharp, stabbing, allodynia, and/or hyperalgesia. Given to the complexity of the mechanisms involved, the management of neuropathic pain is extremely complicated as the available treatments have only moderate benefits. Thus, the development of effective strategies against neuropathic pain constitutes an urgent medical need. Therefore, to contribute to this objective requiring important global efforts, the present paper has critically reviewed and discussed basic, preclinical and translational evidence suggesting that neurosteroids may represent a relevant tool to exploit for the development of effective treatments against neuropathic pain.

2. Historical evidence in favor of a key modulatory action of steroidal compounds on pain sensation

For several years, the production of endogenous steroids was exclusively ascribed to the adrenals and gonads but it is now well established that various other tissues such as the bowel, liver, prostate and nervous system may synthesize or metabolize steroids (Baulieu et al., 1999; Belanger et al., 1989; Dalla-Valle et al., 1992; Martel et al., 1994). The chemical nature of steroids allows them to behave as lipophilic molecules, particularly when they are free or non-conjugated to sulfate radicals. Therefore, unconjugated steroids synthesized by peripheral glands may reach or act on several tissues in the body including the **PNS and CNS**

since free steroids are capable of crossing the blood-brain barrier. Consequently, numerous studies indicate that steroid hormones exert a large array of biological effects including the control of sex behaviors, reproduction, development, stress and the regulation of the activity of various important physiological systems such as the immune, cardiovascular, respiratory and nervous systems (for reviews, Balthazart et al., 2018; Barnes, 1998; Belvisi and Hele, 2003; De Nicola et al., 2018; Fakhri et al., 2002; Firestein et al., 1991; McEwen, 1994; Melcangi and Mensah-Nyagan, 2006). Receptors of various steroid hormones are expressed in several neural structures allowing steroids to crucially control the development, growth, maturation, differentiation and plasticity of the CNS and PNS (Balthazart et al., 2018; Green et al., 1997; Jones, 1993; Kimonides et al., 1998; McEwen, 1994; Rudolph et al., 2016; Sapolsky, 1996; Seckl, 1997; Uno et al., 1989; Yu, 1989).

Because of their pleiotropic potential and diverse effects on the CNS and PNS, steroids have early been suspected to modulate pain sensation. Indeed, since 1927, Cashin and Moravsek observed that intravenous injections of cholesterol were able to suppress pain sensation by exerting anesthetic effects in mammals. Afterwards, Selye (1942) demonstrated that certain pregnane steroids such as PROG and deoxycorticosterone can induce sedation and anesthesia in rat. Together, these observations paved the way for the development of various synthetic analogs of pregnane steroids which reduced pain through allosteric activation of GABA_A receptors (Belelli and Lambert, 2005; Holzbauer, 1976; Kraulis et al., 1975; Majewska, 1992; Olsen, 2018; Purdy et al., 1991; Taleb et al., 2018). Nowadays, the therapeutic use of glucocorticosteroids and their analogs is considered as the most effective strategy against inflammatory pain in spite of the occurrence of diverse side effects (for reviews, Barnes, 1998; Belvisi and Hele, 2003). Glucocorticosteroids reduce inflammatory pain by inducing anti-inflammatory actions on the damaged peripheral or central tissue which activates nociceptive mechanisms and generate pain sensation. Anti-inflammatory effects of glucocorticosteroids result from their

ability to inhibit the expression of collagenase (the key enzyme involved in tissue degeneration during inflammatory mechanisms) and pro-inflammatory cytokines or to stimulate the synthesis of lipocortin which blocks the production of eicosanoids (Barnes and Adcock, 1993; Fakhri et al., 2002; Firestein et al., 1991; Sibilio, 2003). There is also clinical evidence supporting the use of glucocorticoids in the treatment of chronic neuropathic pain (Devor et al., 1985; Kingery, 1997; Lussier et al., 2004; Wareham, 2004). Experimental investigations in animals suggest that glucocorticoids may inhibit the initiation of neuropathic pain states or attenuate this pain but the mechanisms of action are unknown (Clatworthy et al., 1995; Devor et al., 1985; Johansson and Bennett, 1997; Kingery et al., 1999; Takeda et al., 2004). It is usually thought that anti-inflammatory actions of glucocorticosteroids may contribute to the inhibition of neuroinflammatory component of neuropathic pain but there is no specific evidence supporting this hypothesis. As an interesting finding revealed that the endoneurial expression of pro-inflammatory cytokines may have a role in the genesis of neuropathic pain, glucocorticosteroids may reduce this pain through the modulation of neuroimmune interactions (Sommer and Kress, 2004). In support of this idea, a recent study showed that the glucocorticoid triamcinolone, which reduced the neuropathic pain seen in the model of post-traumatic peripheral neuropathy, also decreased the number of endoneurial mast cells expressing (in the injured nerve) the pro-inflammatory cytokine tumor necrosis factor- α (Hayashi et al., 2008). Altogether, the findings recapitulated above strongly support the existence of key actions of endogenous and synthetic steroids in the modulation of inflammatory and neuropathic pain.

3. Endogenous neurosteroids and neuropathic pain modulation

The demonstration of the occurrence of neurosteroidogenesis in a neural center/structure requires the localization in that structure of active forms of key steroidogenic

enzymes such as P450scc, P450c17, 3 α -HSOR, 3 β -HSD and 5 α -R (Baulieu et al., 1999; Compagnone and Mellon, 2000; Mensah-Nyagan et al., 1996a, 1996b). Among these enzymes, P450scc plays a crucial role since it catalyzes cholesterol conversion into PREG, the first pivotal step for neurosteroid biosynthesis (Baulieu et al., 1999; Le Goascogne et al., 1987). The finding that the CNS and PNS have the ability to locally produce key neuroactive steroids (Baulieu et al., 1999; Le Goascogne et al., 1987; Mellon and Griffin, 2002; Mensah-Nyagan et al., 1999) raises a great hope for the development of novel therapies based on the use of neurosteroids to improve the treatment of various neural disorders.

Based on the principle that neurosteroids act mainly through autocrine or paracrine mechanisms, endogenous neurosteroid involvement in the regulation of a neurobiological process is plausible when neurosteroids are locally synthesized in the neural circuit controlling this process. It is true that neurosteroids modulate GABAA, NMDA and P2X receptors which play a crucial role in the regulation of pain (Baulieu et al., 1999; Mensah-Nyagan et al., 2008a; Millan, 1999, 2002; Tuem and Atey, 2017; Wang, 2011) but the local synthesis of neurosteroids near their sites of actions in pain neural centers is a prerequisite to render credible the possible involvement of endogenous neurosteroids in pain modulation. Therefore, we performed a series of studies to investigate whether the spinal cord which pivotally controls pain transmission and also contains potential sites of action for neurosteroids (Haines et al., 1997; Millan, 1999, 2002) may have the enzymatic machinery to locally synthesize neurosteroids. We demonstrated that the rat spinal dorsal horn (DH), which contains various key steroid-synthesizing enzymes such as P450scc, P450c17, 5 α -R and 3 α -HSOR, is an active center producing neurosteroids including PREG, DHEA, PROG, dihydroprogesterone (DHP) and 3 α ,5 α -THP (Kibaly et al., 2005, 2008; Mensah-Nyagan et al., 2008b; Meyer et al., 2008; Patte-Mensah et al., 2003,2004a, 2004b, 2005, 2006). In addition, we observed that substance P, a major nociceptive neuropeptide released by primary afferents,

inhibited in a dose-dependent manner $3\alpha,5\alpha$ -THP biosynthesis in the DH (Patte-Mensah et al., 2005). As the neurosteroid $3\alpha,5\alpha$ -THP is a potent allosteric stimulator of GABAA receptors, our observation suggested that substance P, by reducing $3\alpha,5\alpha$ -THP production, may indirectly decrease the spinal inhibitory tone and therefore facilitate noxious signal transmission. To further investigate the possible role of neurosteroids endogenously produced in the DH in pain modulation, we performed a multidisciplinary study using the rat experimental model of neuropathic pain generated by sciatic nerve ligatures (Bennett and Xie, 1988). Molecular and biochemical investigations (quantitative real time polymerase chain reaction after reverse transcription, western blot, radioimmunoassay, pulse-chase experiments, high performance liquid chromatography and continuous flow scintillation detection) revealed an up-regulation of enzymatic pathways (P450scc and 3α -HSOR) leading to $3\alpha,5\alpha$ -THP biosynthesis in the DH (Meyer et al., 2008; Patte-Mensah et al., 2004a, 2004b). Because $3\alpha,5\alpha$ -THP is well known as a potent activator of the central inhibitory system (Belelli and Lambert, 2005), the observation of an upregulation of $3\alpha,5\alpha$ -THP production in the DH of neuropathic rats suggested that the increased synthesis of $3\alpha,5\alpha$ -THP might be involved in endogenous mechanisms triggered by neuropathic animals to cope with their chronic pain condition. However, the endogenous adaptive mechanisms or the level of $3\alpha,5\alpha$ -THP endogenously produced in the DH may not be sufficient to suppress painful symptoms that is the reason why, in the absence of treatments, neuropathic rats remained allodynic and/or hyperalgesic (Meyer et al., 2008). These data suggest that intrathecal injection of exogenous/synthetic $3\alpha,5\alpha$ -THP as an external contribution to increase the insufficient endogenous level in the DH may represent an effective strategy to suppress pain symptoms in neuropathic rats. In support of this hypothesis, behavioral studies using the plantar test (thermal nociceptive threshold) and the von Frey filament test (mechanical nociceptive threshold) showed that intrathecal administration of $3\alpha,5\alpha$ -THP in the lumbar spinal cord

induced analgesia in neuropathic pain rats by suppressing the thermal hyperalgesia and mechanical allodynia characterizing these animals. Unlike $3\alpha,5\alpha$ -THP, intrathecal injection of Provera (3α -HSOR inhibitor) potentiated both thermal hyperalgesia and mechanical allodynia in neuropathic rats (Meyer et al., 2008; Patte-Mensah et al., 2014; Table 1). Contrary to what is observed for $3\alpha,5\alpha$ -THP production, the biosynthetic pathway (P450c17) producing DHEA was down-regulated in neuropathic rat DH (Kibaly et al., 2005, 2008). Acute DHEA treatment exerted a rapid pro-nociceptive and a delayed anti-nociceptive action. Inhibition of DHEA biosynthesis in the DH by intrathecally administered ketoconazole (P450c17 inhibitor) induced analgesia in neuropathic rats. Chronic treatment of DHEA increased and maintained elevated the basal pain thresholds in neuropathic and control rats, suggesting that androgenic metabolites generated from daily injected DHEA exerted analgesic effects while DHEA itself (before being metabolized) induced a rapid pro-nociceptive action (Kibaly et al., 2008).

In agreement with our findings showing endogenous neurosteroid involvement in pain modulation, various other investigations using synthetic analogs of $3\alpha,5\alpha$ -THP also revealed antinociceptive properties of neurosteroids in humans and animals (Gambhir et al., 2002; Goodchild et al., 2000, 2001; Nadeson and Goodchild, 2000, 2001). Interestingly, it has been shown that analgesic and antinociceptive effects of neurosteroids are reversed *in vivo* by the GABAA receptor antagonist bicuculline confirming therefore the involvement of this receptor considered as a major neurosteroid target (Belelli and Lambert, 2005; Hosie et al., 2006; Majewska, 1992) in the mediation of neurosteroid actions against pain sensation (Nadeson and Goodchild, 2000, 2001). Furthermore, it has been reported that neurosteroid $3\alpha,5\alpha$ -THP is capable of suppressing efficiently median nerve injury-induced mechanical hypersensitivity and glial extracellular signal-regulated kinase activation through GABAA receptor modulation in the rat cuneate nucleus (Huang et al., 2016).

Other important contributions are the works which investigated the interactions of 5α - and 5β -reduced steroid analogs with VGCCs and suggested that neurosteroids having combined inhibitory effects on low- and high-voltage-activated (LVA and HVA) calcium currents may be suitable to develop novel therapies against neuropathic pain (Joksimovic et al., 2018a; Pathirathna et al., 2005; Todorovic et al., 1998). Indeed, VGCCs, composed of HVA or sustained currents and LVA or transient (T-type) currents, are expressed in pain neural pathways and pivotally control cellular excitability and synaptic transmission (Catterall, 2000). Inhibition of peripheral T-channels led to potent antinociception in models of somatic (Choi et al., 2007; Todorovic et al., 2001, 2002, 2003) and visceral pain (Kim et al., 2003). Concordantly, it has been shown that 5α -reduced neurosteroids, which interact with both GABA_A receptors and T-channels in peripheral nociceptive neurons, induce potent analgesic effects (Pathirathna et al., 2005). Because the upregulation of CaV3.2 isoform of T-channels in dorsal root ganglion sensory neurons caused hyperalgesia after surgical incision, additional studies demonstrated that the endogenous 5β -reduced neurosteroid epipregnanolone which inhibited the CaV3.2 isoform, without modulating GABA_A-mediated currents, exerted a potent analgesia in healthy rodents (Ayoola et al., 2014; Joksimovic et al., 2018b; Todorovic et al., 2004). Local intraplantar injection of epipregnanolone decreased both thermal and mechanical nociception in the wild type mice, but was ineffective in CaV3.2 knock-out mice and this observation confirmed the idea that the blockade of CaV3.2 T-type calcium channels in peripheral sensory neurons was indeed involved in analgesia induced by epipregnanolone (Ayoola et al., 2014). Also, intrathecal administration of epipregnanolone that reduced thermal and mechanical nociception in healthy rats, decreased mechanical hyperalgesia in neuropathic animals *in vivo* after surgery; *in vitro* verifications revealed that epipregnanolone blocked isolated HVA currents in nociceptive sensory neurons with in a G-protein-dependent fashion (Joksimovic et al., 2019). Altogether, the aforementioned data

strongly support the idea that VGCCs may represent promising targets to explore for the development of neurosteroid-based strategies against neuropathic pain.

