UNIVERSITÀ DEGLI STUDI DI MILANO

PhD School in Integrative Biomedical Research

Department of Pharmacological and Biomolecular Sciences

Curriculum: Neuroscience

Molecular basis for the development of innovative therapies for peripheral neuropathies treatment: role and cross-regulation of the GABAergic system and neuroactive steroids

SDD BIO/09 - Physiology

Luca Franco Castelnovo

Badge number R10402

PhD Tutor: Prof. Valerio Magnaghi

PhD School Coordinator: Prof. Chiarella Sforza

Academic year 2015/2016

INDEX

Abstract

Peripheral neuropathies are a heterogeneous group of pathologies with a high prevalence worldwide, which are characterized by alterations of peripheral nerves structure and function. Their treatment is currently a challenge for clinicians. Indeed, even if continuous progresses are made in the study of the basic mechanisms underlying these pathologies, etiology is still unknown in a significant number of cases. Different compounds, such as, growth factors, adhesion proteins neurotransmitters, enzymes, peptides and neuroactive steroids, have been proposed to play important roles in the patho-physiology of the peripheral nervous system. Therefore, most of the research is addressed to identify the molecules that might represent the more promising therapy for this set of pathologies.

This thesis focuses on some aspects of the patho-physiological role of the GABAergic system and neuroactive steroids in the peripheral nervous system.

Several papers in literature strongly support the hypothesis that they are both present and active in the peripheral nervous system, in particular in Schwann cells, the myelinating cells of the peripheral nervous system. These cells are indeed able to synthesize GABA and neuroactive steroids and express both the ionotropic GABA-A and the metabotropic GABA-B receptor. In order to deepen the knowledge on this topic, four research lines were pursued in my PhD program and are described in this thesis. The first line regarded the analysis of the effects of specific GABA-B ligands on nerve regeneration in a model of neuropathic pain caused by nerve ligation. These studies showed that the specific GABA-B antagonist CGP56433 was able to recover some morphological, functional and biochemical parameters in peripheral nerves. Surprisingly, some of these effects were potentiated by the co-treatment with GABA-B specific agonist baclofen,

suggesting the co-activation of possible central and peripheral mechanisms. The second research line regarded the analysis of different GABA-A subunits in dorsal root ganglia (DRG) neurons of a model of conditional knockout mice, in which the GABA-B1 receptor is specifically deleted in Schwann cells. The results showed a modulation of different GABA-A subunits, pointing to a down-regulation of GABA-A receptors, mainly regarding the synaptic ones. This evidence may contribute to understand some of the alterations that were previously observed in this conditional knockout mouse model. The third research line dealt with the study of the modulation of protein kinase C-type ε (PKCε), an important neuropathic pain mediator, and its possible cross-talk with the GABA-A receptor and the neuroactive steroid allopregnanolone. The results showed that allopregnanolone down-modulates PKCε expression in Schwann cells, but the direct treatment on DRG neurons did not lead to any significant effect. However, Schwann cells conditioned medium was able to induce a significant up-regulation of PKCε gene expression in DRG neurons. Also the membrane expression of PKCε phosphorylated form resulted to be modulated in similar way. These findings suggest a possible involvement of PKCε in the GABA-A mediated control of pain transmission exerted by allopregnanolone, also pointing out to a Schwann cell-mediated process. Finally, the fourth research line regarded the identification of a novel family of progestogen receptors localized on the cell membrane (mPRs) and PGRMC1 in Schwann cells; moreover, their putative role in the modulation of Schwann cell physiology was also investigated. The data demonstrated the expression of these receptors in Schwann cell plasma membrane. The treatment with a specific mPR agonist proved able to induce cell migration at short time points (2-4 hours) and

increased the expression of myelin associated glycoprotein (MAG) at longer time points (24-36 hours), giving a first demonstration of a role for these receptors in Schwann cells. The identification of this new signaling pathway will allow a better understanding of progestogen actions in Schwann cells.

In conclusion, the results presented in this thesis shed some light on some basic mechanism controlling the patho-physiology of the peripheral nervous system, whose comprehension may lead to the identification of new more specific drugs for peripheral neuropathies treatment.

Abbreviations list

5-HT3: 5-hydroxytryptamine type 3. **AR**: androgen receptor. **BDNF**: brain derived neurotrophic factor. **Ca^{++}**: calcium ion. **cAMP**: cyclic adenosine mono-phosphate. **Cl^-**: chloride ion. **CMT**: Charcot-Marie-Tooth disease. **CRPS-II**: complex regional pain syndrome type II.**DAG**: diacylglycerol. **DHP:** 3α-dihydroprogesterone. **DRG**: dorsal root ganglia. **ER**: estrogen receptor. **GABA**: γ-amino butyric acid. **GABA-T**: GABA transaminase. **GAD**: glutamic acid decarboxylase. **GAP 43**: growth associated protein 43. **GDNF**: glial cell line derived neurotrophic factor. **GFAP**: glial fibrillary acidic protein. **GPCR**: G-protein coupled receptor. **HMSN**: hereditary motor and sensory neuropathy. **HSD**: hydroxysteroid dehydrogenase. **IASP**: International Association for the Study of Pain. **IGF2**: insulin-like growth factor 2. **IP_3**: inositol trisphosphate. **$IPSB_B$**: slow inhibitory post-synaptic potential. **K^+**: potassium ion. **MAG**: myelin associated glycoprotein. **MAPK**: MAP kinase. **MBP**: myelin basic protein. **mPR**: membrane progesterone receptor. **mTOR**: mammalian target of rapamycin. **Na^+**: sodium ion. **NGF**: nerve growth factor. **NL2**: neuroligin 2. **NMDA**: N-Methyl-d-aspartate. **NRG-1**: Neuregulin 1. **NT3**: neurotrophin 3. **NT4/5**: neurotrophin 4/5. **O2**: 10-ethenyl-19-norprogesterone. **P0**: myelin protein zero. **P450 SCC**: P450 cholesterol side-chain cleavage enzyme. **PAQR**: progestin and adipoQ receptor. **PDGF-BB:** platelet derived neurotrophic factor BB. **PGRMC1**: progesterone receptor membrane component 1. **PIP_2**: phosphatidylinositol. **PKA**: protein kinase A. **PKC**: protein kinase C. **PKs**: protein kinases. **PLC**: phospholipase C. **PLP**: pyridoxal phosphate. **PMP22**: peripheral myelin protein of 22 KDa. **pPKCε**: PKCε phosphorilated form. **PR:** progesterone receptor. **PXR:** pregnane X-receptor. **qRT-PCR**: quantitative Real Time polymerase chain reaction. **R5020**: promegestone. **RPA**: RNAse protection assay.

RT-PCR: retro transcription-PCR. **Serbp1**: serpine mRNA binding protein 1. **SREBP**: sterol regulatory element-binding protein. **SSA**: succinate semialdehyde. **StAR**: steroidogenic acute regulatory protein. **THDOC**: tetrahydro-deoxycorticosterone. **THP**: 3α,5α-tetrahydroprogesterone. **TRPV1**: transient receptor potential cation channel subfamily V member 1. **TSPO**: translocator protein of 18 KDa. **VGCC**: voltage-gated calcium channels. **VOCC**: voltage-operated Ca^{++} channels. **YY1**: Yin Yang.

Introduction

PERIPHERAL NERVOUS SYSTEM

GENERAL CONCEPTS

The nervous system is the morpho–functional unity deputed to receive internal or external inputs, to elaborate these stimuli and to generate a response. It is composed by neuronal and glial cells, blood vessels and connective tissue. It can be anatomically divided into central and peripheral nervous system.

The central nervous system can be furtherly divided into encephalon and spinal cord. It is responsible for different important physiological functions, such as intelligence, memory, learning, emotions, the analysis and coordination of sensory data and motor output.

In the implementation of all these crucial functions, it cooperates with the peripheral nervous system. The two systems are indeed anatomically and functionally correlated. The function of the peripheral nervous system is to link the central nervous system and periphery. It collects inputs, internal and external to the body, directing them to the central nervous system through sensitive fibers. Once this information has been elaborated by the central nervous system and a response has been generated, it is sent towards periphery (Marieb and Hoen, 2007).

From a physiological point of view, the peripheral nervous system can be divided into somatic and autonomous. The somatic peripheral nervous system is formed by motor neurons which originate from spinal cord gray matter and make contact with skeletal muscles, controlling voluntary movements. The autonomous nervous system, instead, controls involuntary body responses, making contact with the

heart, visceral smooth muscles, blood vessels and glands. It can be further divided into sympathetic and parasympathetic nervous system, both directly originating from the spinal cord. In particular, sympathetic branches originate from the turaco-lumbar portion of the spinal cord, while the parasympathetic branches originate from the cervical and sacral sections. The two systems exert actions that are often opposite each other, acting on most organs of the body. The sympathetic compartment is mostly activated under stressful or dangerous conditions (the so called "fight or flight" response), while the parasympathetic system is prevalent in resting situations (Marieb and Hoen, 2007).

Nerves, which are formed by fascicles of nervous fibers, are the anatomical structure responsible for signal conduction. There are sensitive, motor/effector and mixed nerves, the latter being the most common in the peripheral nervous system (Figure 1). Nerve fibers are generally formed by two different components, the axon (that is the cellular process originating from the neuronal soma) and the myelin sheath formed by Schwann cells, the glial cells of the peripheral nervous system, that wrap around nerves in a 1:1 ratio. These fibers are called myelinated fibers, and are characterized by a significantly faster conductance. Not all fibers have the myelin sheath. In this case, they are called unmyelinated fibers, and they are organized in structures known as Remak bundles, in which a single Schwann cell envelopes multiple axons without the myelin formation (Monk et al., 2015; Feltri et al., 2016).

Macroscopically, a nerve appears like a white cordlike structure with a thickness between 0.2 μM and 1 cm. Peripheral nerves are formed by myelinated and unmyelinated fibers grouped to form fascicles, separated by connective laminae that

also contain arterial, venous and lymphatic vessels. Single fascicles may be formed only by myelinated or unmyelinated fibers, or can have both type. There are three different connective sheaths in a nerve. Single fibers are enveloped by a sheath called endoneurium, several fascicles are enveloped by the perineurium, and the whole nerve is ensheathed by the epineurium (Marieb and Hoen, 2007).

The cell bodies of neurons forming motor/effector nerves are located in the ventral horns of the spinal cord. Sensitive nerves cell bodies, instead, are grouped in anatomical structures called dorsal root ganglia (DRG), located outside the spinal cord, paravertebral to the spinal column (Figure 1).