Moreover, other previous studies have reported that neurosteroids, including PROG, DHP and $3\alpha,5\alpha$ -THP, improved Na^+K^+ ATPase activity, myelin protein zero and peripheral myelin protein 22 expression levels, thermal nociceptive threshold, skin innervation density and nerve conduction velocity in neuropathic animals (Leonelli et al., 2007). PROG and/or DHP, which counteracted sciatic nerve crush-induced decrease of Na^+K^+ ATPase pump activity and myelin protein expression, also restored normal pain thresholds (Roglio et al., 2008). More importantly, by using a model of incomplete spinal cord injury (SCI)-evoked neuropathic pain, Coronel and coworkers showed that SCI induced an increase in the spinal expression of mitochondrial cholesterol translocator protein (TSPO) and 5α -R that pivotally contribute to endogenous neurosteroid synthesis (Coronel et al., 2016). Previous data have also revealed that PROG and $3\alpha,5\alpha$ -THP levels were locally increased in the spinal cord 75 h after injury (Labombarda et al., 2006). Because a significant decrease of 5α -R mRNA was determined 28 days after the initial SCI, coinciding with the presence of allodynic behaviors, it has been suggested that the early increase in neurosteroidogenic pathway may represent a primary endogenous protective mechanism tending to control pain development (Coronel et al., 2016). Concordantly, PROG prevented sciatic nerve injury-induced increase expression of key players of pain genesis such as the NR1 subunit of NMDA receptor and the gamma isoform of protein kinase C or PKC γ (Coronel et al., 2011). Also, the early onset and maintenance of PROG therapy for a critical period counteracted peripheral nerve injury-evoked neuropathic pain symptoms (Dableh and Henry, 2011).

Because diabetic neuropathy is also associated with neuropathic pain in about 50% of diabetic patients, various preclinical studies have investigated the potential role of neurosteroid in the modulation of diabetes-induced neuropathic pain. We found an up-

regulation of the gene encoding for the key neurosteroidogenic enzyme 3 β -HSD as well as an increase of the amount of [³H]PROG newly synthesized in the spinal cord of streptozotocin-induced diabetic rats (Saredi et al., 2005). By using the same animal model, another study, which analyzed testosterone metabolites, dihydrotestosterone (DHT) and 3 α - androstanediol (3 α -DIOL), on nociceptive and allodynia thresholds showed that diabetes induced a significant decrease in DHT levels in the spinal cord that was reverted by DHT or 3 α -DIOL treatments; furthermore, 3 α -DIOL systemic therapy promoted 3 α -DIOL increase in the spinal cord and both DHT and 3 α -DIOL exerted analgesic effects on diabetic neuropathic pain (Calabrese et al., 2014). Interestingly, DHT counteracted the effect of diabetes on mechanical nociceptive threshold, pre- and post-synaptic components, glutamate release, astrocyte immunostaining, and interleukin-1 β (IL-1 β) expression, while 3 α -DIOL was effective on tactile allodynia threshold, glutamate release, astrocyte immunolabeling and the expression of substance P, toll-like receptor 4, tumor necrosis factor- α , transforming growth factor β -1, IL-1 β and TSPO (Calabrese et al., 2014). Together, these data suggested that the increase of 3 β -HSD expression and PROG production in the spinal cord of diabetic rats (which is insufficient *per se* to suppress pain symptoms) seems also to be an adaptive or compensatory mechanism to reduce the deficit of testosterone metabolites evidenced in these animals since PROG may be a precursor for testosterone synthesis. The data also indicate that testosterone metabolites may potentially constitute beneficial substances for the treatment of diabetic neuropathic pain. Moreover, it has been shown that chronic treatment with 3 α ,5 α -THP prevented diabetes-induced spinal down-regulation of γ 2 subunit of GABAA receptor and counteracted thermal hyperalgesia, as well as motor impairment (Afrazi and Esmaeili-Mahani, 2014).

The beneficial role of neurosteroids in the treatment of neuropathic pain symptoms was also evidenced significantly in preclinical conditions of anticancer drug-induced painful

peripheral neuropathy. Indeed, it is well known that the therapeutic efficacy of anticancer drugs is limited by a major dose-dependent side effect which is painful peripheral neuropathy (Antoine and Camdessanche, 2007; Brewer et al., 2016; Cavaletti and Marmiroli, 2015; Dougherty et al., 2007; Fehrenbacher, 2015; Hershman et al., 2014; Ling et al., 2007a, 2007b; Polomano and Bennett, 2001; Seretny et al., 2014). Vincristine (VINC) and oxaliplatin (OXAL) are two antineoplastics widely used for the treatment of various malignancies including leukemia, ovarian, breast, lung and colorectal cancers (Antoine and Camdessanche, 2007; Baker, 2003; Brewer et al., 2016; Cavaletti and Marmiroli, 2015; Dougherty et al., 2007; Fehrenbacher, 2015; Ling et al., 2007a, 2007b; Polomano and Bennett, 2001; Seretny et al., 2014). However, both VINC and OXAL induce neuropathic pain symptoms such as mechanical hyperalgesia, cold-allodynia and mechanical allodynia (Antoine and Camdessanche, 2007; Dougherty et al., 2007; Hou et al., 2018; Ling et al., 2007a, 2007b; Polomano and Bennett, 2001). These painful symptoms hamper the success of VINC- or OXAL-based therapy as patients, who cannot complete optimal treatment schedules, must also face chronic discomfort and decreased quality of life. Neuroprotective compounds including thiols, neurotrophic factors, anticonvulsants and antioxidants were used to prevent antineoplastic-evoked neuropathy but the clinical data are controversial and these neuroprotective agents induce themselves adverse effects including nausea, reflex dysfunctions, treatment emergent nervousness, insomnia, anorexia or stomach burning (Durand et al., 2005; Finnerup et al., 2015, 2016; Gamelin et al., 2004; Hershman et al., 2014; Hou et al., 2018; Lang et al., 1996; Ling et al., 2007a, 2007b; Marchand et al., 2003). Therefore, it remains crucial to identify other molecules which may treat effectively anticancer drug-evoked neuropathic pain without evoking themselves undesirable effects. Because $3\alpha,5\alpha$ -THP is a natural neurosteroid exerting various beneficial actions (neuroprotection, neurogenesis, anxiolysis and analgesia) with no toxic side-effects (Bansal

and Singh, 2017; Belelli and Lambert, 2005; Bitran et al., 1991; De Nicola et al., 2018; Joksimovic et al., 2018a; Meyer et al., 2008; Pathirathna et al., 2005; Patte-Mensah et al., 2005; Timby et al., 2006; Uzunova et al., 1998; Wang et al., 2010; Zamora-Sanchez et al., 2017), we hypothesized that $3\alpha,5\alpha$ -THP may be an interesting molecule to develop effective strategies against anticancer drug-induced painful neuropathy (Meyer et al., 2010, 2011). To check this hypothesis, we evaluated the effects of $3\alpha,5\alpha$ -THP on behavioral, electrophysiological, histological and neurochemical parameters altered by VINC or OXAL treatment. Our results revealed that the prophylactic or corrective $3\alpha,5\alpha$ -THP treatment (4mg/kg/2days) respectively prevented or abolished VINC or OXAL-induced mechanical hyperalgesia, mechanical allodynia and cold-allodynia (Meyer et al., 2010, 2011; Patte-Mensah et al., 2014; Fig. 1). Electrophysiological analyses allowed the determination of the effects of VINC or OXAL treatment on key functional parameters of the rat sciatic nerve including the conduction velocity (CV), the nerve action potential (NAP) onset and peak amplitude. We observed that VINC induced about 25% CV reduction without any significant change in the NAP peak amplitude. In contrast, the main effect of OXAL treatment was seen at the peak amplitude level which was reduced by about 62%. OXAL also decreased the nerve CV but the significant effect was only observed for the fastest conducting fibers. Prophylactic treatment with $3\alpha,5\alpha$ -THP prevented VINC- or OXAL-induced sciatic nerve functional alterations. In addition, when VINC- or OXAL-evoked nerve dysfunctions are well established before the onset of $3\alpha,5\alpha$ -THP treatment, the administration of $3\alpha,5\alpha$ -THP corrective treatment (4mg/kg/2days) during one week was able to restore normal or control values of nerve CV and NAP peak amplitude in VINC- and OXAL-treated rats (Meyer et al., 2010, 2011; Patte-Mensah et al., 2014). In the line of previous histological studies describing various nerve tissue damages induced by anticancer drug treatments (Authier et al., 2009; Jamieson et al., 2009; Lauria et al., 2005; Ravula et al., 2007; Siau et al., 2006), we have used

the indirect immunofluorescence approach and laser scanning confocal microscopic quantifications to show that VINC or OXAL treatment, which dramatically decreased the density of **intraepidermal nerve fibers (IENF)** in hind paw intraplantar skins, also repressed neurofilament 200kDa expression level in sciatic nerve axons and dorsal root ganglion neurons (Meyer et al., 2010, 2011; Patte-Mensah et al., 2014). The occurrence of such cellular alterations was prevented by prophylactic administration of $3\alpha,5\alpha$ -THP. Furthermore, corrective $3\alpha,5\alpha$ -THP treatment repaired VINC or OXAL-induced nerve tissue damages by restoring normal density of **IENF** in hind paw intraplantar skins and control expression level of neurofilament 200kDa in VINC- or OXAL-treated rat dorsal root ganglia and sciatic nerves (Meyer et al., 2010; Patte-Mensah et al., 2014).

More importantly, our studies have evidenced the fact that the local activity of neurosteroidogenic enzymes in pain neural pathways is pivotal for the induction of a beneficial action of neurosteroids against anticancer drug-evoked neuropathic pain. Indeed, the inhibition of PROG conversion into 5α -reduced metabolites (DHP and $3\alpha,5\alpha$ -THP) by finasteride (Finn et al., 2006; Stoner, 1990) completely blocked antinociceptive, analgesic and neuroprotective effects exerted by PROG against VINC-induced painful neuropathy. In fact, in the absence of finasteride, PROG may be converted to the potent PROG nuclear receptor agonist DHP (Brann et al., 1990) and to the neuroprotector $3\alpha,5\alpha$ -THP (Melcangi et al., 2008), allowing therefore a strong stimulation or optimal activities of biochemical and neuroanatomical/neurochemical components in peripheral nerves such as CNPase, axonal constitutive proteins/transporters and **IENF**. In the presence of finasteride, PROG (or its non- 5α -reduced metabolites), exerted only 3% increase on CNPase expression in control rat nerves and this slight stimulatory action failed to reverse the severe CNPase repression evoked by VINC treatment (Meyer et al., 2010). In agreement with our observations, other investigations have shown that DHP restored to normal values decreased levels of myelin-

associated glycoprotein, peripheral myelin protein 22 and myelin basic protein in sciatic nerves of docetaxel-treated rats while PROG rescue effect was weak or undetectable (Roglio et al., 2009). Moreover, by using a model of brain traumatism *in vivo*, Sayeed et al. (Sayeed et al., 2006) have shown that 3 α ,5 α -THP is more effective than PROG in reducing cortical damages after transient middle cerebral artery occlusion. Interestingly, it is well demonstrated that DHP may be inter-converted into 3 α ,5 α -THP thanks to the enzymatic activity of 3 α -HSOR abundantly expressed in the nervous system (Patte-Mensah et al., 2004b, 2005). Collectively, these data indicate that the conversion into 5 α ,3 α -reduced metabolites is critical for the expression of PROG analgesic and neuroprotective effects. During our studies which revealed an efficient effect of 3 α ,5 α -THP against anticancer-induced neuropathic pain, we did not measure plasma levels of 3 α ,5 α -THP and its metabolites after the prophylactic or corrective 3 α ,5 α -THP treatment (Meyer et al., 2010, 2011). However, a recent report has shown that subcutaneous injection of 3 α ,5 α -THP to male Wistar rats at the dose of 1.25 or 2.5 mg/kg led to the increase of 3 α ,5 α -THP plasma level which reached 97 or 252 nM, respectively (Holmberg et al., 2013). Therefore, it appears reasonable to estimate that 3 α ,5 α -THP dose regimen investigated in our studies, namely 4mg/kg/2days, may generate similar plasma concentration ranges as those detected by Holmberg and coworkers (Holmberg et al., 2013; Meyer et al., 2010, 2011). Moreover, it has been demonstrated that 3 β -hydroxylated isopregnanolone or epipregnanolone did not affect formalin-induced pain and that the antinociceptive effect of 5 α - and 5 β -DHP in the formalin test is dependent on their conversion into 3 α ,5 α -THP or PREG (Ocvirk et al., 2008). Altogether, these results, which confirm the crucial role played by 5 α ,3 α -reduced neurosteroids in pain modulation, also suggest that 3 α ,5 α -THP may offer an interesting possibility to develop effective strategy against neuropathic pain. 3 α ,5 α -THP treatment also successfully corrected motor behaviors altered by

OXAL-chemotherapy, indicating that $3\alpha,5\alpha$ -THP-based therapy may be relevant for the treatment of both painful/sensory and motor peripheral neuropathies (Taleb et al., 2017).

Among the family of $3\alpha,5\alpha$ -neurosteroids is also 3α -androstenediol or 3α -DIOL synthesized from the 5α -reduction of testosterone into DHT followed by the conversion of DHT into 3α -DIOL by the enzyme 3α -HSOR. Similarly to $3\alpha,5\alpha$ -THP, 3α -DIOL also activates allosterically the GABAA receptor and potentiates the central inhibition (Belelli and Lambert, 2005; Bitran et al., 1996; Edinger and Frye, 2004; Frye et al., 1996, 2007; Frye and Seliga, 2001; Hosie et al., 2006; Olsen, 2018; Reddy, 2008; Rhodes and Frye, 2004). While $3\alpha,5\alpha$ -THP has extensively been investigated in several experimental models and in humans as a neuroprotective, neurogenic, anaesthetic, anxiolytic or analgesic compound, the neurosteroid 3α -DIOL has received little attention (Akwa et al., 1999; Belelli and Lambert, 2005; Ciriza et al., 2004, 2006; Griffin et al., 2004; Meyer et al., 2008; Patte-Mensah et al., 2006; Uzunova et al., 1998; Wang et al., 2010). We have therefore investigated whether 3α -DIOL may also be a neuroprotective neurosteroid offering effective possibilities for the treatment of chemotherapy-induced painful neuropathy and neuropathological symptoms (Meyer et al., 2013). Thus, we assessed the potential of 3α -DIOL to prevent or suppress paclitaxel (PAC)-evoked neurochemical and functional abnormalities in rat peripheral nerves. Prophylactic or corrective 3α -DIOL treatment (4 mg/kg/2days) prevented or suppressed PAC-evoked heat-thermal hyperalgesia, cold-allodynia and mechanical allodynia/hyperalgesia, by reversing to normal, decreased thermal and mechanical pain thresholds of PAC-treated rats. Electrophysiological studies demonstrated that 3α -DIOL restored control values of nerve conduction velocity and action potential peak amplitude significantly altered by PAC-treatment. 3α -DIOL also repaired PAC-induced nerve damages by restoring normal neurofilament-200 level in peripheral axons and control amount of 2',3'-cyclic-nucleotide-3'-phosphodiesterase in myelin sheaths. Decreased density of IENF evoked by PAC-therapy was

also counteracted by 3 α -DIOL treatment. More importantly, 3 α -DIOL beneficial effects were not sedation-dependent but resulted from its neuroprotective ability, nerve tissue repairing capacity and long-term analgesic action. Interestingly, previous works reported that 3 α -DIOL may exert estrogenic activity by interacting with estrogen receptors or may increase intracellular cAMP levels through binding to the sex hormone binding globulin (Ding et al., 1998; Pettersson et al., 2009). It has also been shown that the stimulatory effect of testosterone on learning and memory may partially be mediated through actions of its metabolite 3 α -DIOL at estrogen receptors in the dorsal hippocampus (Edinger and Frye, 2007). Estrogens acting via classical and/or non classical receptors/targets have recognized neuroprotective properties in several experimental models of neurodegenerative/neurological disorders (for reviews, Arevalo et al., 2015; Baez-Jurado et al., 2019). Furthermore, we have previously observed that neurosteroid estradiol endogenously produced in the spinal pain circuit is pivotal for the protection of dorsal root ganglion sensory neurons against sciatic nerve chronic constriction injury-induced apoptosis (Schaeffer et al., 2010a, 2010b). Therefore, the aforementioned beneficial effects exerted by 3 α -DIOL against PAC-evoked peripheral nerve damages and neuropathic pain may involve interactions of 3 α -DIOL with various targets including estrogen and GABAA receptors.

Altogether, these data confirmed the idea that 5 α ,3 α -reduced neurosteroids may offer promising options for the management of neuropathic pain evoked by chemotherapeutic drugs, spinal cord or peripheral nerve injuries.

4. Neurosteroids and sex dimorphism in pain sensitivity?

The ability of the nervous system to synthesize neurosteroids has been demonstrated in both male and female but the occurrence of sex dimorphism in neurosteroidogenic pathways remains a matter of speculation (for reviews Baulieu et al., 1999; Melcangi et al., 2008;

Mensah-Nyagan et al., 1999). A recent study, which investigated sex differences in the brain expression of steroidogenic molecules under basal conditions and after gonadectomy, suggested that mRNA expression of steroidogenic molecules in the adult rat brain is sexually dimorphic and presents regional specificity, both under basal condition and after gonadectomy (Giatti et al., 2019). Although protein levels were not investigated in this study, it raises interestingly the hypothesis that local neurosteroidogenesis may contribute to sex and regional differences in brain levels of neurosteroids and may cause sex differences in the adult brain function (Giatti et al., 2019). Most investigations showing a key role of endogenously-produced neurosteroids in the modulation of neuropathic pain symptoms essentially used male animals and only few females have been tested to confirm the fact that the local manipulation of neurosteroidogenesis affects pain sensitivity thresholds in both sexes (Gonzalez et al., 2019; Meyer et al., 2008, 2010, 2011, 2013; Patte-Mensah et al., 2010, 2014; Schaeffer et al., 2010a, 2010b). However, whether selective differences may exist in the local production of neurosteroids in pain neural pathways of males versus females and whether these differences may sexually determine the efficacy of each neurosteroid in preventing pain symptoms remains an open question. Future investigations will certainly clarify this point in order to establish, in comparison with gonadal steroids, the specific contribution of endogenous neurosteroids (locally produced in the brain, spinal cord or peripheral nervous system) to sex differences in pain perception. Indeed, several lines of clinical and basic evidence showed that female and male gonadal steroids are key factors accounting for the gender differences in pain and analgesia (for reviews, Aloisi and Bonifazi, 2006; Aloisi et al., 2003; Arendt-Nielsen et al., 2004; Chakrabarti et al., 2010; Craft et al., 2004; Fillingim and Gear, 2004). Variations in sex steroid levels, receptor expression and mechanisms of action in the nervous system have been correlated with the development of chronic pain (Aloisi, 2003; Craft et al., 2004). Androgens, particularly testosterone, which are higher in males exert

analgesic effects in humans and experimental models while estrogens were found to have both hyperalgesic and analgesic effects depending on the experimental conditions (Aloisi and Bonifazi, 2006; Aloisi et al., 2004; Ceccarelli et al., 2003; Hau et al., 2004). In addition, direct evidence has been provided for the occurrence of sex differences in the expression of steroid receptors in the periaqueductal gray that pivotally controls nociception and responsiveness to pain in male and female through interactions with the rostral ventromedial medulla which in turn projects to the spinal DH to elaborate the descending pathway driving pain inhibition (Lloyd and Murphy, 2006, 2008). The differential expression of steroid receptors in the male and female brain and/or pain neural circuits may probably contribute to sex dimorphism in endogenous neurosteroid-induced antinociceptive and/or analgesic effects. Moreover, sex differences have also been well identified in both basal and stress -induced activity of the hypothalamic-pituitary-adrenal axis (HPA) which is more responsive to physical and psychological stressors in females than males (Critchlow et al., 1963; Frederic et al., 1993; Goel and Bale, 2008; Iwasaki-Sekino et al., 2009; Mitsushima et al., 2003; Seale et al., 2004). Stress can exert a complex modulatory action on pain leading either to an adaptive (stress-induced analgesia) or a maladaptive (stress-induced hyperalgesia) response depending on the nature, intensity, and duration of the stressful or aversive stimulus (Ferdousi and Finn, 2018). In particular, psychological stress in early life has been evidenced as a predisposing factor for the development of chronic pain and various studies showed that early-life stress is associated with long-term changes in the activity of the HPA axis which exhibits dysfunctions in chronic pain patients (Burke et al., 2017; Catley et al., 2000; Heim et al., 2008; Maccari et al., 2014; Riva et al., 2010; Vachon-Preseau et al., 2013). Because stress stimulates the secretion of adrenal steroids that cross the blood-brain-barrier to serve as precursors for the production of brain neurosteroids which can modulate the HPA axis function, a recent work investigated whether neurosteroid levels in the brain display sex- and/or region-specific differences under

basal and acute stress conditions (Sze et al., 2018). This study which showed the existence of sex and regional differences in the brain concentrations of 5α -reduced steroids following acute stress, also suggested a sex-specific expression of steroidogenic enzymes in the brain (Sze et al., 2018). Thus, it appears that differential neurosteroidogenesis may contribute to sex differences in HPA axis responses to stress which in turns influence pain sensations. Therefore, future sex-based comparative investigations and elucidation of the specific role of neurosteroids endogenously synthesized in the female and male brain and/or spinal cord in normal and pathological pain conditions will certainly provide important insight to improve the current knowledge on sex dimorphism in pain sensitivity.

5. Conclusion

The data reviewed in the present paper show that $5\alpha,3\alpha$ -reduced neurosteroids, which possess a high therapeutic potential and a good toxicological profile, may be used to develop effective and safe strategies against neuropathic pain. Moreover, steroidal compounds in general, including hormonal steroids, neurosteroids and synthetic analogs of neuroactive steroids that control various mechanisms involved in pain sensation, deserve further attention to develop effective steroid-based strategies against neuropathic and chronic pain. In particular, several works that revealed a pivotal role of GABAA receptors (Gambhir et al., 2002; Goodchild et al., 2000, 2001; Nadeson and Goodchild, 2000, 2001) and VGCCs (Joksimovic et al., 2018a, 2018b, 2019; Pathirathna et al., 2005; Todorovic et al., 1998) in the mediation of analgesic and antinociceptive effects of neurosteroids suggest that further preclinical and clinical exploration of interactions between neurosteroids and these receptors/channels in various pain models/conditions may offer interesting opportunities to develop effective neurosteroid-based therapies against neuropathic pain. Finally, it is well known that mitochondrial dysfunction represents a major source of oxidative imbalance in

persistent pain conditions (Flatters, 2015; Grace et al., 2016; Guo et al., 2013; Gwak et al., 2013; Lee et al., 2007; Park et al., 2006; Schwartz et al., 2008; Sui et al., 2013; Xiao and Bennett, 2012). Therefore, pharmacological strategies aim to protect mitochondrial function may also represent an effective strategy to alleviate deleterious consequences of oxidative stress and promote functional neuroprotection in patients at risk for neuropathic pain. Interestingly, various natural neurosteroids or newly characterized analogs of neurosteroids have recently been shown to improve mitochondrial bioenergetics/activities and to increase neuronal survival under pathological conditions (Grimm et al., 2014, 2016; Karout et al., 2016; Lejri et al., 2017; Taleb et al., 2018). Altogether, these data open interesting perspectives for the therapeutic exploitation of neurosteroids for the effective management of neuropathic pain.

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Conflict of interest

Authors declare that they have no conflict of interest.