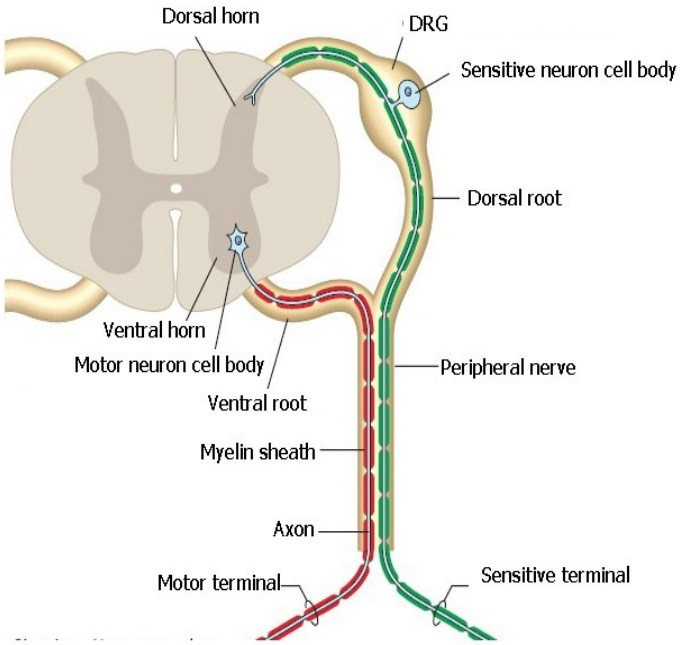


Figure 1 – Typical structure of a mixed peripheral nerve. Sensitive and motor fibers, whose cell bodies are localized in respectively in DRG and the ventral horn, both contribute to form the peripheral nerve, which originates both sensitive and motor terminals.

Generally, DRG are formed by pseudo-unipolar neurons, presenting a single axon originating from the soma that divides in a "T" shape in a peripheral and a central branch. The peripheral branch represents afferent fiber, which collect information from the periphery, while the central branch projects to the dorsal horn of the spinal cord (Figure 1). Neuronal soma in DRG are surrounded by a type of glial cells called satellite cells. They are homologous to Schwann cells and have the function to give structural and biochemical support to ganglia neurons (Hanani, 2005).

As better detailed below, Schwann cells in the peripheral nervous system play a pivotal role in many processes besides the formation of the myelin sheath, being fundamental in different patho-physiological processes. Indeed, they are important in the development of the peripheral nervous system (Feltri et al., 2016), they are involved in a complex cross-talk with neurons (Taveggia, 2016; Salzer¸ 2015; Faroni et al., 2014), and drive the nerve regeneration process after nerve damage (Faroni et al., 2015; Glenn and Talbot, 2013b).

SENSORY SYSTEM AND NOCICEPTIVE FIBERS

As previously mentioned, peripheral nerve sensitive fibers originate from DRG neurons, whose axon divide into a central and a peripheral branch. The central branch projects towards the spinal cord dorsal horn, while the peripheral branch goes towards the periphery, originating sensitive terminals that can be more or less specific in detecting particular stimuli, such as the nociceptive one (Basbaum et al., 2009).

The definition of pain, as formulated in 1986 by the International Association for the Study of Pain (IASP), defines it as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Based on this definition, pain presents a perceptive component, linked to sensory and nociceptive functions, and a subjective component, determined by cognitive, emotional and affective state (Merskey, 1994).

Nociception is the process by which intense thermal, mechanical, or chemical stimuli are detected by a subpopulation of peripheral nerve fibers, called nociceptors (Basbaum and Jessell, 2000; Babaum et al., 2009). Pain can be differentiated into acute and chronic pain. Acute pain is of short duration and it disappears as the pathological or harmful phenomena that caused it improves or heals. Chronic pain, instead, can be perceived for long periods of time and it is often caused by chronical pathological processes (Basbaum et al., 2009).

Pain propagation process begins with the activation of specific receptors known as nociceptors. They are free nerve endings that are the distal portion of afferent neurons. Sensitive nerve fibers, from which they originate, can be classified by a neurophysiological point of view into three types, based on structure, fiber diameter and conduction speed (Meyer et al., 2008). The three types of nociceptive fibers are C, Aδ and Aβ. C fibers are unmyelinated, their diameter spans between 0.4 and 1.2 μm and have a conduction speed of 0.5-2.0 m/s. Aδ fibers are lightly myelinated, with a diameter between 2.0 and 6.0 μm and a conduction speed of 12 up to 30 m/s. Aβ fibers are well myelinated, have diameter greater than 10 μm and high conduction speed, between 30 and 100 m/s. This latter category is lowly involved

in normal pain stimuli conduction, but it is important segmental pain suppression mechanisms (Millan, 1999).

These three types of fibers show different patterns of pain propagation, depending of the mechanical, thermal or chemical origin of harmful stimuli they face. Aδ fibers rapidly transmit unimodal information generated by high intensity stimuli. They are responsible for the beginning of acute pain sensation, leading to the removal or "flight" reflex. C fibers, instead, are polymodal and carry out information slower. They are responsible for second pain perception, and the prolonged potentials they generate can sum up leading to chronic pain. This distinction is very clear in particular for what regards skin, but it is not necessarily so clear in every organ (Meyer et al., 2008).

Aδ fibers can be divided into two groups: type I and type II fibers. Type I fibers are characterized by high-threshold mechanoreceptors, responding mainly to high intensity mechanical stimuli, with low sensitivity for chemical and thermal stimuli. Type II fibers, instead, are characterized by mechanical-thermal receptors, that respond to high (over 45°C) and low (beneath -15°C) temperature and to intense mechanical stimuli (Millan, 1999; Basbaum et al., 2009).

As already mentioned, C fibers are usually polymodal, responding to different kind of stimuli (Perl, 2007), such as thermal and low-threshold mechanical ones, and specific receptors for particular algetic compounds such as K^+, acetylcholine, proteolytic enzymes, serotonin, substance P, prostaglandins and histamine. There are also high-threshold C fibers, responsible for pain response following burns. Lastly, there are slow-conduction C fibers insensible to mechanical stimuli that

activate only in case of histamine-mediated inflammatory response (Millan, 1999; Basbaum et al., 2009).

In pathological conditions, two different painful non-physiological conditions can arise: allodynia and hyperalgesia. The first is defined as a pain response generated by normally non-painful stimuli, the second is an excessive response to a normally painful stimulus (Basbaum et al., 2009).

SCHWANN CELLS AND MYELINATION

Schwann cells are highly specialized cells whose main function is the formation of the myelin sheath in the peripheral nervous system.

Schwann cells isolating properties are fundamental for the transmission of the signal in myelinated fibers. Indeed, they allow saltatory conduction, electrically isolating the axon, except in areas comprised between two adjacent Schwann cells, known as nodes of Ranvier. However, Schwann cells functions go much beyond that, being involved in an important cross-interaction with neuronal cells and having a fundamental role in axonal normal development and long-term survival (Taveggia, 2016). They have also an important role in regenerative processes following nerve lesions, being able to differentiate and stimulate nerve fibers regeneration (Glenn and Talbot, 2013b; Faroni et al., 2015). On the other hand, axons provide signals that regulate Schwann cell proliferation, survival and differentiation, as well as myelin formation (Bozzali and Wrabetz, 2004; Simons and Trajkovic, 2006; Woodhoo and Sommer, 2008; Taveggia et al., 2010).

Schwann cells derive from cell precursors which originate from the neural crest during embryogenesis (Le Douarin et al., 1991, Figure 2). A fundamental determinant in Schwann cell lineage determination is NRG-1, since it suppresses neuronal differentiation and promotes glial differentiation (Shah et al., 2004). Schwann cell precursors are migratory and proliferative (Monk et al., 2015), and rely on axonal signals for survival (Dong et al., 1995). They are characterized by the expression of specific differentiation markers, like the growth associated protein 43 (GAP 43) and F-Spondin (Debby-Brafman et al., 1999; Byrstyn-Cohen et al., 1998).

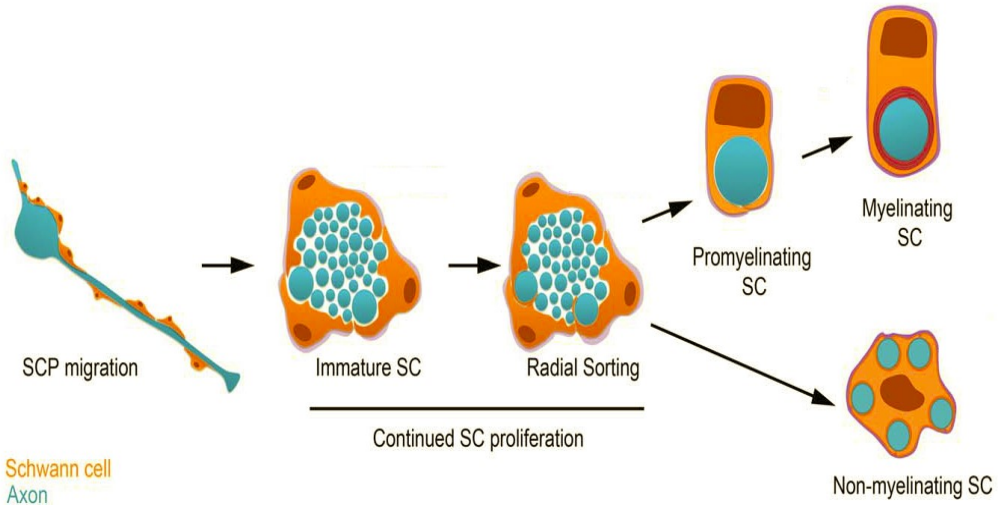


Figure 2 – Steps of Schwann differentiation. Schwann cell precursors, deriving from the neural crest, differentiate into immature Schwann cells, that are responsible for the radial sorting process. Once radial sorting is complete, immature Schwann cell differentiate into the myelinating or non-myelinating phenotype. Adapted from Monk et al., 2015.

The following differentiation step leads to the formation of immature Schwann cells (Figure 2). Notch signaling has been reported to be a key regulator of this process, indeed its inactivation decreases Schwann cell precursor proliferation and their

transition to immature Schwann cells, while its over activation up-regulates both processes (Woodhoo et al., 2009). In this differentiation step, cells stop to migrate and become less dependent on axonal signaling, since their survival now depends on autocrine factors. Among them, an important role is played by growth factors, such as insulin-like growth factor 2 (IGF2), neurotrophin 3 (NT3), and platelet derived neurotrophic factor BB (PDGF-BB, Meier et al., 1999; Jessen and Mirsky, 2005; Monk et al., 2015, Figure 2).

During development, immature Schwann cells regulate the radial sorting of axons, that is the physiological process by which axons are separated based on their caliber. Bigger axons are radially segregated to the periphery (Feltri et al., 2016). After the segregation is complete, immature Schwann cells in contact with bigger axons stablish a 1:1 ratio with them, developing into pro-myelinating Schwann cells that subsequently become myelinating Schwann cells, that wrap these high diameter axons forming the myelin sheath. The others differentiate into non-myelinating Schwann cells, which envelope several small caliber axons, without forming a real myelin sheath, originating the so called Remak bundles (Monk et al., 2015, Feltri et al., 2016, Figure 2). Anyway, Schwann cells are characterized by remarkable plasticity, and can switch from a differentiation state to the other based on the situation, in particular during pathology (Feltri et al., 2016).

Several transcription factors have been reported to be important in different differentiation phases (Svaren and Meijer, 2008; Salzer 2015, Figure 3). Sox2, NF-kB and Egr1 are expressed in the first differentiation stages. When axons are segregated and Schwann cells start to differentiate towards the myelinating phenotype, NF-kB, Pou3f1 (Oct6/SCIP/Tst1), Pou3f2 (Brn2), Sox10, NFATc4, Yin

Yang (YY1), and Egr2/Krox20 are regulated and/or activated (Svaren and Meijer 2008; Kao et al. 2009; He et al., 2010). In particular, when Schwann cells pass from the pro-myelinating to the myelinating phenotype, Pou3f1 and Pou3f2 are no more expressed, while Krox20 is up-regulated (Salzer, 2015). Krox 20 expression is promoted by Pou3f1, Pou3f2, NFATc4, YY1 and Sox10 (Jaegle et al. 2003; Kao et al. 2009; He et al. 2010). Also the sterol regulatory element-binding protein (SREBP) transcription factors are up-regulated and essential for myelination, in line with the up-regulation of lipids synthesis during myelination (Camargo et al., 2009). The myelinating phenotype requires the contemporary expression of Krox20 and Sox10, indeed conditional inactivation of one of the two genes leads to de-differentiation (Decker et al. 2006; Bremer et al. 2011).

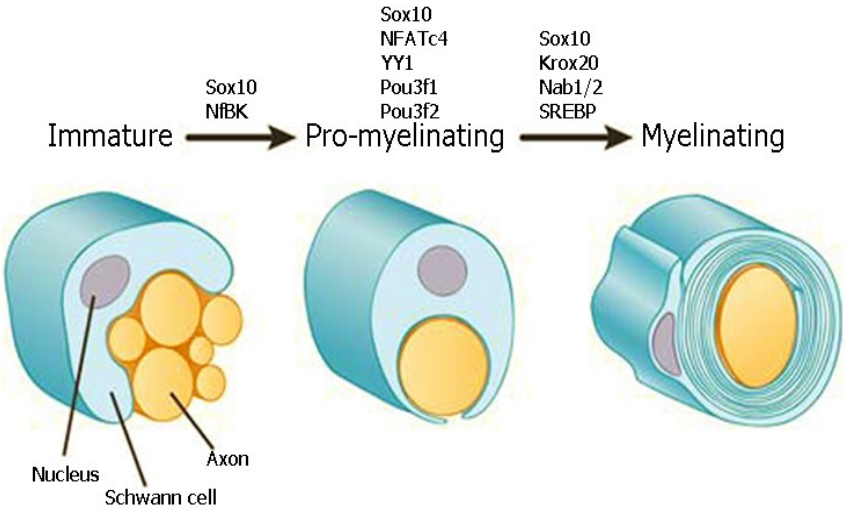


Figure 3 – Main transcription factors involved in Schwann cell differentiation from the immature to the myelinating phenotype. Adapted from Salzer, 2015.

Krox20 is considered a master regulator of myelination (Topilko et al. 1994; Le et al. 2005b, Salzer, 2015). Indeed, its expression is cross-repressed with the expression of two important determinants of non-myelinating Schwann cells, Sox2 and c-Jun (Le et al. 2005a; Parkinson et al. 2008; Jessen et al. 2015), and this cross-regulation is fundamental in the differentiation of Schwann cells into one of the two states.

Several extrinsic signals are also implied in the regulation of the myelination process. Among them, Neuregulin 1 (NRG-1), in particular the type III, is the key axonal molecule controlling myelination. Indeed, its expression level on axonal surface determines their myelination fate, being more expressed on high caliber neurons and lowly expressed on smaller ones (Taveggia et al., 2005). NRG-1 has an important role in virtually every aspect of Schwann cell lineage, including axon segregation during radial sorting (Taveggia et al., 2005; Michailov et al., 2004; Jessen et al., 2015, Raphael et al., 2011). There are six major classes of NRG-1 (types I-VI), differing in the ectodomains splicing pattern (Salzer, 2015). Type III is the predominant type in peripheral axons (Mei and Xiong, 2008). NRG-1 exerts its action through the binding with the ErbB family of tyrosine kinase receptors, that in Schwann cells are mainly present in the form of the ErbB2/ErbB3 heterodimer (Newbern and Birchmeier, 2010). Although NRG-1/ErbB signaling plays a fundamental role during the myelination process, it is not necessary to maintain myelination (Atanasoski et al., 2006; Fricker et al., 2011), nor for re-myelination after injury, this because mature Schwann cells are able to secrete a soluble NRG isoform (NRG-1 type I) that promotes re-myelination in an autocrine fashion (Stassart et al., 2013).

Recently, the interaction between Lgi4 secreted by Schwann cells and Adam22 expressed by axons has been demonstrated to be necessary for Schwann cells to differentiate beyond the pro-myelinating stage (Kegel et al., 2013), although the mechanism by which this interaction influences the myelination process is still unknown.

Also the extra-cellular matrix has a role in the control of myelination. Different matrix components, such as laminin, are synthesized in Schwann cells and function as autocrine signals (Chernousov et al., 2008; Salzer, 2015). Laminin is a trimer formed by different α, β and γ chains. Laminin 2 is the main endoneurial form, while laminin 411 and 511 are minor forms (Chernousov et al., 2008). All this forms have been reported to promote radial sorting and myelination (Wallquist et al., 2005; Yang et al., 2005). Laminin exerts these effects binding to different receptors expressed on the abaxonal (outer) membrane of the myelin sheath, such as β1-integrins and dystroglycan (Salzer, 2015).

Another mediator of the myelination process whose importance has lately been underlined is the G protein-coupled receptor GPR126 (Monk et al., 2009), that has been reported to be absolutely necessary for myelination and to play a role in Schwann cell development and in the radial sorting process (Mogha et al., 2013). It exerts its action modulating cyclic adenosine mono-phosphate (cAMP) intracellular levels, an essential signal for Schwann cell myelination, directly elevating cAMP levels or activating the protein kinase A (PKA) (Mogha et al., 2013; Glenn and Talbot, 2013a, b). Recent data show that laminin 211 is a physiological ligand of the receptor (Petersen et al., 2015). As for NRG-1/ErbB signaling, its activation is not necessary for the maintenance of myelination (Glenn and Talbot, 2013a, b).

Several evidence in literature support the hypothesis that an important role may also be played by other mediators, such as neuroactive steroids (as detailed below) and neurotrophins, some produced by Schwann cells, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), NT3, neurotrophin 4/5 (NT4/5) and glial cell line derived neurotrophic factor (GDNF, Chan et al., 2001; Cheng et al., 1999; Hoke et al., 2003; Schumacher et al., 2004). Numerous studies suggest that also neurotransmitters, such as purines, acetylcholine and GABA (detailed below) are involved in the peripheral nervous system biology and in Schwann cells functionality (Fields and Burnstock, 2006; Loreti et al., 2007; Stevens et al., 2004). Different intracellular pathways have been reported to be activated by the mediators mentioned above. Among them, the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway seems to have a key role is Schwann cell development (Maurel and Salzer, 2000; Ogata et al., 2006). Indeed, hyper activation of PI3K leads to increased Schwann cell wrapping and hypermyelination of axons (Goebbels et al., 2010) and these effects can be reverted with mTOR inhibitor rapamycin, suggesting that they are mediated by PI3K activation of the serine/threonine kinase Akt, which its responsible for mTOR activation (Goebbels et al., 2012; Salzer, 2015). NRG-1 strongly activates mTOR, that, in accordance, is localized in sites that are in contact with the axon. Therefore, mTOR role in myelination is probably linked to its regulation of protein translation (Laplante and Sabatini, 2013; Salzer, 2015).

Another intracellular pathway that has been linked to NRG-1 effects is the phospholipase C (PLC)-γ pathway (Kao et al., 2009). Indeed, NRG-1 binding to ErbB receptors directly activates PLC-γ, leading to increased intracellular calcium ion (Ca^{++}) levels and subsequent calcineurin B activation, leading to a series of

intracellular events that activate Sox10 and Krox20 and up-regulate P0 expression (Kao et a., 2009; Lazarevic et al., 2009).

Several data support the hypothesis that also the MAP kinase (MAPK) pathway has a critical role in the regulation of early Schwann cell differentiation, mediating the pro-myelinating activity of NRG-1. Indeed, it has been shown that the inactivation of this pathway mimics ErbB2 knockout (Grossmann et al., 2009). Genetic Erk1/2 ablation during development leads to impaired differentiation of Schwann cells and defective myelination (Newbern et al., 2011). Conditional expression of an activate MAPK form (Mek1) leads to increased myelination even in cells deficient in ErbB3 (Sheean et al., 2014). MAPK activates the pro-myelinating transcription factor YY1 (He et al., 2010), but its effect in myelination may depend mainly on its stimulation of mTOR-dependent and independent protein translation. The fact that the activation of this pathway was able to rescue myelination, although the NRG-1 activation of PI3K and PLC-γ was impossible because of ErbB3 absence suggests that the MAPK pathway may be able to activate the other two pathways or to grant a sufficient myelination level by itself (Sheean et al., 2014).

Lastly, cAMP is known to promote and maintain Schwann cell myelinating phenotype (Stewart et al. 1991; Monje et al. 2010), likely with different mechanisms. Indeed, it converts Schwann cell response to NRG-1 from proliferation to differentiation (Arthur-Farraj et al., 2011) and increases Pou3f1 and Krox20 expression (Monuki et al., 1989: Kipanyula et al., 2013). Moreover, it activates cAMP-dependent PKA, which in turns activates by phosphorylation several transcription factors, such as NFkB and some members of the CREB family, such as CREB1, CREM and ATF1 (Tasken and Aandahl, 2004), which may have

an important role in cAMP-mediated effects on myelination (Arthur-Farraj et al., 2011).

When Schwann cells myelinate axons, they organize in a longitudinally and radially polarized fashion, with distinct membrane domains, specific arrays of proteins and communicating sets of cytoplasmatic compartments (Pereira et al., 2012, Salzer, 2015). Longitudinal polarity leads to the organization of the myelinated fiber into nodal, paranodal, juxtparanodal and internodal compartments, while radial polarity consists in the presence of adaxonal (inner) and abaxonal membrane surfaces. The adaxonal membrane comprises the paranodal, juxtparanodal and internodal domain, with the latter being by far the larger, around 99% of myelinating Schwann cells. Proteins typical of this area are the myelin associated glycoprotein (MAG), CADM1 and CADM4 (Maurel et al. 2007; Spiegel et al. 2007).