References

- Afrazi, S. and Esmaeili-Mahani, S., 2014. Allopregnanolone suppresses diabetes-induced neuropathic pain and motor deficit through inhibition of GABAA receptor down-regulation in the spinal cord of diabetic rats. *Iran J Basic Med Sci* 17, 312-317.
- Akwa, Y., Purdy, R.H., Koob, G.F. and Britton, K.T., 1999. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. *Behav Brain Res* 106, 119-125.
- Aloisi, A.M., 2003. Gonadal hormones and sex differences in pain reactivity. *Clin. J. Pain* 19, 168-174.
- Aloisi, A.M. and Bonifazi, M., 2006. Sex hormones, central nervous system and pain. *Horm Behav* 50, 1-7. <https://doi.org/10.1016/j.yhbeh.2005.12.002>
- Aloisi, A.M., Ceccarelli, I. and Fiorenzani, P., 2003. Gonadectomy affects hormonal and behavioral responses to repetitive nociceptive stimulation in male rats. *Ann. N Y Acad. Sci.* 1007, 232-237. <https://doi.org/10.1196/annals.1286.022>
- Aloisi, A.M., Ceccarelli, I., Fiorenzani, P., De Padova, A.M. and Massafra, C., 2004. Testosterone affects formalin-induced responses differently in male and female rats. *Neurosci Lett* 361, 262-264. <https://doi.org/10.1016/j.neulet.2003.12.023>
- Antoine, J.C. and Camdessanche, J.P., 2007. Peripheral nervous system involvement in patients with cancer. *Lancet Neurol* 6, 75-86. [https://doi.org/10.1016/S1474-4422\(06\)70679-2](https://doi.org/10.1016/S1474-4422(06)70679-2)
- Arendt-Nielsen, L., Bajaj, P. and Drewes, A.M., 2004. Visceral pain: gender differences in response to experimental and clinical pain. *Eur J Pain* 8, 465-472. <https://doi.org/10.1016/j.ejpain.2004.03.001>

Arevalo, M.A., Azcoitia, I. and Garcia-Segura, L.M., 2015. The neuroprotective actions of oestradiol and oestrogen receptors. *Nat Rev Neurosci* 16, 17-29. <https://doi.org/10.1038/nrn3856>

Authier, N., Balayssac, D., Marchand, F., Ling, B., Zangarelli, A., Descoeur, J., Coudore, F., Bourinet, E. and Eschalier, A., 2009. Animal models of chemotherapy-evoked painful peripheral neuropathies. *Neurotherapeutics* 6, 620-629. <https://doi.org/10.1016/j.nurt.2009.07.003>

Ayoola, C., Hwang, S.M., Hong, S.J., Rose, K.E., Boyd, C., Bozic, N., Park, J.Y., Osuru, H.P., DiGrucio, M.R., Covey, D.F., Jevtovic-Todorovic, V. and Todorovic, S.M., 2014. Inhibition of CaV3.2 T-type calcium channels in peripheral sensory neurons contributes to analgesic properties of epipregnanolone. *Psychopharmacol. (Berl)* 231, 3503-3515. <https://doi.org/10.1007/s00213-014-3588-0>

Baez-Jurado, E., Rincon-Benavides, M.A., Hidalgo-Lanussa, O., Guio-Vega, G., Ashraf, G.M., Sahebkar, A., Echeverria, V., Garcia-Segura, L.M. and Barreto, G.E., 2019. Molecular mechanisms involved in the protective actions of Selective Estrogen Receptor Modulators in brain cells. *Front Neuroendocrinol* 52, 44-64. <https://doi.org/10.1016/j.yfrne.2018.09.001>

Baker, D.E., 2003. Oxaliplatin: a new drug for the treatment of metastatic carcinoma of the colon or rectum. *Rev Gastroenteron Disord* 3, 31-38.

Balthazart, J., Choleris, E. and Remage-Healey, L., 2018. Steroids and the brain: 50years of research, conceptual shifts and the ascent of non-classical and membrane-initiated actions. *Horm Behav* 99, 1-8. <https://doi.org/10.1016/j.yhbeh.2018.01.002>

Bansal, R. and Singh, R., 2017. Exploring the potential of natural and synthetic neuroprotective steroids against neurodegenerative disorders: A literature review. *Med Res Rev*. <https://doi.org/10.1002/med.21458>

- Barnes, P.J., 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)* 94, 557-572.
- Barnes, P.J. and Adcock, I., 1993. Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol Sci* 14, 436-441.
- Baulieu, E.E., Robel, P. and Schumacher, M., 1999. Neurosteroids: A new regulatory function in the nervous system (*Contemporary Endocrinology*), Humana Press, Totowa.
- Belanger, B., Belanger, A., Labrie, F., Dupont, A., Cusan, L. and Monfette, G., 1989. Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. *J Steroid Biochem* 32, 695-698.
- Belelli, D. and Lambert, J.J., 2005. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci* 6, 565-575. <https://doi.org/10.1038/nrn1703>
- Belvisi, M.G. and Hele, D.J., 2003. Soft steroids: a new approach to the treatment of inflammatory airways diseases. *Pulm Pharmacol Ther* 16, 321-325. [https://doi.org/10.1016/S1094-5539\(03\)00105-6](https://doi.org/10.1016/S1094-5539(03)00105-6)
- Bennett, G.J. and Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33, 87-107.
- Bitran, D., Hilvers, R.J., Frye, C.A. and Erskine, M.S., 1996. Chronic anabolic-androgenic steroid treatment affects brain GABA(A) receptor-gated chloride ion transport. *Life Sci* 58, 573-583. [https://doi.org/10.1016/0024-3205\(95\)02326-7](https://doi.org/10.1016/0024-3205(95)02326-7)
- Bitran, D., Hilvers, R.J. and Kellogg, C.K., 1991. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. *Brain Res* 561, 157-161. [https://doi.org/10.1016/0006-8993\(91\)90761-j](https://doi.org/10.1016/0006-8993(91)90761-j)

Brann, D.W., Putnam, C.D. and Mahesh, V.B., 1990. Dose-Related Effects of Progesterone and 5 Alpha-Dihydroprogesterone upon Estrogen-Induced Prolactin Release. *J Neuroendocrinol* 2, 341-345. <https://doi.org/10.1111/j.1365-2826.1990.tb00415.x>

Brewer, J.R., Morrison, G., Dolan, M.E. and Fleming, G.F., 2016. Chemotherapy-induced peripheral neuropathy: Current status and progress. *Gynecol Oncol* 140, 176-183. <https://doi.org/10.1016/j.ygyno.2015.11.011>

Burke, N.N., Finn, D.P., McGuire, B.E. and Roche, M., 2017. Psychological stress in early life as a predisposing factor for the development of chronic pain: Clinical and preclinical evidence and neurobiological mechanisms. *J Neurosci Res* 95, 1257-1270. <https://doi.org/10.1002/jnr.23802>

Calabrese, D., Giatti, S., Romano, S., Porretta-Serapiglia, C., Bianchi, R., Milanese, M., Bonanno, G., Caruso, D., Viviani, B., Gardoni, F., Garcia-Segura, L.M. and Melcangi, R.C., 2014. Diabetic neuropathic pain: a role for testosterone metabolites. *J Endocrinol* 221, 1-13. <https://doi.org/10.1530/JOE-13-0541>

Cashin, M. and Moravsek, V., 1927. The physiological action of cholesterol. *Am J Physiol* 82, 294-298.

Catley, D., Kaell, A.T., Kirschbaum, C. and Stone, A.A., 2000. A naturalistic evaluation of cortisol secretion in persons with fibromyalgia and rheumatoid arthritis. *Arthritis Care Res* 13, 51-61.

Catterall, W.A., 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 26, 13-25. [https://doi.org/10.1016/s0896-6273\(00\)81133-2](https://doi.org/10.1016/s0896-6273(00)81133-2)

Cavaletti, G. and Marmiroli, P., 2015. Chemotherapy-induced peripheral neurotoxicity. *Curr Opin Neurol* 28, 500-507. <https://doi.org/10.1097/WCO.0000000000000234>

- Ceccarelli, I., Fiorenzani, P., Massafra, C. and Aloisi, A.M., 2003. Long-term ovariectomy changes formalin-induced licking in female rats: the role of estrogens. *Reprod Biol Endocrinol* 1, 24.
- Chakrabarti, S., Liu, N.J. and Gintzler, A.R., 2010. Formation of mu-/kappa-opioid receptor heterodimer is sex-dependent and mediates female-specific opioid analgesia. *Proc. Natl. Acad. Sci. U S A* 107, 20115-20119. <https://doi.org/10.1073/pnas.1009923107>
- Choi, S., Na, H.S., Kim, J., Lee, J., Lee, S., Kim, D., Park, J., Chen, C.C., Campbell, K.P. and Shin, H.S., 2007. Attenuated pain responses in mice lacking Ca(V)_{3.2} T-type channels. *Genes Brain Behav* 6, 425-431. <https://doi.org/10.1111/j.1601-183X.2006.00268.x>
- Ciriza, I., Azcoitia, I. and Garcia-Segura, L.M., 2004. Reduced progesterone metabolites protect rat hippocampal neurones from kainic acid excitotoxicity in vivo. *J Neuroendocrinol* 16, 58-63.
- Ciriza, I., Carrero, P., Frye, C.A. and Garcia-Segura, L.M., 2006. Reduced metabolites mediate neuroprotective effects of progesterone in the adult rat hippocampus. The synthetic progestin medroxyprogesterone acetate (Provera) is not neuroprotective. *J Neurobiol.* 66, 916-928. <https://doi.org/10.1002/neu.20293>
- Clatworthy, A.L., Illich, P.A., Castro, G.A. and Walters, E.T., 1995. Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain. *Neurosci Lett* 184, 5-8. [https://doi.org/10.1016/0304-3940\(94\)11154-b](https://doi.org/10.1016/0304-3940(94)11154-b)
- Colloca, L., Ludman, T., Bouhassira, D., Baron, R., Dickenson, A.H., Yarnitsky, D., Freeman, R., Truini, A., Attal, N., Finnerup, N.B., Eccleston, C., Kalso, E., Bennett, D.L., Dworkin, R.H. and Raja, S.N., 2017. Neuropathic pain. *Nat Rev Dis Primers* 3, 17002. <https://doi.org/10.1038/nrdp.2017.2>

- Compagnone, N.A. and Mellon, S.H., 2000. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front Neuroendocrinol* 21, 1-56. <https://doi.org/10.1006/frne.1999.0188>
- Coronel, M.F., Labombarda, F., Roig, P., Villar, M.J., De Nicola, A.F. and Gonzalez, S.L., 2011. Progesterone prevents nerve injury-induced allodynia and spinal NMDA receptor upregulation in rats. *Pain Med* 12, 1249-1261. <https://doi.org/10.1111/j.1526-4637.2011.01178.x>
- Coronel, M.F., Sanchez Granel, M.L., Raggio, M.C., Adler, N.S., De Nicola, A.F., Labombarda, F. and Gonzalez, S.L., 2016. Temporal changes in the expression of the translocator protein TSPO and the steroidogenic enzyme 5 α -reductase in the dorsal spinal cord of animals with neuropathic pain: Effects of progesterone administration. *Neurosci Lett* 624, 23-28. <https://doi.org/10.1016/j.neulet.2016.04.067>
- Craft, R.M., Mogil, J.S. and Aloisi, A.M., 2004. Sex differences in pain and analgesia: the role of gonadal hormones. *Eur J Pain* 8, 397-411. <https://doi.org/10.1016/j.ejpain.2004.01.003>
- Critchlow, V., Liebelt, R.A., Bar-Sela, M., Mountcastle, W. and Lipscomb, H.S., 1963. Sex difference in resting pituitary-adrenal function in the rat. *Am J Physiol* 205, 807-815. <https://doi.org/10.1152/ajplegacy.1963.205.5.807>
- Cruccu, G. and Truini, A., 2017. A review of Neuropathic Pain: From Guidelines to Clinical Practice. *Pain Ther* 6, 35-42. <https://doi.org/10.1007/s40122-017-0087-0>
- Dableh, L.J. and Henry, J.L., 2011. Progesterone prevents development of neuropathic pain in a rat model: Timing and duration of treatment are critical. *J Pain Res* 4, 91-101. <https://doi.org/10.2147/JPR.S17009>

- Dalla-Valle, L., Belvedere, P., Simontacchi, C. and Colombo, L., 1992. Extraglandular hormonal steroidogenesis in aged rats. *J Steroid Biochem Mol Biol* 43, 1095-1098. [https://doi.org/10.1016/0960-0760\(92\)90337-I](https://doi.org/10.1016/0960-0760(92)90337-I)
- De Nicola, A.F., Garay, L.I., Meyer, M., Guennoun, R., Sitruk-Ware, R., Schumacher, M. and Gonzalez Deniselle, M.C., 2018. Neurosteroidogenesis and progesterone anti-inflammatory/neuroprotective effects. *J Neuroendocrinol* 30. <https://doi.org/10.1111/jne.12502>
- Devor, M., Govrin-Lippmann, R. and Raber, P., 1985. Corticosteroids suppress ectopic neural discharge originating in experimental neuromas. *Pain* 22, 127-137.
- Ding, V.D., Moller, D.E., Feeney, W.P., Didolkar, V., Nakhla, A.M., Rhodes, L., Rosner, W. and Smith, R.G., 1998. Sex hormone-binding globulin mediates prostate androgen receptor action via a novel signaling pathway. *Endocrinology* 139, 213-218. <https://doi.org/10.1210/endo.139.1.5681>
- Dougherty, P.M., Cata, J.P., Burton, A.W., Vu, K. and Weng, H.R., 2007. Dysfunction in multiple primary afferent fiber subtypes revealed by quantitative sensory testing in patients with chronic vincristine-induced pain. *J Pain Symptom Manage* 33, 166-179. <https://doi.org/10.1016/j.jpainsymman.2006.08.006>
- Durand, J.P., Alexandre, J., Guillevin, L. and Goldwasser, F., 2005. Clinical activity of venlafaxine and topiramate against oxaliplatin-induced disabling permanent neuropathy. *Anticancer Drugs* 16, 587-591.
- Edinger, K.L. and Frye, C.A., 2004. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. *Behav Neurosci* 118, 1352-1364. <https://doi.org/10.1037/0735-7044.118.6.1352>

Edinger, K.L. and Frye, C.A., 2007. Androgens' effects to enhance learning may be mediated in part through actions at estrogen receptor-beta in the hippocampus. *Neurobiol Learn Mem* 87, 78-85. <https://doi.org/10.1016/j.nlm.2006.07.001>

Fakih, M., Johnson, C.S. and Trump, D.L., 2002. Glucocorticoids and treatment of prostate cancer: a preclinical and clinical review. *Urology* 60, 553-561. [https://doi.org/10.1016/s0090-4295\(02\)01741-7](https://doi.org/10.1016/s0090-4295(02)01741-7)