The compacted myelin sheath is comprised between the adaxonal and abaxonal membranes. It derives from Schwann cell wrapping around axons. As previously mentioned, myelin provides electrical isolation to axons, allowing saltatory conduction. Observing electron microscopy images of compact myelin, it is possible to appreciate the alternation of intraperiod lines and major dense lines. The first are the areas where there is apposition of extracellular leaflets, the latter are the areas of cytoplasmic leaflets apposition (Salzer, 2015, Figure 4).

Myelin is characterized by an extremely high plasma membrane lipid content, roughly 70%, being particularly enriched in galactosphingolipids, saturated long-chain fatty acids and, in particular, cholesterol, that is essential for the assembly of the myelin sheath (Saher and Simons, 2010).

The main protein components of compact myelin, fundamental for the compaction process, are myelin protein zero (P0), myelin basic protein (MBP) and the peripheral myelin protein of 22 KDa (PMP22). P0 is a transmembrane glycoprotein, which promotes the apposition of extracellular leaflets (Filbin et al. 1990; Shapiro et al., 1996). In cooperaration with MBP, it promotes also intracellular leaflets apposition, giving major dense lines (Martini et al., 1995, Figure 4).

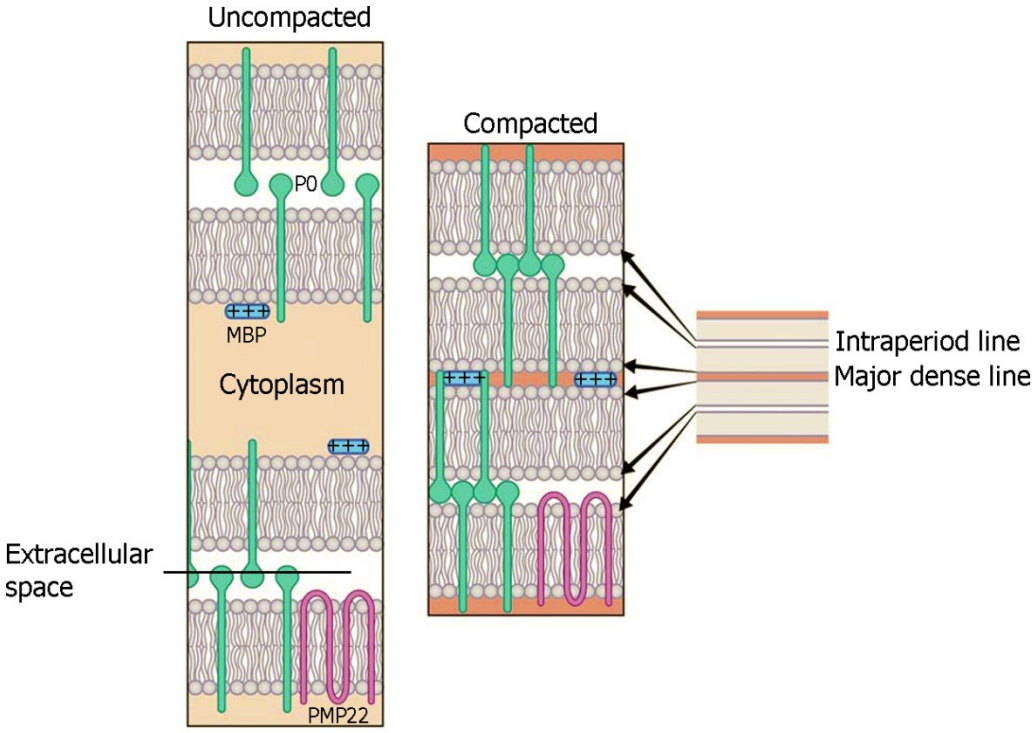


Figure 4 – Basic structure of uncompacted and compacted myelin sheath. The main myelin proteins P0, MBP and PMP22 contribute to the compaction process and to the formation of the myelin sheath. Adapted from Salzer, 2015.

Among compact myelin sheaths layers there are clefts with thin cytoplasmic insert, known as Schmidt-Lanterman incisures. These clefts have long been thought to provide a communication conduit between inner and outer adjacent membranes (Balice-Gordon et al., 1998). However, recent studies suggest they may also be site

of autocrine signaling (Heller et al., 2014). They are enriched in the tyrosin-kinase Src (Terada et al., 2013) and in many adhesion molecules present in the adaxonal membrane, such as MAG and CADMs (Maurel et al., 2007; Spiegel et al., 2007), suggesting a common topology.

Mutations in genes encoding for myelin proteins are an important cause of hereditary peripheral neuropathies. Indeed, mutations to P0 and PMP2, for example, can originate the Charcot-Marie-Tooth disease (CMT) in its different variants (Martini et al., 1995; Shy, 2006; Suter and Scherer, 2003; Berger et al., 2006; Pareyson et al., 2013).

PERIPHERAL NEUROPATHIES

GENERAL CONCEPTS

Peripheral neuropathies are a heterogeneous group of pathologies, characterized by alterations of peripheral nerves structure or function. These highly debilitating pathologies present a high prevalence (14,8%) in Western countries, and their incidence grows with age, being particularly high in people affected by dysmetabolic diseases (Gregg et al., 2004).

From a patho-physiological point of view, peripheral neuropathies can be classified as hereditary or acquired, axonal or demyelinating. Hereditary peripheral neuropathies, which can be either axonal or de-myelinating, are very common neurological diseases, often with high incidence (Saporta and Shy, 2013). Some examples are the CMT, whose prevalence, 1:2500, is very high (Braathen et al., 2011), or the hereditary motor and sensory neuropathy (HMSN, Lupski et al., 2001; Dyck et al., 1993). Acquired peripheral neuropathies can derive from chronic (such as diabetes, Singh et al., 2014) or infective diseases (such as leprosy, Masaki et al., 2013), environmental toxins (Little and Albers, 2015), alcoholism (de la Monte and Kril, 2014), nutritional deficit (D'Amour and Butterworth, 1994), autoimmune diseases (Bourque et al., 2015) but they are often due to iatrogenic causes (Sposato and Faustinoni, 2014) or traumatic events, most commonly attributable to direct mechanical trauma, and less frequently to surgical resection secondary to tumor excision (Faroni et al., 2015).

The symptomatology can be very heterogeneous, depending on the kind of nervous district (sensitive, motor or vegetative) that is involved. The main debilitating conditions include lack of motor control and proprioceptive sensitivity. However, some common clinical manifestations can be identified in the presence of sensory symptoms (like numbness or tingling), weakness, autonomic symptoms (such as satiety, impotence, orthostatic hypotension and sweat abnormalities), or the onset of neuropathic pain (Watson and Dyck, 2015). The etiology of peripheral neuropathies is generally unknown in 50% of the cases (Imreovà and Pura, 2005). Therefore, therapies are based mainly on symptomatic and palliative pharmacological treatments (Watson and Dyck, 2015), making the identification of more specific pharmacological targets or alternative bioengineered approaches a strong medical need.

NEUROPATHIC PAIN

Neuropathic pain is one of the main clinical sign of peripheral neuropathies. Peripheral neuropathic pain derives from lesions to the peripheral nervous system, which can be caused by mechanical trauma, metabolic disease, neurotoxins, infections or tumor invasion, and may determine different physiological changes both in peripheral and central nervous systems (Dworkin et al., 2003, Woolf and Mannion, 1999). Neuropathic pain is classified and treated on the basis of the underlying disease (Dworkin et al., 2007).

The two major general contributors in the development of neuropathic pain seem to be the altered balance between compensatory and de-compensatory responses of

the nervous system to damage and the genetic background, which seems able to have a role in the establishment of neuropathic pain (Costigan et al., 2009).

A typical characteristic of neuropathic pain is the absence of an identifiable stimulus. Pain arises spontaneously from ectopic action potentials in nociceptive pathways, without a stimulus at the level of peripheral terminals. Several evidence suggest that ectopic activity is generated mainly in primary sensory neurons. Indeed, after peripheral nerve damage, spontaneous activity may originate at different sites, such as the neuroma (that is the site of injury with aborted axon growth), DRG cell bodies (Amir et al., 2005), and uninjured afferent fibers near the lesion (Wu et al., 2002). The spontaneous pain can derive both from ectopic activity in nociceptors (Bostock et al., 2005) and from low-threshold large myelinated afferents (Campbell et al., 1988).

Even if several approaches are used or are currently studied in order to promote nerve regeneration (as detailed below), most of the times none of these methods leads to satisfactory nerve repair, and current therapies are mainly addressed to the control of painful symptoms rather than to treat nerve degeneration and/or to promote regeneration. Therefore, new strategies that simultaneously promote nerve regeneration controlling neuropathic pain are needed.

NERVE REGENERATION

It is well established that, differently from central nervous system neurons, peripheral nerves can regenerate after a damage. It is also known that Schwann cells support nerve regeneration, while oligodendrocytes of the central nervous system

do not (Fenrich and Gordon, 2004; Faroni et al., 2015; Taveggia, 2016). Indeed, when axons lose contact with their cell bodies after a lesion, axonal transport in the stumps stops, starting the Wallerian degeneration process. During this process, both axon and myelin in the distal stump degenerate, and macrophages are recruited in order to contribute to debris clearance (Chen et al., 2007, Figure 5).

Schwann cells shift from the myelinating to the non-myelinating state and start to proliferate and migrate (Fu and Gordon, 1997; Gaudet et al., 2011). During this transition, Schwann cells express many regeneration-associated genes, such as neurotrophic factors (NGF, BDNF, GDNF, pleiotrophin, Boyd and Gordon, 2003; Hoke et al., 2006) and transcription factors like Notch and c-Jun (Jessen and Mirsky, 2005; Jessen et al., 2008; Fontana et al., 2012, Figure 5). Also the neuronal compartment undergoes some modifications, reflecting a transition from a transmission to a growth mode, in a process called chromatolysis (Lieberman, 1971; Gordon and English, 2015). Pro-regeneration genes, like tubulin and actin, are up-regulated, whereas transmission-related genes, such as acetylcholine and choline acetyltransferase are down-regulated (Fu and Gordon, 1997; Zigmond, 2012).

During the regeneration process, all axons that are still in contact with their cell body are reported to emit sprouts (Gordon and English., 2015). A study performed using transgenic mice expressing green fluorescent protein in their neurons has shown that after seven days of regeneration, in proximity of the surgical site mice presented a highly disorganized extracellular matrix with few Schwann cells (Witzel et al., 2005). Indeed, laminin becomes organized after 10 days circa, and only after that Schwann cells can move into the surgical site and align forming the

bands of Burgner, which guide axon sprouts through the surgical repair site (Gordon and English, 2015, Figure 5).

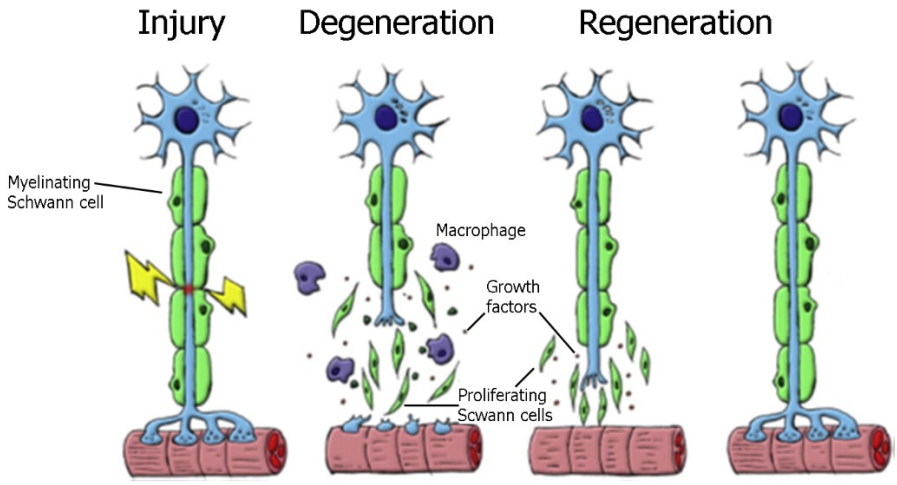


Figure 5 – Schematic representation of the events following nerve damage. Wallerian degeneration. Following injury, Schwann cells detach from the axons, start proliferating and help the recruited macrophages to clear the cellular and myelin debris. At the same time, expression of stimulating factors by SCs create a favorable environment for nerve regrowth towards the target organ. Adapted from Faroni et al., 2015.

After injury, the regeneration outcome relates to the age of patient, the mechanism of injury and, in particular, to the proximity of the injury to the nerve cell body (Faroni et al., 2015). Distal digital nerve injuries, indeed, have usually much better outcome than proximal brachial plexus avulsions, which may be functionally devastating, leading to serious problems to perform activities of daily living, as well as preventing return to work (Brushart, 2011).

The surgical treatment of choice in case of nerve injury is microsurgical repair by tensionless epineurial sutures, and in case the gap distance prevents end-to-end suturing, autologous nerve grafting remains the gold standard (Millesi, 2007). An

important factor is time, since delayed repair demonstrated to be significantly detrimental to satisfactory sensory and motor recovery (Jivan et al., 2009, Gordon et al., 2011). Even if, in recent years, the knowledge of peripheral nerve injury patho-physiology strongly improved, clinical outcomes are still often poor (Lundborg, 2000; Faroni et al., 2015). There is therefore a strong need for the identification of new strategies to promote nerve regeneration, such as nerve guidance conduits, pharmacotherapy and cell therapy (Faroni et al., 2015).

THE GABAERGIC SYSTEM

GABA

γ-amino butyric acid (GABA) is the main inhibitory neurotransmitter of the nervous system. After its first characterization in 1950 (Roberts and Frankel, 1950) many studies demonstrated its role in the patho-physiology of the nervous system.

Glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, is not equally distributed in the central nervous system. Indeed, it is present in higher concentration in districts like substancia nigra, globus pallido, hypothalamus, hippocampus, quadrigeminal bodies, cerebral cortex, cerebellum and the spinal cord. Lower concentrations have been detected in the pons, the medulla oblongata and the white matter (Erlander et al., 1991). GAD synthesizes GABA through glutamate decarboxylation in a highly specific interaction. The enzyme requires pyridoxal phosphate (PLP) as a cofactor, and so can be inhibited using PLP antagonists, such as hydrazinic compounds. GAD is present in different isoforms, the main two being GAD 67 and GAD 65 (Erlander et al., 1991). GAD 67 is evenly distributed throughout the cell, while GAD 65 is mainly localized in the cytoplasm of axon terminals (Pinal and Tobin, 1998).

After synthesis, GABA is stored in vesicles by the action of a specific transporter coupled to a vesicular protonic pump. GABA release in the synaptic space requires vesicles to fuse with plasma membrane (Figure 6). This event can occur spontaneously or following de-polarization caused by Ca^{++} influx (Hablitz et al., 2010).

Once released, GABA can bind its specific receptors (described later) or it can go towards re-uptake by neurons and glia, by the action of specific transporters GAT1-3, that co-transport GABA with sodium (Na^+) and chloride (Cl^-) ions (Carver and Reddy, 2013, Figure 6). There is evidence suggesting that the glial transporter is more important in the maintenance of low GABA synaptic levels. Once re-uptaken by neurons and glia, it is degraded by the enzyme GABA transaminase (GABA-T), present both in pre- and post-synaptic neurons and in glia, that catalyzes its de-amination to succinate semialdehyde (SSA, Figure 6). This chemical compound is subsequently oxidized by a NAD-dependent succinate semi aldehyde dehydrogenase leading to the formation of succinic acid, which enters Krebs cycle. GABA-T transfers the amino group from GABA to a molecule of α-ketoglutarate, leading to the formation of new glutamate (Petroff, 2002; Carver and Reddy, 2013). GABA exerts its effects through the binding with its receptors, the ionotropic receptor GABA-A and the metabotropic receptor GABA-B.

GABA-A RECEPTORS

GABA-A receptors are hetero-oligomeric receptors formed by different combinations of 5 subunits. GABA binding with these receptors leads to the opening of a Cl^- channel that usually leads to an inward current inducing hyper-polarization.

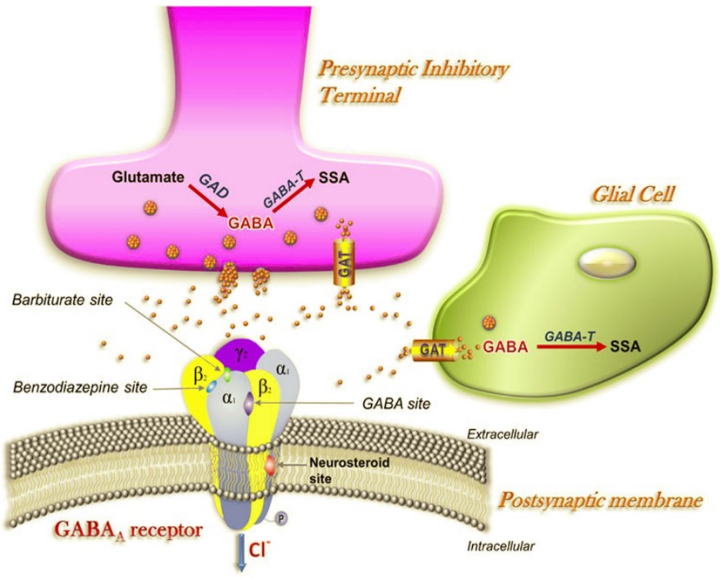


Figure 6 – Representation of a GABAergic synapse. GAD converts glutamate to GABA, that is stored in vesicles before release. Once released, it can bind one of its receptors (in this example, a post-synaptic GABA-A receptor), or it can go towards GAT-mediated re-uptake in neuronal and glial cells, when it is converted to SSA by the action of GABA-T. Adapted from Carver and Reddy, 2013.

However, in some cases, the Cl^- channel opening leads to depolarization; this happens in neuronal cells (mostly immature) which present an intracellular Cl^- concentration higher than the extracellular one (Lu et al., 2014).

Nineteen genes encoding for GABA-A subunits have been identified so far: α1–α6, β1–β3, γ1–γ3, δ, ε, π, τ, ρ1–ρ3 (Fritschy and Panzanelli, 2014). Different subunit composition leads to the formation of different receptors which differ in subcellular localization, distribution and function. The majority of GABA-A receptors are formed by the combination of 2 α, 2 β and a γ, δ or ε subunit (Boileau et al., 2005; Olsen and Sieghart, 2008; Patel et al., 2014; Jacob et al., 2008, Figure 7a).

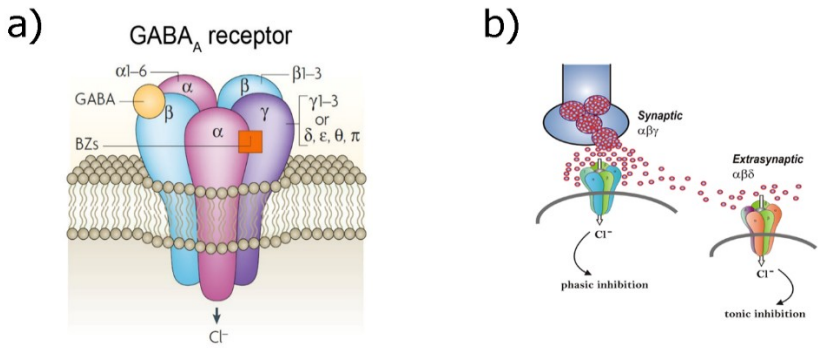


Figure 7 – a) Typical subunit composition of most GABA-A receptors and b) typical subunits composition and localization of classical synaptic and extra synaptic receptors. Adapted from Jacob et al., 2008 and Reddy, 2011.

An important distinction can be made between synaptic and extra synaptic GABA-A receptors. The first are localized at post-synaptic terminals, and mediate the classical phasic inhibition upon GABA release from pre-synaptic terminals. The second receptor sub-type is instead localized aside synaptic position, and is responsible for tonic GABA-A inhibition (Belelli et al., 2009; Reddy, 2011, Figure 7b). An interesting characteristic of this latter receptors, in particular the δ subunit-containing ones, is that, at least in some specific brain areas, they resulted to be particularly responsive to neuroactive steroids (Belelli et al., 2002; Wohlfarth et al., 2002. Mihalek et al., 1999).

In general, β subunits composition is not particularly indicative of the synaptic or extra-synaptic nature of a given GABA-A receptor. α1, α2, α3 and γ2 subunits typically form synaptic receptors, while α4, α5, α6 are typical extra-synaptic receptor subunits, with the γ2 subunit often substituted by δ (Fritschy and Panzanelli 2014). In particular, it is generally correct to say that a GABA-A receptor

that presents a δ subunit is extra-synaptic, but not all extra-synaptic receptors present the δ subunit (Belelli et al., 2009).

Tonic inhibition is generally thought to be due to receptor activation by ambient GABA. However, there is evidence that spontaneous opening of GABA-A receptors may contribute to most of tonic currents recorded in the dentate gyrus (Wlodarczyk et al., 2013).

Several extra-synaptic receptors, presenting the δ subunit, have high affinity for GABA and mediate most of the actions of neuroactive steroids, in particular the progestogen allopregnanolone (described in more detail below), modulating brain activity when their synthesis is up-regulated, such as in condition of stress, delivery and ethanol intoxication (Sarkar et al., 2011; Carver and Reddy, 2013). They present a specific pharmacological profile, indeed they are modulated by the super agonist gaboxadol, but are insensitive to classical benzodiazepines (Mortensen et al., 2010).