Fehrenbacher, J.C., 2015. Chemotherapy-induced peripheral neuropathy. *Prog Mol Biol Transl Sci* 131, 471-508. <https://doi.org/10.1016/bs.pmbts.2014.12.002>

Ferdousi, M. and Finn, D.P., 2018. Stress-induced modulation of pain: Role of the endogenous opioid system. *Prog Brain Res* 239, 121-177. <https://doi.org/10.1016/bs.pbr.2018.07.002>

Fillingim, R.B. and Gear, R.W., 2004. Sex differences in opioid analgesia: clinical and experimental findings. *Eur J Pain* 8, 413-425. <https://doi.org/10.1016/j.ejpain.2004.01.007>

Finn, D.A., Beadles-Bohling, A.S., Beckley, E.H., Ford, M.M., Gililand, K.R., Gorin-Meyer, R.E. and Wiren, K.M., 2006. A new look at the 5alpha-reductase inhibitor Finasteride. *CNS Drug Reviews* 12, 53-76. <https://doi.org/10.1111/j.1527-3458.2006.00053.x>

Finnerup, N.B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R.H., Gilron, I., Haanpaa, M., Hansson, P., Jensen, T.S., Kamerman, P.R., Lund, K., Moore, A., Raja, S.N., Rice, A.S., Rowbotham, M., Sena, E., Siddall, P., Smith, B.H. and Wallace, M., 2015. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 14, 162-173. [https://doi.org/10.1016/S1474-4422\(14\)70251-0](https://doi.org/10.1016/S1474-4422(14)70251-0)

Finnerup, N.B., Haroutounian, S., Kamerman, P., Baron, R., Bennett, D.L., Bouhassira, D., Cruccu, G., Freeman, R., Hansson, P., Nurmikko, T., Raja, S.N., Rice, A.S., Serra, J., Smith, B.H., Treede, R.D. and Jensen, T.S., 2016. Neuropathic pain: an updated grading

- system for research and clinical practice. *Pain* 157, 1599-1606.
<https://doi.org/10.1097/j.pain.0000000000000492>
- Firestein, G.S., Paine, M.M. and Littman, B.H., 1991. Gene expression (collagenase, tissue inhibitor of metalloproteinases, complement, and HLA-DR) in rheumatoid arthritis and osteoarthritis synovium. Quantitative analysis and effect of intraarticular corticosteroids. *Arthritis Rheum* 34, 1094-1105. <https://doi.org/10.1002/art.1780340905>
- Flatters, S.J., 2015. The contribution of mitochondria to sensory processing and pain. *Prog Mol Biol Transl Sci* 131, 119-146. <https://doi.org/10.1016/bs.pmbts.2014.12.004>
- Frederic, F., Oliver, C., Wollman, E., Delhaye-Bouchaud, N. and Mariani, J., 1993. IL-1 and LPS induce a sexually dimorphic response of the hypothalamo-pituitary-adrenal axis in several mouse strains. *Eur Cytokine Netw* 4, 321-329.
- Frye, C.A., Babson, A. and Walf, A.A., 2007. Self-administration of 3alpha-androstanediol increases locomotion and analgesia and decreases aggressive behavior of male hamsters. *Pharmacol. Biochem. Behav.* 86, 415-421. <https://doi.org/10.1016/j.pbb.2006.05.025>
- Frye, C.A., Duncan, J.E., Basham, M. and Erskine, M.S., 1996. Behavioral effects of 3 alpha-androstanediol. II: Hypothalamic and preoptic area actions via a GABAergic mechanism. *Behav Brain Res* 79, 119-130. [https://doi.org/10.1016/0166-4328\(96\)00005-8](https://doi.org/10.1016/0166-4328(96)00005-8)
- Frye, C.A. and Seliga, A.M., 2001. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. *Cogn Affect Behav Neurosci* 1, 371-381.
- Gambhir, M., Mediratta, P.K. and Sharma, K.K., 2002. Evaluation of the analgesic effect of neurosteroids and their possible mechanism of action. *Indian J Physiol Pharmacol* 46, 202-208.
- Gamelin, L., Boisdron-Celle, M., Delva, R., Guerin-Meyer, V., Ifrah, N., Morel, A. and Gamelin, E., 2004. Prevention of oxaliplatin-related neurotoxicity by calcium and magnesium infusions: a retrospective study of 161 patients receiving oxaliplatin

- combined with 5-Fluorouracil and leucovorin for advanced colorectal cancer. *Clin Cancer Res* 10, 4055-4061. <https://doi.org/10.1158/1078-0432.CCR-03-0666>
- Giatti, S., Diviccaro, S., Garcia-Segura, L.M. and Melcangi, R.C., 2019. Sex differences in the brain expression of steroidogenic molecules under basal conditions and after gonadectomy. *J Neuroendocrinol* 31, e12736. <https://doi.org/10.1111/jne.12736>
- Goel, N. and Bale, T.L., 2008. Organizational and activational effects of testosterone on masculinization of female physiological and behavioral stress responses. *Endocrinology* 149, 6399-6405. <https://doi.org/10.1210/en.2008-0433>
- Gonzalez, S.L., Meyer, L., Raggio, M.C., Taleb, O., Coronel, M.F., Patte-Mensah, C. and Mensah-Nyagan, A.G., 2019. Allopregnanolone and Progesterone in Experimental Neuropathic Pain: Former and New Insights with a Translational Perspective. *Cell Mol Neurobiol* 39, 523-537. <https://doi.org/10.1007/s10571-018-0618-1>
- Goodchild, C.S., Guo, Z. and Nadeson, R., 2000. Antinociceptive properties of neurosteroids I. Spinally-mediated antinociceptive effects of water-soluble aminosteroids. *Pain* 88, 23-29. [https://doi.org/10.1016/S0304-3959\(00\)00301-8](https://doi.org/10.1016/S0304-3959(00)00301-8)
- Goodchild, C.S., Robinson, A. and Nadeson, R., 2001. Antinociceptive properties of neurosteroids IV: pilot study demonstrating the analgesic effects of alphadolone administered orally to humans. *Br J Anaesth* 86, 528-534. <https://doi.org/10.1093/bja/86.4.528>
- Grace, P.M., Strand, K.A., Galer, E.L., Urban, D.J., Wang, X., Baratta, M.V., Fabisiak, T.J., Anderson, N.D., Cheng, K., Greene, L.I., Berkelhammer, D., Zhang, Y., Ellis, A.L., Yin, H.H., Campeau, S., Rice, K.C., Roth, B.L., Maier, S.F. and Watkins, L.R., 2016. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. *Proc. Natl. Acad. Sci. U S A* 113, E3441-3450. <https://doi.org/10.1073/pnas.1602070113>

- Green, P.S., Bishop, J. and Simpkins, J.W., 1997. 17 alpha-estradiol exerts neuroprotective effects on SK-N-SH cells. *J Neurosci* 17, 511-515. <https://doi.org/doi.org/10.1523/JNEUROSCI.17-02-00511.1997>
- Griffin, L.D., Gong, W., Verot, L. and Mellon, S.H., 2004. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. *Nat Med* 10, 704-711. <https://doi.org/10.1038/nm1073>
- Grimm, A., Mensah-Nyagan, A.G. and Eckert, A., 2016. Alzheimer, mitochondria and gender. *Neurosci. Biobehav. Rev.* 67, 89-101. <https://doi.org/10.1016/j.neubiorev.2016.04.012>
- Grimm, A., Schmitt, K., Lang, U.E., Mensah-Nyagan, A.G. and Eckert, A., 2014. Improvement of neuronal bioenergetics by neurosteroids: implications for age-related neurodegenerative disorders. *Biochim Biophys Acta* 1842, 2427-2438. <https://doi.org/10.1016/j.bbadis.2014.09.013>
- Gunn, B.G., Cunningham, L., Mitchell, S.G., Swinny, J.D., Lambert, J.J. and Belelli, D., 2015. GABAA receptor-acting neurosteroids: a role in the development and regulation of the stress response. *Front Neuroendocrinol* 36, 28-48. <https://doi.org/10.1016/j.yfrne.2014.06.001>
- Guo, B.L., Sui, B.D., Wang, X.Y., Wei, Y.Y., Huang, J., Chen, J., Wu, S.X., Li, Y.Q., Wang, Y.Y. and Yang, Y.L., 2013. Significant changes in mitochondrial distribution in different pain models of mice. *Mitochondrion* 13, 292-297. <https://doi.org/10.1016/j.mito.2013.03.007>
- Gwak, Y.S., Hassler, S.E. and Hulsebosch, C.E., 2013. Reactive oxygen species contribute to neuropathic pain and locomotor dysfunction via activation of CamKII in remote segments following spinal cord contusion injury in rats. *Pain* 154, 1699-1708. <https://doi.org/10.1016/j.pain.2013.05.018>

- Haines, D.E., Mihailoff, G.A. and Yeziarski, R.P. (1997). The Spinal Cord. *Fundamental Neuroscience*. Haines, D.E. New York, Churchill Livingstone Inc.: 129-141.
- Hau, M., Dominguez, O.A. and Evrard, H.C., 2004. Testosterone reduces responsiveness to nociceptive stimuli in a wild bird. *Horm Behav* 46, 165-170. <https://doi.org/10.1016/j.yhbeh.2004.02.007>
- Hayashi, R., Xiao, W., Kawamoto, M., Yuge, O. and Bennett, G.J., 2008. Systemic glucocorticoid therapy reduces pain and the number of endoneurial tumor necrosis factor-alpha (TNFalpha)-positive mast cells in rats with a painful peripheral neuropathy. *J Pharmacol Sci* 106, 559-565.
- Heim, C., Newport, D.J., Mletzko, T., Miller, A.H. and Nemeroff, C.B., 2008. The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinol.* 33, 693-710. <https://doi.org/10.1016/j.psyneuen.2008.03.008>
- Hershman, D.L., Lacchetti, C., Dworkin, R.H., Lavoie Smith, E.M., Bleeker, J., Cavaletti, G., Chauhan, C., Gavin, P., Lavino, A., Lustberg, M.B., Paice, J., Schneider, B., Smith, M.L., Smith, T., Terstriep, S., Wagner-Johnston, N., Bak, K. and Loprinzi, C.L., 2014. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol* 32, 1941-1967. <https://doi.org/10.1200/JCO.2013.54.0914>
- Higashi, T., Nishio, T., Yokoi, H., Ninomiya, Y. and Shimada, K., 2007. Studies on Neurosteroids XXI: an improved liquid chromatography-tandem mass spectrometric method for determination of 5alpha-androstane-3alpha,17beta-diol in rat brains. *Anal Sci* 23, 1015-1019. <https://doi.org/10.2116/analsci.23.1015>
- Holmberg, E., Backstrom, T., Johansson, M., Lofgren, M. and Haage, D., 2013. Allopregnanolone induces a diurnally dependent hyperphagic effect and alters feeding

latency and duration in male Wistar rats. *Acta Physiol (Oxf)* 208, 400-409.
<https://doi.org/10.1111/apha.12100>

Holzbauer, M., 1976. Proceedings: Physiological aspects of the hypnotic properties of steroid hormones. *Br J Pharmacol* 56, 382P-382P

Hosie, A.M., Wilkins, M.E., da Silva, H.M. and Smart, T.G., 2006. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. *Nature* 444, 486-489. <https://doi.org/10.1038/nature05324>

Hou, S., Huh, B., Kim, H.K., Kim, K.H. and Abdi, S., 2018. Treatment of Chemotherapy-Induced Peripheral Neuropathy: Systematic Review and Recommendations. *Pain Physician* 21, 571-592

Huang, C.T., Chen, S.H., Lue, J.H., Chang, C.F., Wen, W.H. and Tsai, Y.J., 2016. Neurosteroid Allopregnanolone Suppresses Median Nerve Injury-induced Mechanical Hypersensitivity and Glial Extracellular Signal-regulated Kinase Activation through gamma-Aminobutyric Acid Type A Receptor Modulation in the Rat Cuneate Nucleus. *Anesthesiology* 125, 1202-1218. <https://doi.org/10.1097/ALN.0000000000001360>

Iwasaki-Sekino, A., Mano-Otagiri, A., Ohata, H., Yamauchi, N. and Shibasaki, T., 2009. Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. *Psychoneuroendocrinol.* 34, 226-237. <https://doi.org/10.1016/j.psyneuen.2008.09.003>

Jamieson, S.M., Subramaniam, J., Liu, J.J., Jong, N.N., Ip, V., Connor, B. and McKeage, M.J., 2009. Oxaliplatin-induced loss of phosphorylated heavy neurofilament subunit neuronal immunoreactivity in rat DRG tissue. *Mol. Pain* 5, 66. <https://doi.org/10.1186/1744-8069-5-66>