Some data suggest that surface expression of these receptors is modulated, in dentate gyrus and thalamus, by PKA and protein kinase C (PKC) activity, targeting α4 containing GABA-A receptors (Connelly et al., 2013; Tao et al., 2013). In particular, cell surface expression of these receptors has been reported to be mediated by α4 subunit's Ser443 phosphorylation mediated by PKC (Abramian et al., 2010).

Although α4/β/δ receptors seem to be the most prominent extra synaptic population, also α5 containing GABA-A extra-synaptic receptors seem to be expressed in the hippocampus, olfactory bulb and cerebral cortex. In these receptors, the α5 subunit

is most probably linked with β3 and γ2 subunits. They respond to diazepam but are insensitive to zolpidem.

Other diazepam-sensitive (γ2 containing) receptors have been proved to be located extra-synaptically. In particular, α3-containing extra synaptic receptors have been detected in the amygdala and in the inferior olivary nucleus (Marowsky et al., 2012; Devor et al., 2001), even if most α3 containing receptors have been proved to be synaptic (Studer et al., 2006; Schneider Gasser et al., 2007; Notter et al., 2014). The mechanisms that determine synaptic or extra-synaptic localization of these receptors are not yet completely understood, probably involving interaction with gephryn. Something similar has been reported to happen also with α5-containing GABA-A receptors, which can locate synaptically in the hippocampal formation (Serwanski et al., 2006). Extra-synaptic localization in this case seems to be correlated with interaction with radixin, an ezrin-radixin-meosin family member which interacts with the cytoskeleton (Loebrich et al., 2006).

Different studies, in particular using immunoprecipitations techniques, allowed to determine GABA-A subunits composition in several regions of the central nervous system. Even if the most common stoichiometric ratio is two α, two β and one γ, δ or ε, there is also evidence suggesting the existence of receptor containing only α and β subunits, or with different stoichiometry (such as 2 α, 1 β and 2 γ or 2 α, 1 β and 2 ε, Jones and Henderson, 2007). The most classical GABA-A receptor composition, showing the pharmacological profile of native receptors, presents two α, two β and one γ2 subunits. A and β subunits can be identical or different among them (Fritschy and Panzanelli, 2014). Γ2 subunit can be substituted by γ1, γ3 (not common in the central nervous system), δ and ε subunits. So far, at least 36 different

GABA-A subtypes have been identified in the central nervous system (Olsen and Sieghart, 2008).

Immunohistochemical studies on the distribution of 10 subunits (α1-α6, β2, β3, γ2 and δ, Fritschy and Mohler, 1995; Nusser et al., 1999; Peng et al., 2002; Chandra et al., 2006; Hortnagl et al., 2013) suggest that, in general, the six α subunits may correspond to distinct GABA-A subtypes (even if a substantial fraction of GABA-A receptors may contain two different α subunits, Balic et al., 2009) with a specific distribution pattern, partially overlapping with others. β2 and β3 subunits mostly overlap with α1 and α2, while the β1 subunit is expressed at lower levels in different brain regions (Fritschy and Panzanelli, 2014). Among γ subunits, γ2 is ubiquitously expressed, while γ1 has a specific expression pattern, mainly restricted to hypothalamus, amygdala, basal ganglia and the inferior olivary nucleus. The δ subunit, as already mentioned typical of extra-synaptic receptors, is co-expressed with the α4 subunit in the forebrain and with the α6 in the cerebellum.

More specific distribution data are available for some regions, such as the hippocampus (in particular the CA1 subfield), the neocortex, the olfactory bulb, parts of the thalamus, the cerebellum and the spinal cord dorsal horn. Analyzing staining patterns, it is sometimes possible to distinguish between synaptic and extra-synaptic receptors. The first ones appear as brightly stained clusters, co-localizing with post-synaptic markers like gephryn and neuroligin 2 (NL2). The latter ones do not form clusters, with uniform staining, suggesting widespread distribution on dendritic branches. Moreover, they do not co-localize with gephryn (Fritschy and Panzanelli, 2014).

Even if not clearly detectable from immunohistochemical studies, strong functional evidence suggests that there is a sub-population of receptors localized in axonal pre-synaptic terminals (Grasshoff et al., 2007; Trigo et al., 2008; Long et al., 2009; Witschi et al., 2011), which may represent a particular subset of extra-synaptic receptors with specialized functions. They are supposed to have a role in the modulation of potential transmission, neuronal synchronization, transmitters release and presynaptic afferent depolarization (Trigo et al., 2008; Long et al., 2009; Ruiz et al., 2010; Wakita et al., 2013).

Lastly, data obtained analyzing the mRNA expression of 18 different GABA-A subtypes in mouse brain allow to sort out the GABA-A subunits distribution in the central nervous system, once combined with previous data (Fritschy and Panzanelli, 2014). This data confirms previous studies in the distribution of more abundant subunits (α1–α6, β1–β3, and γ2, Laurie et al., 1992; Wisden et al., 1992), but also give new insights on lowly expressed ones, such as ε, whose expression seems to be mostly limited to amygdala, basal forebrain, locus coeruleus and other noradrenergic sites. P1 and ρ2 subunits (which form a subtype of GABA-A receptors once known as GABA-C) are expressed only on the superficial layers of the superior colliculus, and the σ subunit is undetectable in the adult brain.

Different studies conducted with knock-in mice for different GABA-A subunits helped to better understand their specific role in GABA-A receptors assembly. The deletion of specific subunits proved to be able to modify the expression pattern of other subunits, indicating the possible presence of compensatory mechanisms. For example, α1 knockout led to up-regulation of both α2 and α3 subunits (Kralic et al., 2006; Zeller et al., 2008). δ knockout, instead, led to an up-regulation of α4 subunit

in association with γ2, leading also to altered subcellular distribution (Peng et al., 2002).

Generally, there is no simple replacement of the missing subunit with other ones, in particular in neurons which express both synaptic and extra synaptic receptors. Indeed, deletion of synaptic α subunits may lead to pot-synaptic receptors disappearance and loss of post-synaptic currents. When this happens, extra synaptic currents can remain unaltered or be increase (Kralic et al., 2006; Peden et al., 2008), suggesting that the inability of α4 receptors to cluster at post-synaptic sites is not linked to competition with other receptors. Also, in neurons expressing different sub-types of synaptic receptors, there are specific rearrangements but without replacement of the missing subunit. This phenomenon was observed for example in CA1 pyramidal cells of knockout mice for the α2 subunit, where all GABA-A receptors were unchanged in perisomatic synapses, but disappeared from the initial segment of the axon (Panzanelli et al., 2011). In some sporadic cases, however, some forms of compensative mechanisms have been reported. For example, in thalamic reticular neurons of knockout mice for the α3 subunit there is an apparent loss of post-synaptic GABA-A receptors, but post-synaptic currents were higher than in control mice (Schofield et al., 2009).

Taken together, these data suggest that different GABA-A subtypes, defined by their different subunit composition, have different roles and characteristics, being not interchangeable within a given neuronal type (Fritschy and Panzanelli, 2014).

Several studies have been conducted with a model of knock-in mice (H101R), ideated to remove the diazepam binding site at the α-γ interface of the pentamer without altering other features (Rudolph et al., 1999; Low et al., 2000; Crestani et

al., 2002; Yee et al., 2005). Behavioral analysis of different variants of the model revealed different loss of specific diazepam effects, allowing a better understanding of each subunits contribution to diazepam effects. Evidence from these experiments suggests that sedative effects depend on α1 containing receptors, while anxiolysis is mediated by allosteric modulation of α2 containing receptors, with a partial contribution from α3-containing ones in stressful conditions (Rudolph and Mohler, 2004). As previously stated, it is in generally complicated to distinguish different GABA-A receptors based on β subunits composition, mainly because each β subunit can be associated with different α ones. However, some data suggest that β3-subunit containing receptors are responsible for the action of intravenous general anesthetics and of some effect of pentobarbital (Jurd et al., 2003, Zeller et al., 2007). Moreover, neuron-specific deletion of the subunit decreases survival of most mutant mice after early post-natal age (Ferguson et al., 2007).

GABA-A receptors have also been found to be expressed in glial cells, including astrocytes (Berger et al.,1992; Israel et al., 2003; Kettenmann et al.,1987), oligodendrocytes and Schwann cells. GABA-A expression in Schwann cells is detailed below and in the results. Both oligodendrocyte precursor cells and mature oligodendrocytes have been reported to express the GABA-A receptor (Hoppe and Kettenmann 1989; Von Blankenfeld et al., 1991; Berger et al., 1992). A recent study analyzed the subunits composition of GABA-A expressed in mature oligodendrocytes cultivated alone or in co-culture with DRG neurons. Combining pharmacological and immunofluorescence studies, the authors suggest that GABA-A receptors in oligodendrocytes do not express the δ subunit (as suggested by the

low sensitivity for allopregnanolone), suggesting that the most important subunits present in oligodendrocytes are α3, β2, β3, γ1 and γ3 (Arellano et al., 2016).

GABA-B RECEPTORS

The GABA-B receptor was initially identified by in the early 80's (Bowery et al., 1980). In the following years, different studies contributed to demonstrate its presence in mammalians central nervous system, both in neurons and glia (Kozlov et al., 2006; Serrano et al., 2006; Kuhn et al., 2004; Luyt et al., 2007). In particular, some pharmacological and biochemical studies showed that these receptors were not responsive to treatments with bicuculline or GABA-mimetics, but were specifically activated by GABA and β-p-clorofenil-GABA or baclofen. Moreover, it was demonstrated that GABA-B receptors are not coupled to Cl^- channels and are not responsive to barbiturates or benzodiazepines.

At synaptic level, GABA-B receptors are localized both at pre- and post-synaptic level (Figure 8). Pre-synaptic localization is much more common, where GABA-B receptors play an important role in the regulation of the release of different receptors, including GABA itself. They are commonly called auto-receptors when localized on GABAergic pre-synaptic terminals, and hetero-receptors when localized on other terminals (Gassmann and Bettler, 2012).

Two sub-types of the GABA-B receptor have been identified so far, named GABA-B1 and GABA-B2. GABA-B1 has been characterized at least in 3 different isoforms, named GABA-B1a, b, and c (Kaupmann et al., 1997, Isomoto et al., 1998, Pfaff et al. 1999, Gassmann and Bettler, 2012). The second sub-type, named

GABA-B2, was identified simultaneously by different groups (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999). Different studies made using recombinant receptors have clearly shown that the active form of the GABA-B receptor is the GABA-B1/GABA-B2 heterodimer, with the two subunits in a 1:1 stoichiometric ratio (Charles et al., 2003). Different models of mice lacking the GABA-B1 gene confirmed that it is essential for GABA-B assembly (Schuler et al., 2001). The interaction between the two subunits takes place at their carboxyl-terminal domains, leading to the formation of a α-helix coiled coin structure. The GABA-B1 subunit is responsible for the binding with GABA and other specific ligands, while GABA-B2 is responsible to the functionality of the heterodimer, for the co-regulation of some transcription factors (such as ATF1 and CREB-2) and for the interaction with auxiliary subunits, belonging to the KCTD family, which bind to GABA-B2 as part of a stable receptor complex at the cell surface (Charles et al., 2003; Gassmann and Bettler, 2012).