- Jensen, M.P., Moore, M.R., Bockow, T.B., Ehde, D.M. and Engel, J.M., 2011. Psychosocial factors and adjustment to chronic pain in persons with physical disabilities: a systematic review. *Arch Phys Med Rehabil* 92, 146-160. <https://doi.org/10.1016/j.apmr.2010.09.021>
- Johansson, A. and Bennett, G.J., 1997. Effect of local methylprednisolone on pain in a nerve injury model. A pilot study. *Reg Anesth* 22, 59-65.
- Joksimovic, S.L., Covey, D.F., Jevtovic-Todorovic, V. and Todorovic, S.M., 2018a. Neurosteroids in Pain Management: A New Perspective on an Old Player. *Front Pharmacol* 9, 1127. <https://doi.org/10.3389/fphar.2018.01127>
- Joksimovic, S.L., Donald, R.R., Park, J.Y. and Todorovic, S.M., 2019. Inhibition of multiple voltage-gated calcium channels may contribute to spinally mediated analgesia by epipregnanolone in a rat model of surgical paw incision. *Channels (Austin)* 13, 48-61. <https://doi.org/10.1080/19336950.2018.1564420>
- Joksimovic, S.L., Joksimovic, S.M., Tesic, V., Garcia-Caballero, A., Feseha, S., Zamponi, G.W., Jevtovic-Todorovic, V. and Todorovic, S.M., 2018b. Selective inhibition of CaV3.2 channels reverses hyperexcitability of peripheral nociceptors and alleviates postsurgical pain. *Sci Signal* 11. <https://doi.org/10.1126/scisignal.aao4425>
- Jones, K.J., 1993. Gonadal steroids and neuronal regeneration. A therapeutic role. *Adv Neurol* 59, 227-240.
- Karout, M., Miesch, M., Geoffroy, P., Kraft, S., Hofmann, H.D., Mensah-Nyagan, A.G. and Kirsch, M., 2016. Novel analogs of allopregnanolone show improved efficiency and specificity in neuroprotection and stimulation of proliferation. *J. Neurochem.* 139, 782-794. <https://doi.org/10.1111/jnc.13693>
- Kibaly, C., Meyer, L., Patte-Mensah, C. and Mensah-Nyagan, A.G., 2008. Biochemical and functional evidence for the control of pain mechanisms by dehydroepiandrosterone

- endogenously synthesized in the spinal cord. *Faseb J.* 22, 93-104.
<https://doi.org/10.1096/fj.07-8930com>
- Kibaly, C., Patte-Mensah, C. and Mensah-Nyagan, A.G., 2005. Molecular and neurochemical evidence for the biosynthesis of dehydroepiandrosterone in the adult rat spinal cord. *J. Neurochem.* 93, 1220-1230. <https://doi.org/10.1111/j.1471-4159.2005.03113.x>
- Kim, D., Park, D., Choi, S., Lee, S., Sun, M., Kim, C. and Shin, H.S., 2003. Thalamic control of visceral nociception mediated by T-type Ca²⁺ channels. *Science* 302, 117-119.
<https://doi.org/10.1126/science.1088886>
- Kimonides, V.G., Khatibi, N.H., Svendsen, C.N., Sofroniew, M.V. and Herbert, J., 1998. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. *Proc. Natl. Acad. Sci. U S A* 95, 1852-1857. <https://doi.org/10.1073/pnas.95.4.1852>
- Kingery, W.S., 1997. A critical review of controlled clinical trials for peripheral neuropathic pain and complex regional pain syndromes. *Pain* 73, 123-139.
[https://doi.org/10.1016/S0304-3959\(97\)00049-3](https://doi.org/10.1016/S0304-3959(97)00049-3)
- Kingery, W.S., Castellote, J.M. and Maze, M., 1999. Methylprednisolone prevents the development of autotomy and neuropathic edema in rats, but has no effect on nociceptive thresholds. *Pain* 80, 555-566. [https://doi.org/10.1016/S0304-3959\(98\)00251-6](https://doi.org/10.1016/S0304-3959(98)00251-6)
- Kraulis, I., Foldes, G., Traikov, H., Dubrovsky, B. and Birmingham, 1975. Distribution, metabolism and biological activity of deoxycorticosterone in the central nervous system. *Brain Res* 88, 1-14. [https://doi.org/10.1016/0006-8993\(75\)90942-7](https://doi.org/10.1016/0006-8993(75)90942-7)
- Labombarda, F., Pianos, A., Liere, P., Eychenne, B., Gonzalez, S., Cambourg, A., De Nicola, A.F., Schumacher, M. and Guennoun, R., 2006. Injury elicited increase in spinal cord neurosteroid content analyzed by gas chromatography mass spectrometry. *Endocrinology* 147, 1847-1859. <https://doi.org/10.1210/en.2005-0955>

- Lang, E., Hord, A.H. and Denson, D., 1996. Venlafaxine hydrochloride (Effexor) relieves thermal hyperalgesia in rats with an experimental mononeuropathy. *Pain* 68, 151-155.
- Lauria, G., Cornblath, D.R., Johansson, O., McArthur, J.C., Mellgren, S.I., Nolano, M., Rosenberg, N. and Sommer, C., 2005. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. *Eur J Neurol* 12, 747-758. <https://doi.org/10.1111/j.1468-1331.2005.01260.x>
- Le Goascogne, C., Robel, P., Gouezou, M., Sananes, N., Baulieu, E.E. and Waterman, M., 1987. Neurosteroids: cytochrome P-450_{scc} in rat brain. *Science* 237, 1212-1215. <https://doi.org/10.1126/science.3306919>
- Lee, Y.A., Kim, Y.J., Cho, E.J. and Yokozawa, T., 2007. Ameliorative effects of proanthocyanidin on oxidative stress and inflammation in streptozotocin-induced diabetic rats. *J Agric Food Chem* 55, 9395-9400. <https://doi.org/10.1021/jf071523u>
- Lejri, I., Grimm, A., Miesch, M., Geoffroy, P., Eckert, A. and Mensah-Nyagan, A.G., 2017. Allopregnanolone and its analog BR 297 rescue neuronal cells from oxidative stress-induced death through bioenergetic improvement. *Biochim Biophys Acta* 1863, 631-642. <https://doi.org/10.1016/j.bbadis.2016.12.007>
- Leonelli, E., Bianchi, R., Cavaletti, G., Caruso, D., Crippa, D., Garcia-Segura, L.M., Lauria, G., Magnaghi, V., Roglio, I. and Melcangi, R.C., 2007. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. *Neuroscience* 144, 1293-1304. <https://doi.org/10.1016/j.neuroscience.2006.11.014>
- Ling, B., Authier, N., Balayssac, D., Eschalier, A. and Coudore, F., 2007a. Behavioral and pharmacological description of oxaliplatin-induced painful neuropathy in rat. *Pain* 128, 225-234. <https://doi.org/10.1016/j.pain.2006.09.016>
- Ling, B., Coudore-Civiale, M.A., Balayssac, D., Eschalier, A., Coudore, F. and Authier, N., 2007b. Behavioral and immunohistological assessment of painful neuropathy induced by

a single oxaliplatin injection in the rat. *Toxicology* 234, 176-184.
<https://doi.org/10.1016/j.tox.2007.02.013>

Loyd, D.R. and Murphy, A.Z., 2006. Sex differences in the anatomical and functional organization of the periaqueductal gray-rostral ventromedial medullary pathway in the rat: a potential circuit mediating the sexually dimorphic actions of morphine. *J Comp Neurol* 496, 723-738. <https://doi.org/10.1002/cne.20962>

Loyd, D.R. and Murphy, A.Z., 2008. Androgen and estrogen (alpha) receptor localization on periaqueductal gray neurons projecting to the rostral ventromedial medulla in the male and female rat. *J Chem Neuroanat* 36, 216-226. <https://doi.org/10.1016/j.jchemneu.2008.08.001>

Lussier, D., Huskey, A.G. and Portenoy, R.K., 2004. Adjuvant analgesics in cancer pain management. *Oncologist* 9, 571-591. <https://doi.org/10.1634/theoncologist.9-5-571>

Maccari, S., Krugers, H.J., Morley-Fletcher, S., Szyf, M. and Brunton, P.J., 2014. The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. *J Neuroendocrinol* 26, 707-723. <https://doi.org/10.1111/jne.12175>

Majewska, M.D., 1992. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Prog. Neurobiol.* 38, 379-395. [https://doi.org/10.1016/0301-0082\(92\)90025-A](https://doi.org/10.1016/0301-0082(92)90025-A)

Marchand, F., Alloui, A., Chapuy, E., Jourdan, D., Pelissier, T., Ardid, D., Hernandez, A. and Eschalier, A., 2003. Evidence for a monoamine mediated, opioid-independent, antihyperalgesic effect of venlafaxine, a non-tricyclic antidepressant, in a neurogenic pain model in rats. *Pain* 103, 229-235. [https://doi.org/10.1016/S0304-3959\(03\)00168-4](https://doi.org/10.1016/S0304-3959(03)00168-4)

Martel, C., Melner, M.H., Gagne, D., Simard, J. and Labrie, F., 1994. Widespread tissue distribution of steroid sulfatase, 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4

- isomerase (3 beta-HSD), 17 beta-HSD 5 alpha-reductase and aromatase activities in the rhesus monkey. *Mol Cell Endocrinol* 104, 103-111
- Maurice, T., Gregoire, C. and Espallergues, J., 2006. Neuro(active)steroids actions at the neuromodulatory sigma1 (sigma1) receptor: biochemical and physiological evidences, consequences in neuroprotection. *Pharmacol. Biochem. Behav.* 84, 581-597.
- McEwen, B.S. (1994). Endocrine effects on the brain and their relationship to behavior. *Basic Neurochemistry*. Siegel, G.J., Agranoff, B.W., Albers, R.W. and Molinoff, B. New York, Raven Press: 1003-1023.
- Melcangi, R.C., Garcia-Segura, L.M. and Mensah-Nyagan, A.G., 2008. Neuroactive steroids: state of the art and new perspectives. *Cell. Mol. Life Sci.* 65, 777-797. <https://doi.org/10.1007/s00018-007-7403-5>
- Melcangi, R.C. and Mensah-Nyagan, A.G., 2006. Neuroprotective effects of neuroactive steroids in the spinal cord and peripheral nerves. *J Mol Neurosci* 28, 1-2. <https://doi.org/10.1385/JMN:30:3:341>
- Mellon, S.H. and Griffin, L.D., 2002. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab* 13, 35-43. [https://doi.org/10.1016/S1043-2760\(01\)00503-3](https://doi.org/10.1016/S1043-2760(01)00503-3)
- Mensah-Nyagan, A.G., Do-Rego, J.L., Beaujean, D., Luu-The, V., Pelletier, G. and Vaudry, H., 1999. Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. *Pharmacol Rev* 51, 63-81.
- Mensah-Nyagan, A.G., Do-Rego, J.L., Feuilleley, M., Marcual, A., Lange, C., Pelletier, G. and Vaudry, H., 1996a. In vivo and in vitro evidence for the biosynthesis of testosterone in the telencephalon of the female frog. *J. Neurochem.* 67, 413-422. <https://doi.org/10.1046/j.1471-4159.1996.67010413.x>
- Mensah-Nyagan, A.G., Feuilleley, M., Do-Rego, J.L., Marcual, A., Lange, C., Tonon, M.C., Pelletier, G. and Vaudry, H., 1996b. Localization of 17beta-hydroxysteroid

- dehydrogenase and characterization of testosterone in the brain of the male frog. *Proc. Natl. Acad. Sci. U S A* 93, 1423-1428. <https://doi.org/10.1073/pnas.93.4.1423>
- Mensah-Nyagan, A.G., Kibaly, C., Schaeffer, V., Venard, C., Meyer, L. and Patte-Mensah, C., 2008a. Endogenous steroid production in the spinal cord and potential involvement in neuropathic pain modulation. *J Steroid Biochem Mol Biol* 109, 286-293. <https://doi.org/10.1016/j.jsbmb.2008.03.002>
- Mensah-Nyagan, A.G., Saredi, S., Schaeffer, V., Kibaly, C., Meyer, L., Melcangi, R.C. and Patte-Mensah, C., 2008b. Assessment of neuroactive steroid formation in diabetic rat spinal cord using high-performance liquid chromatography and continuous flow scintillation detection. *Neurochem. Int.* 52, 554-559. <https://doi.org/10.1016/j.neuint.2007.06.010>
- Meyer, L., Patte-Mensah, C., Taleb, O. and Mensah-Nyagan, A.G., 2010. Cellular and functional evidence for a protective action of neurosteroids against vincristine chemotherapy-induced painful neuropathy. *Cell. Mol. Life Sci.* <https://doi.org/10.1007/s00018-010-0372-0>
- Meyer, L., Patte-Mensah, C., Taleb, O. and Mensah-Nyagan, A.G., 2011. Allopregnanolone prevents and suppresses oxaliplatin-evoked painful neuropathy: multi-parametric assessment and direct evidence. *Pain* 152, 170-181. <https://doi.org/10.1016/j.pain.2010.10.015>
- Meyer, L., Patte-Mensah, C., Taleb, O. and Mensah-Nyagan, A.G., 2013. Neurosteroid 3alpha-androstanediol efficiently counteracts paclitaxel-induced peripheral neuropathy and painful symptoms. *PLoS One* 8, e80915. <https://doi.org/10.1371/journal.pone.0080915>
- Meyer, L., Venard, C., Schaeffer, V., Patte-Mensah, C. and Mensah-Nyagan, A.G., 2008. The biological activity of 3alpha-hydroxysteroid oxido-reductase in the spinal cord regulates