GABA-B1 isoforms are distributed throughout the neuronal and glial compartments in the brain, in the spinal cord (Charles et al., 2001; Margeta-Mitrovic et al., 1999) and in DRG (Campbell et al., 1993; Charles et al., 2001; Dolphin and Scott, 1986; Magnaghi, 2007; Towers et al., 2000), suggesting the involvement of GABA-B receptors in sensory functions (Bowery, 1993; Fromm, 1989; Hering-Hanit and Gadoth, 2001; Sindrup and Jensen, 2002).

GABA-B receptors belong to the G-protein coupled receptor (GPCR) family. When an agonist binds to the receptor, it induces the activation of an inhibitory G protein, belonging to the Gi/G_0 family, which leads to the inhibition of the enzyme adenylyl cyclase. This inhibition causes a reduction in cAMP levels, with reduced PKA

activity and, therefore, reduced phosphorylation levels of PKA-dependent transcription factors (Steiger et al., 2004) and kinases (Diversè-Pierluissi et al., 1997; Ren and Mody, 2003; Gassmann and Bettler, 2012, Figure 8).

These processes are able to modulate neuronal excitability and the release of neurotransmitters. GABA-B activation may also cause a reduced Ca^{++} and increase potassium ion (K^+) conductance. The effect on Ca^{++} is more important at pre-synaptic level, involving mainly voltage-gated calcium channels (VGCC, Figure 8). Reduced Ca^{++} influx in the cell may be the main mechanism responsible for the inhibition of mediators' release, slowing vesicles formation (Sakaba and Neher, 2003). Instead, the effect on K^+ currents is modulated at post-synaptic level. Indeed, the activation of post-synaptic GABA-B receptors leads to a slow inhibitory post-synaptic potential ($IPSB_B$) through the activation of K^+ GIRK channels, whose opening causes membrane hyper-polarization (Gassmann and Bettler, 2012).

The regulation of these channels is modulated by β and γ subunits of the activated G protein (Gassmann and Bettler, 2012, Figure 8). This process is limited to the cell membrane and independent from second messengers, being able to mediate these effects just few seconds after the receptor activation.

GABA-B receptors activity seems to be very important in the modulation of normal physiological processes of the central nervous system. For example, studies performed using knockout mice for the receptor have shown that he animals were hyper-active and presented epileptic episodes (Bettler and Tiao, 2006, Kornau, 2006).

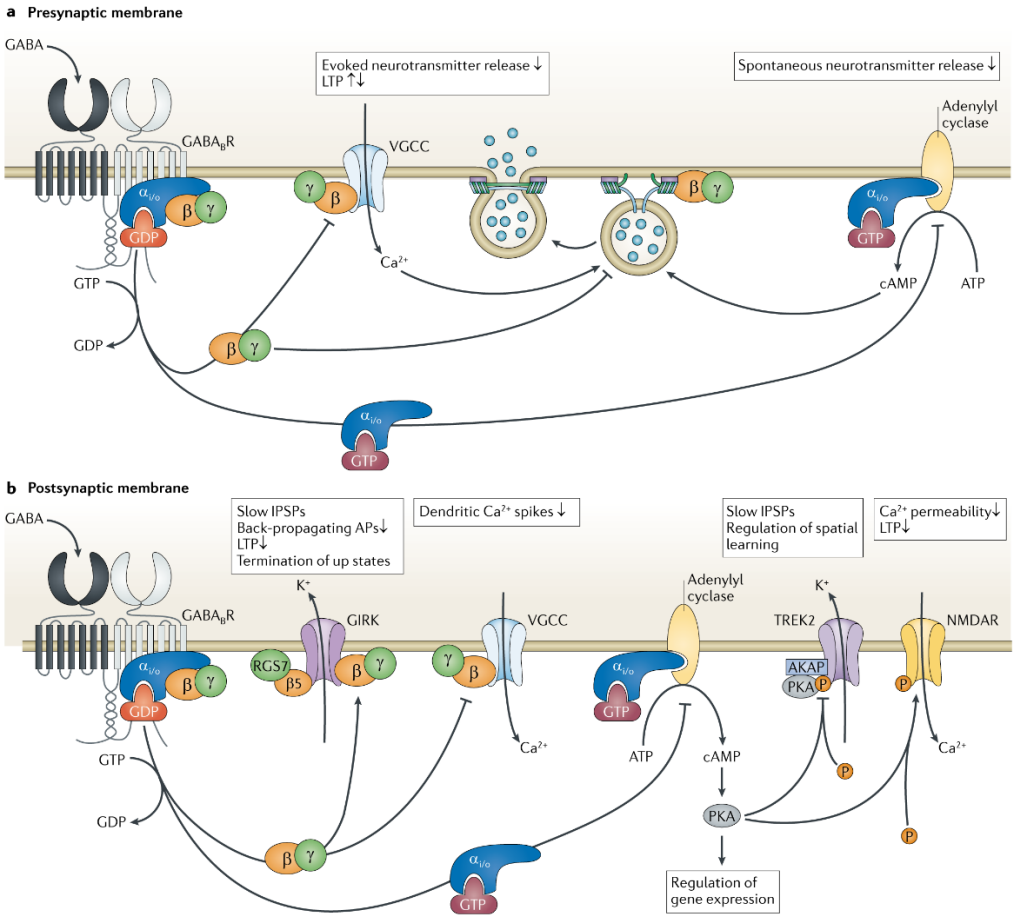


Figure 8 – Pre-synaptic (a) and post-synaptic (b) GABA-B receptors. Both at pre- and post-synaptic level, GABA-B effects are mediated both by the $\alpha_{i/0}$, β and γ subunits of the G protein coupled to the receptor. Those actions are mainly mediated by adenylyl-cyclase down-regulation, but also by modulation of Ca^{++} and K^+ channels. Adapted from Gassmann and Bettler, 2012.

Some results obtained with ultra-structural studies show how most GABA-B receptors are present in excitatory synapses, such as glutamatergic ones (Lacey et al., 2005; Kulik et al., 2006, Gassmann and Bettler, 2012). Some studies, indeed, show how some fibers are able to release GABA following an increase in excitatory transmission, strengthening the hypothesis that it can be co-released by glutamatergic synapses as a control mechanism of their activity (Walker et al.,

2002; Gutierrez, 2002). Another study suggests that GABA-B receptors on glutamatergic synapses may also be activated by the GABA released by dendritic cells (Zilberter et al., 1999).

Most cerebral neurons co-express both GABA-B 1a and 1b isoforms. They derive from the same gene after different splicing of the promoter (Steiger et al., 2004; Gassmann and Bettler, 2012). From a structural point of view, the two isoforms are different in their ectodomains at the level of a couple of sushi domains (Blein et al., 2004). Even if there are no functional or pharmacological differences among the two isoforms *in vitro*, they generate different responses *in vivo*. This may be due to different post-transcriptional modifications the two subunits may face. Indeed, it is reported that GABA-B mediated responses are regulated by phosphorylation (Couve et al., 2002; Kuramoto et al., 2007) and by proteins associated to the receptor (Perroy et al., 2003; Pontier et al., 2006; Kanaide et al., 2007; Balasubramanian et al., 2007). The two isoforms functions were analyzed using mice genetically modified to express just one of them (Perez-Garci et al., 2006; Vigot et al., 2006; Shaban et al., 2006). These studies suggested that the most significant difference is GABA-B1a presence in glutamatergic terminals as shown in hippocampal and neocortical pyramidal cells (Vigot et al., 2006; Shaban et al., 2006). These data were confirmed by imaging studies, localizing GABA-B1a in CA3 pyramidal neurons (Vigot et al., 2006). It is possible that sushi domains, being the only different region among the two subunits, may be able to determine the different axonal localization of the two isoforms.

GABAERGIC SYSTEM IN THE PERIPHERAL NERVOUS SYSTEM

Some studies from different research groups, performed between the 1970's and 80's, provided indication that the GABAergic system is present and active in the peripheral nervous system. Binding studies showed the GABA receptors both in myelinated and unmyelinated fibers as well as in Schwann cells (Brown and Marsh, 1978; Brown et al., 1979; Morris et al., 1983; Olsen et al., 1984; Nagai et al., 1998). More recent studies demonstrated that peripheral nerves, and Schwann cells in particular, express different GABA-A (α2-3, β1-3, γ2) and GABA-B (1a, 1b and 2) subunits (Melcangi et al.,1999; Magnaghi et al., 2001,2006). As already mentioned, Schwann cells are able to synthesize GABA through the action of the GAD67 enzyme. The same studies provided evidence that GABA can control its own synthesis via a GABA-A mediated mechanism through the MAPK/CREB pathway activation (Magnaghi et al., 2009, 2010). As better detailed below, the GABA-A receptor has also an important role in the mediation of several action of the neuroactive steroid allopregnanolone, including the modulation of myelin proteins expression, Schwann cell proliferation and the trafficking of the glutamate EAAC1 transporter (Magnaghi et al., 2001; Perego et al., 2012).

In recent years, some evidence proved the importance of the GABAergic systempresent in Schwann cells in the neuron-glia interaction and in the patho-physiology of the peripheral nervous system. In particular, two papers (Magnaghi et al., 2008; Faroni et al., 2014), demonstrated a role for the GABA-B receptor in peripheral myelination and in pain processing mechanisms. Both GABA-B1 total knockout mice (Magnaghi et al., 2008) and conditional knockout mice, which

present a selective deletion of the GABA-B1 receptor only in Schwann cells (P0-CRE_GABA-B1$^{fl/fl}$ mice, Faroni et al., 2014), present indeed alterations in pain response to thermal and mechanical stimuli and changes both in Schwann cell autonomous and non-autonomous parameters. Specifically, GABA-B1 conditional knockout mice were characterized by morphological alteration, with a significant increase of the percentage of irregular fibers and signs of unloose wrapping of the myelin sheath, revealing defective myelination. At the molecular level, the gene expression of myelin proteins P0 and PMP22 was increased, and P0 expression was higher in conditional knockout animals also at the protein level in 3 months old animals. The study of intracellular pathways revealed that in three months old animals the NRG-1 typeIII-ErbB2/ErbB3-Erk2 pathway, that, as already mentioned, is considered one of the main pathway involved in myelination control (Feltri et al., 2016; Salzer, 2015; Taveggia, 2016), are modulated. This evidence strongly suggests a role for GABA-B receptors in its modulation. A detailed analysis of these fibers type showed that conditional knockout mice presented an increased percentage of small axons and a higher presence of Remak bundles and unmyelinated fibers, pointing to an increased presence of C fibers, mostly involved in pain transmission. Accordingly, the number of small cell bodies in DRG, from which these fibers originate, resulted to be increased. Those morphological evaluations were in line with observed alterations in pain sensitivity, indeed both 3 and 6 months old animals resulted to be hyperalgesic following thermal stimuli, and younger animals also shown mechanical allodynia. Interesting, in a paper in which a similar model was used to obtain a selective knockout of GABA-B receptors in DRG, only slight modifications in pain sensitivity where observed (Gangadharan et

al., 2009). Experimental animals also presented gait alterations, showing functional locomotor impairment, probably ascribable both to myelination disorders and altered pain processing (Faroni et al., 2014).