- thermal and mechanical pain thresholds after sciatic nerve injury. *Neurobiol Dis* 30, 30-41. <https://doi.org/10.1016/j.nbd.2007.12.001>
- Millan, M.J., 1999. The induction of pain: an integrative review. *Prog. Neurobiol.* 57, 1-164. [https://doi.org/10.1016/S0301-0082\(98\)00048-3](https://doi.org/10.1016/S0301-0082(98)00048-3)
- Millan, M.J., 2002. Descending control of pain. *Prog. Neurobiol.* 66, 355-474. [https://doi.org/10.1016/S0301-0082\(02\)00009-6](https://doi.org/10.1016/S0301-0082(02)00009-6)
- Mitsushima, D., Masuda, J. and Kimura, F., 2003. Sex differences in the stress-induced release of acetylcholine in the hippocampus and corticosterone from the adrenal cortex in rats. *Neuroendocrinology* 78, 234-240. <https://doi.org/10.1159/000073707>
- Nadeson, R. and Goodchild, C.S., 2000. Antinociceptive properties of neurosteroids II. Experiments with Saffan and its components alphaxalone and alphadolone to reveal separation of anaesthetic and antinociceptive effects and the involvement of spinal cord GABA(A) receptors. *Pain* 88, 31-39. [https://doi.org/10.1016/S0304-3959\(00\)00300-6](https://doi.org/10.1016/S0304-3959(00)00300-6)
- Nadeson, R. and Goodchild, C.S., 2001. Antinociceptive properties of neurosteroids III: experiments with alphadolone given intravenously, intraperitoneally, and intragastrically. *Br J Anaesth* 86, 704-708. <https://doi.org/10.1093/bja/86.5.704>
- Ocvirk, R., Pearson Murphy, B.E., Franklin, K.B. and Abbott, F.V., 2008. Antinociceptive profile of ring A-reduced progesterone metabolites in the formalin test. *Pain* 138, 402-409. <https://doi.org/10.1016/j.pain.2008.01.019>
- Olsen, R.W., 2018. GABAA receptor: Positive and negative allosteric modulators. *Neuropharmacol.* 136, 10-22. <https://doi.org/10.1016/j.neuropharm.2018.01.036>
- Park, E.S., Gao, X., Chung, J.M. and Chung, K., 2006. Levels of mitochondrial reactive oxygen species increase in rat neuropathic spinal dorsal horn neurons. *Neurosci Lett* 391, 108-111. <https://doi.org/10.1016/j.neulet.2005.08.055>

- Pathirathna, S., Brimelow, B.C., Jagodic, M.M., Krishnan, K., Jiang, X., Zorumski, C.F., Mennerick, S., Covey, D.F., Todorovic, S.M. and Jevtovic-Todorovic, V., 2005. New evidence that both T-type calcium channels and GABAA channels are responsible for the potent peripheral analgesic effects of 5alpha-reduced neuroactive steroids. *Pain* 114, 429-443. <https://doi.org/10.1016/j.pain.2005.01.009>
- Patte-Mensah, C., Kappes, V., Freund-Mercier, M.J., Tsutsui, K. and Mensah-Nyagan, A.G., 2003. Cellular distribution and bioactivity of the key steroidogenic enzyme, cytochrome P450side chain cleavage, in sensory neural pathways. *J. Neurochem.* 86, 1233-1246. <https://doi.org/10.1046/j.1471-4159.2003.01935.x>
- Patte-Mensah, C., Kibaly, C., Boudard, D., Schaeffer, V., Begle, A., Saredi, S., Meyer, L. and Mensah-Nyagan, A.G., 2006. Neurogenic pain and steroid synthesis in the spinal cord. *J Mol Neurosci* 28, 17-31. <https://doi.org/10.1385/JMN:30:3:341>
- Patte-Mensah, C., Kibaly, C. and Mensah-Nyagan, A.G., 2005. Substance P inhibits progesterone conversion to neuroactive metabolites in spinal sensory circuit: a potential component of nociception. *Proc. Natl. Acad. Sci. U S A* 102, 9044-9049. <https://doi.org/10.1073/pnas.0502968102>
- Patte-Mensah, C., Li, S. and Mensah-Nyagan, A.G., 2004a. Impact of neuropathic pain on the gene expression and activity of cytochrome P450side-chain-cleavage in sensory neural networks. *Cell. Mol. Life Sci.* 61, 2274-2284. <https://doi.org/10.1007/s00018-004-4235-4>
- Patte-Mensah, C. and Mensah-Nyagan, A.G., 2008. Peripheral neuropathy and neurosteroid formation in the central nervous system. *Brain Res Rev* 57, 454-459. <https://doi.org/10.1016/j.brainresrev.2007.05.007>
- Patte-Mensah, C., Meyer, L., Kibaly, C. and Mensah-Nyagan, A.G., 2010. Regulatory effect of dehydroepiandrosterone on spinal cord nociceptive function. *Front Biosci (Elite Ed)* 2, 1528-1537. <https://doi.org/10.2741/212>

- Patte-Mensah, C., Meyer, L., Taleb, O. and Mensah-Nyagan, A.G., 2014. Potential role of allopregnanolone for a safe and effective therapy of neuropathic pain. *Prog. Neurobiol.* 113, 70-78. <https://doi.org/10.1016/j.pneurobio.2013.07.004>
- Patte-Mensah, C., Penning, T.M. and Mensah-Nyagan, A.G., 2004b. Anatomical and cellular localization of neuroactive 5 alpha/3 alpha-reduced steroid-synthesizing enzymes in the spinal cord. *J Comp Neurol* 477, 286-299. <https://doi.org/10.1002/cne.20251>
- Pettersson, H., Lundqvist, J., Oliw, E. and Norlin, M., 2009. CYP7B1-mediated metabolism of 5alpha-androstane-3alpha,17beta-diol (3alpha-Adiol): a novel pathway for potential regulation of the cellular levels of androgens and neurosteroids. *Biochim Biophys Acta* 1791, 1206-1215. <https://doi.org/10.1016/j.bbailip.2009.08.010>
- Polomano, R.C. and Bennett, G.J., 2001. Chemotherapy-evoked painful peripheral neuropathy. *Pain Med* 2, 8-14. <https://doi.org/10.1046/j.1526-4637.2001.002001008.x>
- Purdy, R.H., Morrow, A.L., Moore, P.H., Jr. and Paul, S.M., 1991. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc. Natl. Acad. Sci. U S A* 88, 4553-4557. <https://doi.org/10.1073/pnas.88.10.4553>
- Ravula, S.K., Wang, M.S., McClain, M.A., Asress, S.A., Frazier, B. and Glass, J.D., 2007. Spatiotemporal localization of injury potentials in DRG neurons during vincristine-induced axonal degeneration. *Neurosci Lett* 415, 34-39. <https://doi.org/10.1016/j.neulet.2007.01.009>
- Reddy, D.S., 2008. Mass spectrometric assay and physiological-pharmacological activity of androgenic neurosteroids. *Neurochem. Int.* 52, 541-553. <https://doi.org/10.1016/j.neuint.2007.05.019>
- Rhodes, M.E. and Frye, C.A., 2004. Androgens in the hippocampus can alter, and be altered by, ictal activity. *Pharmacol. Biochem. Behav.* 78, 483-493. <https://doi.org/10.1016/j.pbb.2004.04.020>

Riva, R., Mork, P.J., Westgaard, R.H., Ro, M. and Lundberg, U., 2010. Fibromyalgia syndrome is associated with hypocortisolism. *Int J Behav Med* 17, 223-233. <https://doi.org/10.1007/s12529-010-9097-6>

Roglio, I., Bianchi, R., Camozzi, F., Carozzi, V., Cervellini, I., Crippa, D., Lauria, G., Cavaletti, G. and Melcangi, R.C., 2009. Docetaxel-induced peripheral neuropathy: protective effects of dihydroprogesterone and progesterone in an experimental model. *J Peripher Nerv Syst* 14, 36-44. <https://doi.org/10.1111/j.1529-8027.2009.00204.x>

Roglio, I., Bianchi, R., Gotti, S., Scurati, S., Giatti, S., Pesaresi, M., Caruso, D., Panzica, G.C. and Melcangi, R.C., 2008. Neuroprotective effects of dihydroprogesterone and progesterone in an experimental model of nerve crush injury. *Neuroscience* 155, 673-685. <https://doi.org/10.1016/j.neuroscience.2008.06.034>

Rudolph, L.M., Cornil, C.A., Mittelman-Smith, M.A., Rainville, J.R., Remage-Healey, L., Sinchak, K. and Micevych, P.E., 2016. Actions of Steroids: New Neurotransmitters. *J Neurosci* 36, 11449-11458. <https://doi.org/10.1523/JNEUROSCI.2473-16.2016>.

Sapolsky, R.M., 1996. Stress, Glucocorticoids, and Damage to the Nervous System: The Current State of Confusion. *Stress* 1, 1-19

Saredi, S., Patte-Mensah, C., Melcangi, R.C. and Mensah-Nyagan, A.G., 2005. Effect of streptozotocin-induced diabetes on the gene expression and biological activity of 3beta-hydroxysteroid dehydrogenase in the rat spinal cord. *Neuroscience* 135, 869-877. <https://doi.org/10.1016/j.neuroscience.2005.06.033>

Sayed, I., Guo, Q., Hoffman, S.W. and Stein, D.G., 2006. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med* 47, 381-389. <https://doi.org/10.1016/j.annemergmed.2005.12.011>

- Schaeffer, V., Meyer, L., Patte-Mensah, C., Eckert, A. and Mensah-Nyagan, A.G., 2010a. Sciatic nerve injury induces apoptosis of dorsal root ganglion satellite glial cells and selectively modifies neurosteroidogenesis in sensory neurons. *Glia* 58, 169-180. <https://doi.org/10.1002/glia.20910>
- Schaeffer, V., Meyer, L., Patte-Mensah, C. and Mensah-Nyagan, A.G., 2010b. Progress in dorsal root ganglion neurosteroidogenic activity: basic evidence and pathophysiological correlation. *Prog. Neurobiol.* 92, 33-41. <https://doi.org/10.1016/j.pneurobio.2010.04.009>
- Schwartz, E.S., Lee, I., Chung, K. and Chung, J.M., 2008. Oxidative stress in the spinal cord is an important contributor in capsaicin-induced mechanical secondary hyperalgesia in mice. *Pain* 138, 514-524. <https://doi.org/10.1016/j.pain.2008.01.029>
- Seale, J.V., Wood, S.A., Atkinson, H.C., Bate, E., Lightman, S.L., Ingram, C.D., Jessop, D.S. and Harbuz, M.S., 2004. Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *J Neuroendocrinol* 16, 516-524. <https://doi.org/10.1111/j.1365-2826.2004.01195.x>
- Seckl, J.R., 1997. 11beta-Hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? *Front Neuroendocrinol* 18, 49-99. <https://doi.org/10.1006/frne.1996.0143>
- Selye, H. and Masson, G., 1942. Additional Steroids with Luteoid Activity. *Science* 96, 358. <https://doi.org/10.1126/science.96.2494.358>
- Seretny, M., Currie, G.L., Sena, E.S., Ramnarine, S., Grant, R., MacLeod, M.R., Colvin, L.A. and Fallon, M., 2014. Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. *Pain* 155, 2461-2470. <https://doi.org/10.1016/j.pain.2014.09.020>

- Siau, C., Xiao, W. and Bennett, G.J., 2006. Paclitaxel- and vincristine-evoked painful peripheral neuropathies: loss of epidermal innervation and activation of Langerhans cells. *Exp Neurol* 201, 507-514. <https://doi.org/10.1016/j.expneurol.2006.05.007>
- Sibilia, J., 2003. Corticosteroids and inflammation. *Rev Prat* 53, 495-501.
- Sommer, C. and Kress, M., 2004. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett* 361, 184-187. <https://doi.org/10.1016/j.neulet.2003.12.007>
- Stoner, E., 1990. The clinical development of a 5alpha-reductase inhibitor, finasteride. *J Steroid Biochem Molec Biol* 37, 375-378.
- Sui, B.D., Xu, T.Q., Liu, J.W., Wei, W., Zheng, C.X., Guo, B.L., Wang, Y.Y. and Yang, Y.L., 2013. Understanding the role of mitochondria in the pathogenesis of chronic pain. *Postgrad Med J* 89, 709-714. <https://doi.org/10.1136/postgradmedj-2012-131068>
- Sze, Y., Gill, A.C. and Brunton, P.J., 2018. Sex-dependent changes in neuroactive steroid concentrations in the rat brain following acute swim stress. *J Neuroendocrinol* 30, e12644. <https://doi.org/10.1111/jne.12644>
- Takeda, K., Sawamura, S., Sekiyama, H., Tamai, H. and Hanaoka, K., 2004. Effect of methylprednisolone on neuropathic pain and spinal glial activation in rats. *Anesthesiology* 100, 1249-1257. <https://doi.org/10.1097/00000542-200405000-00029>
- Taleb, O., Bouzobra, F., Tekin-Pala, H., Meyer, L., Mensah-Nyagan, A.G. and Patte-Mensah, C., 2017. Behavioral and electromyographic assessment of oxaliplatin-induced motor dysfunctions: Evidence for a therapeutic effect of allopregnanolone. *Behav Brain Res* 320, 440-449. <https://doi.org/10.1016/j.bbr.2016.10.040>
- Taleb, O., Patte-Mensah, C., Meyer, L., Kemmel, V., Geoffroy, P., Miesch, M. and Mensah-Nyagan, A.G., 2018. Evidence for effective structure-based neuromodulatory effects of

new analogues of neurosteroid allopregnanolone. *J Neuroendocrinol* 30.
<https://doi.org/10.1111/jne.12568>

Timby, E., Balgard, M., Nyberg, S., Spigset, O., Andersson, A., Porankiewicz-Asplund, J., Purdy, R.H., Zhu, D., Backstrom, T. and Poromaa, I.S., 2006. Pharmacokinetic and behavioral effects of allopregnanolone in healthy women. *Psychopharmacol. (Berl)* 186, 414-424. <https://doi.org/10.1007/s00213-005-0148-7>

Todorovic, S.M., Jevtovic-Todorovic, V., Meyenburg, A., Mennerick, S., Perez-Reyes, E., Romano, C., Olney, J.W. and Zorumski, C.F., 2001. Redox modulation of T-type calcium channels in rat peripheral nociceptors. *Neuron* 31, 75-85. [https://doi.org/10.1016/s0896-6273\(01\)00338-5](https://doi.org/10.1016/s0896-6273(01)00338-5)

Todorovic, S.M., Meyenburg, A. and Jevtovic-Todorovic, V., 2002. Mechanical and thermal antinociception in rats following systemic administration of mibefradil, a T-type calcium channel blocker. *Brain Res* 951, 336-340. [https://doi.org/10.1016/s0006-8993\(02\)03350-4](https://doi.org/10.1016/s0006-8993(02)03350-4)

Todorovic, S.M., Pathirathna, S., Brimelow, B.C., Jagodic, M.M., Ko, S.H., Jiang, X., Nilsson, K.R., Zorumski, C.F., Covey, D.F. and Jevtovic-Todorovic, V., 2004. 5beta-reduced neuroactive steroids are novel voltage-dependent blockers of T-type Ca²⁺ channels in rat sensory neurons in vitro and potent peripheral analgesics in vivo. *Mol Pharmacol* 66, 1223-1235. <https://doi.org/10.1124/mol.104.002402>

Todorovic, S.M., Prakriya, M., Nakashima, Y.M., Nilsson, K.R., Han, M., Zorumski, C.F., Covey, D.F. and Lingle, C.J., 1998. Enantioselective blockade of T-type Ca²⁺ current in adult rat sensory neurons by a steroid that lacks gamma-aminobutyric acid-modulatory activity. *Mol Pharmacol* 54, 918-927.

Todorovic, S.M., Rastogi, A.J. and Jevtovic-Todorovic, V., 2003. Potent analgesic effects of anticonvulsants on peripheral thermal nociception in rats. *Br J Pharmacol* 140, 255-260. <https://doi.org/10.1038/sj.bjp.0705434>

Treede, R.D., Jensen, T.S., Campbell, J.N., Cruccu, G., Dostrovsky, J.O., Griffin, J.W., Hansson, P., Hughes, R., Nurmikko, T. and Serra, J., 2008. Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* 70, 1630-1635. <https://doi.org/10.1212/01.wnl.0000282763.29778.59>

Tuem, K.B. and Atey, T.M., 2017. Neuroactive Steroids: Receptor Interactions and Responses. *Front Neurol* 8, 442. <https://doi.org/10.3389/fneur.2017.00442>

Uno, H., Tarara, R., Else, J.G., Suleman, M.A. and Sapolsky, R.M., 1989. Hippocampal damage associated with prolonged and fatal stress in primates. *J Neurosci* 9, 1705-1711. <https://doi.org/10.1523/JNEUROSCI.09-05-01705.1989>

Uzunova, V., Sheline, Y., Davis, J.M., Rasmusson, A., Uzunov, D.P., Costa, E. and Guidotti, A., 1998. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proc. Natl. Acad. Sci. U S A* 95, 3239-3244. <https://doi.org/10.1073/pnas.95.6.3239>

Vachon-Preseau, E., Roy, M., Martel, M.O., Caron, E., Marin, M.F., Chen, J., Albouy, G., Plante, I., Sullivan, M.J., Lupien, S.J. and Rainville, P., 2013. The stress model of chronic pain: evidence from basal cortisol and hippocampal structure and function in humans. *Brain* 136, 815-827. <https://doi.org/10.1093/brain/aws371>

Wang, J.M., Singh, C., Liu, L., Irwin, R.W., Chen, S., Chung, E.J., Thompson, R.F. and Brinton, R.D., 2010. Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U S A* 107, 6498-6503. <https://doi.org/10.1073/pnas.1001422107>

- Wang, M., 2011. Neurosteroids and GABA-A Receptor Function. *Front Endocrinol (Lausanne)* 2, 44. <https://doi.org/10.3389/fendo.2011.00044>
- Wareham, D., 2004. Postherpetic neuralgia. *Clin Evid* 1182-1193.
- Xiao, W.H. and Bennett, G.J., 2012. Effects of mitochondrial poisons on the neuropathic pain produced by the chemotherapeutic agents, paclitaxel and oxaliplatin. *Pain* 153, 704-709. <https://doi.org/10.1016/j.pain.2011.12.011>
- Yu, W.H., 1989. Survival of motoneurons following axotomy is enhanced by lactation or by progesterone treatment. *Brain Res* 491, 379-382. [https://doi.org/10.1016/0006-8993\(89\)90075-9](https://doi.org/10.1016/0006-8993(89)90075-9)
- Zamora-Sanchez, C.J., Hansberg-Pastor, V., Salido-Guadarrama, I., Rodriguez-Dorantes, M. and Camacho-Arroyo, I., 2017. Allopregnanolone promotes proliferation and differential gene expression in human glioblastoma cells. *Steroids* 119, 36-42. <https://doi.org/10.1016/j.steroids.2017.01.004>

Figure Legends

Fig. 1. Effects of 3 α ,5 α -THP (4 mg/kg/2 days) corrective treatment on VINC-induced neuropathic pain symptoms. Corrective 3 α ,5 α -THP treatment, which aimed at suppressing painful neuropathic symptoms persisting after the end of VINC treatment, consisted in starting 3 α ,5 α -THP administration after the two VINC cycles (3 α ,5 α -THP injections started 2 days after VINC cycles and lasted one week). (A, B) Action of 3 α ,5 α -THP against VINC-induced mechanical allodynia (A) and hyperalgesia (B). The curves show the mean + SEM of the percentages of paw withdrawal responses to mechanical stimulation by von Frey filament 4 g (A) or 15 g (B) (n = 6 per group). (C) Effect of 3 α ,5 α -THP against VINC-evoked cold-allodynia. Each point represents the mean + SEM of 6 observations in each of 6 rats. Non-parametric Mann-Whitney *U* test was used for the analysis of the von Frey test results and one-way repeated measures ANOVAs followed by Newman-Keuls *post hoc* comparisons were used for the acetone test. **p* < 0.05, ***p* < 0.01, ****p* < 0.005. * vs (Veh+Veh), # vs (VINC+Veh). Veh: Vehicle

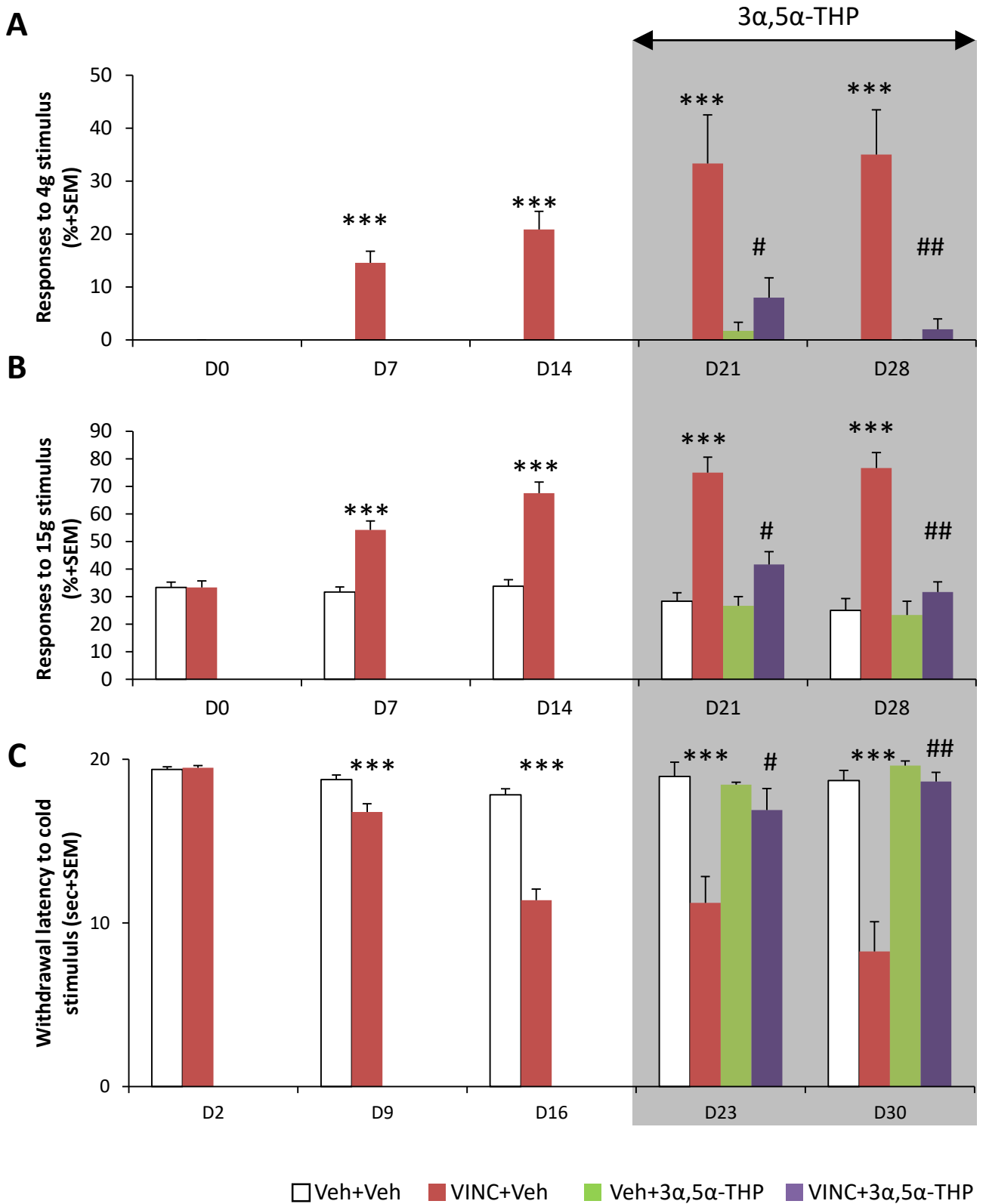


Figure 1

		<u>(A) Pre-injection</u>		Neuropathic	Neuropathic
		Sham-operated	Contralateral paw	Ipsilateral paw	
	Mechanical PWT (g±SEM)	116±15	102±9	13±1 ^{###}	
	Thermal PWL (sec±SEM)	12.25±0.55	11.94±0.49	6.88±0.37 ^{###}	
		<u>(B) Post intrathecal injection</u>		Neuropathic	Neuropathic
		Sham-operated	Contralateral paw	Ipsilateral paw	
Vehicle	Mechanical PWT (g±SEM)	111±15	95±13	15.4±2.6 ^{##}	
	Thermal PWL (sec±SEM)	9.96±0.43	9.06±0.19	6.2±0.28 ^{###}	
3α,5α-THP	Mechanical PWT (g±SEM)	207±35*	262±21**	53.6±7.6 ^{*,##}	
	Thermal PWL (sec±SEM)	11.44±0.34***	11.11±0.49***	8.63±0.26 ^{***,###}	
Provera	Mechanical PWT (g±SEM)	43.5±9*	56.2±5.3*	11.6±1.4 ^{*,##}	
	Thermal PWL (sec±SEM)	7.73±0.37***	7.48±0.36***	5.88±0.24 ^{*,###}	

Table 1: Effects of intrathecal injection of 3α,5α-THP and Provera on the thermal and mechanical pain thresholds of sciatic neuropathic and sham-operated rats. Pre-injection values (A) and responses 150 min (for mechanical thresholds) or 165 min (for thermal thresholds) after intrathecal injections (B) of 3α,5α-THP (5 mg/kg) or Provera (15 mg/kg). Each value represents the mean ± SEM of 5 observations for mechanical paw withdrawal thresholds (PWT) or 3 observations for thermal paw withdrawal latency (PWL) on each paw of 6 sciatic neuropathic and 6 sham-operated rats. Ipsilateral paw withdrawal responses were compared to contralateral paw withdrawal responses (^{##}p<0.01,^{###}p<0.001). Injections of 3α,5α-THP (5 mg/kg) or Provera (15 mg/kg) were compared to the vehicle group (VEH) (*p<0.05; **p<0.01; ***p<0.001).