Another study, which analyzed GABA-B receptors localization *in vitro* and *in vivo* in Schwann cells, suggested that they may be particularly expressed in Schwann cells that are assuming the non-myelinating phenotype (Procacci et al., 2012). Indeed, immunofluorescence evaluations of coronal and longitudinal sections of rat sciatic nerves revealed that GABA-B receptors show some co-localization with glial fibrillary acidic protein (GFAP), a marker of non-myelinating Schwann cells, while there was almost no co-localization with the myelination marker MAG (that, as previously mentioned, is a key component of the adaxonal membrane (Procacci et al., 2012).

Collectively, these lines of evidence suggest that GABA-A and GABA-B receptors may play a dualistic role in Schwann cells (Figure 9). Indeed, as just touched on, GABA-A activation through the neuroactive steroid allopregnanolone is able to positively modulate P0 an PMP22 expression (Melcangi et al., 1999, Magnaghi et al., 2001). Moreover, it is able to modulate the expression and responsiveness of GABA-B receptors (Magnaghi et al., 2006, 2010) and to stimulate Schwann cell proliferation (Perego et al., 2012). GABA-A activation also leads to an increase in the expression levels of GAD67 and, consistently, to increased GABA synthesis (Magnaghi et al., 2010). The trafficking of the EAAC1 transporter, which mediates the uptake of GABA precursor glutamate, is also regulated (Perego et al., 2012). On the other hand, GABA-B activation leads to a decrease in Schwann cell proliferation (Magnaghi et al., 2004) and induces their differentiation. Moreover,

GABA-B activation leads to a decrease in myelin proteins expression (Magnaghi et al., 2004).

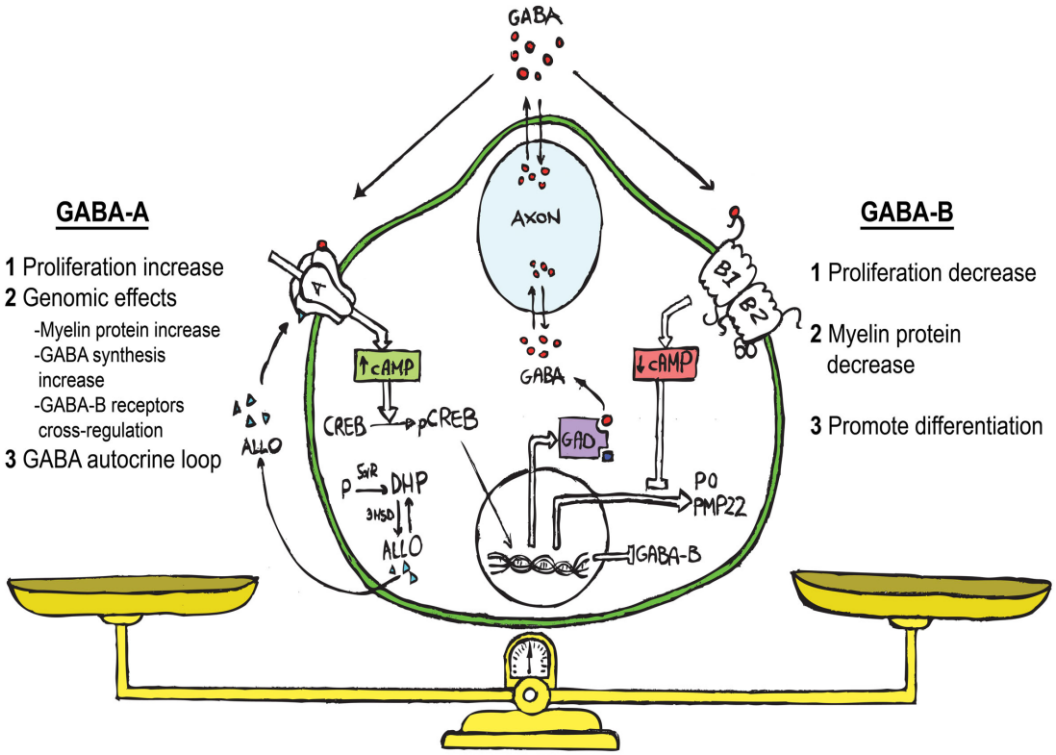


Figure 9 – Cross-interaction and dualistic effect of GABA-B receptors in Schwann cells. Adapted from Faroni and Magnaghi, 2011.

Taken together, those findings suggest that the GABAergic system may play a role in Schwann cell differentiation, with GABA-A mainly involved in the regulation of the myelinating phenotype and GABA-B in the differentiation of non-myelinating Schwann cells (Faroni and Magnaghi 2011; Procacci et al., 2012; Faroni et al., 2014).

PROTEIN KINASE C – TYPE ε

GENERAL CONCEPTS

Protein kinases (PKs) are a family of isoenzymes which are activated as second messengers, that act phosphorylating specific intracellular targets and therefore modulating different pathways. The most important sub-families of PKs are PKA and PKC.

PKA is an isoenzyme that is activated following cAMP increased intracellular concentration, when a GPCR coupled with a Gs protein is activated. In general, the signal cascade following PKA activation leads to the modulation of specific genes' expression.

PKC is activated by phosphorylation and membrane translocation. The phosphorylation can be catalyzed by two different enzymes: diacylglycerol (DAG) and calmodulin. DAG is formed following the activation of GPCRs coupled with a Gq protein, which activates PLC that hydrolyzes phosphatidylinositol (PIP_2) giving DAG and inositol trisphosphate (IP_3).

IP_3 is able to induce Ca^{++} release. Increased intracellular Ca^{++} levels are responsible for the other PKC activation mechanism. Indeed, calmodulin is activated after its binding with Ca^{++} and then phosphorylates PKC, activating it. Therefore, PKC can be activated through a G-protein mediated mechanism, but also by Ca^{++} intracellular fluctuations that can follow other biological events, such as activation of ion channels.

Ten PKC isoforms have been identified so far (Mellor and Parker, 1998) and they are classified into three groups, based on their activation mechanism (Nishizuka, 1995). Conventional PKCs (α, βI, βII and γ) can be activated both by DAG and the Ca^{++}-calmodulin complex. Novel PKC isoforms (δ, ε, η and θ) are activated only by DAG. Atypical PKCs (ξ, λ/ι) do not require any of the two enzymes to be activated, being in this way not under PLC control. Their activation mechanism requires the presence of phosphatidylserine.

Several data suggest that, among other isoforms, PKCε plays an important role in the modulation of neuropathic pain. In the central nervous system, it resulted to be expressed mainly in the cerebral cortex, cerebellum and hippocampus. In small amounts, it is expressed also in peripheral non-nervous tissues (Saito et al., 1993; Chen et al., 2000).

The first evidence pointing to PKCε role in pain modulation was its identification in the brain (Mochly-Rosen et al., 1987; Saito et al., 1998) and in primary peripheral neurons involved in nociception (Aley et al., 2000). Moreover, PKCε potentiates afferent unmyelinated fibers signal (Dray et al., 1988; Rang et al, 1988), in particular C fibers, responsible for thermoception and nociception (Parada et al, 2003; Cesare and McNaughton, 1996). Its activation increases sensory neurons sensitivity (Schepelmann et al., 1993), intensifying and prolonging painful stimuli. Consistently, PKCε inhibition blocks sensory neurons' hyper stimulation (Burgess et al. 1989; McGuirk and Dolphin, 1992). In a model of inflammatory-derived hyperalgesia, temporary PKCε inhibition proved able to block the pathological state, suggesting its implication both in the early phase and in the chronicization of hyperalgesia (Parada et al, 2003).

From a mechanicistic point of view, PKCε exerts its pro-hyperalgesic effect phosphorylating both excitatory (such as voltage-operated Ca^{++} channels, VOCC, and transient receptor potential cation channel subfamily V member 1, TRPV1), and inhibitory (such as GABA-A and opioid μ receptors) channel receptors, activating the first and down-modulating the latter. TRPV1 is a non-specific ion channel activated by different ligands, such as anandamide (Zygmunt et al., 1999), Leukotriene B and other inflammatory mediators (Hwang et al., 2000) and capsaicin. It is believed to be an important mediator in inflammatory pain conditions, indeed it is expressed in nociceptive C fibers (Tominaga et al., 1998). Its physiological function is the transmission of painful stimuli due to high temperature, but in pathological conditions of hyperalgesia its opening can be triggered also at lower temperature. This phenomenon has been reported to be present after PKCε activation (Crandall et al.,2002). PKCε has also been shown to activate this receptor through phosphorylation (Olah et al., 2002) and to facilitate its membrane translocation in sensitive neurons (Morenilla-Palao 2004). Accordingly, PKCε inhibition is able to cause a lower TRPV1 response to capsaicin (Zhou et al., 2001).

As mentioned before, PKCε mediated phosphorylation is also able to de-sensibilize opioid μ receptors (Zhang et al., 1990; Narita et al., 1997) and GABA-A receptors (McMahon and Koltzenburg, 1990; Si et al., 2004; Yamada and Akasu, 1996; Ma et al, 2006), leading to a hyper-excitation condition, typical of neuropathies.

CROSS-TALK WITH ALLOPREGNANOLONE AND GABA-A

Several lines of evidence suggest that PKCε may be involved in a complex mechanism that involves GABA-A and allopregnanolone (Figure 10). Studies conducted with PKCε null mice (Hodge et al., 1999) revealed that these animals are hypersensitive to GABA-A receptor allosteric modulators' behavioral effects (Hodge et al., 2002). As previously stated, allopregnanolone is one of the most important allosteric modulators of the GABA-A receptor. Accordingly, similar results were obtained also *in vitro*, since the peptide-mediated blocking of PKCε translocation in cortical synaptosomes leads to increased sensitivity to allopregnanolone (Khasar et al., 1999, Hodge et al., 1999).

As already mentioned, PKCε exerts its action though the phosphorylation of its substrates. Specifically, its action on the GABA-A receptor is mediated by the phosphorylation of the Serine 327 amino acidic residue of the γ2 subunit (Qi et al., 2007). PKCε is also able to modulate GABA-A receptor trafficking, acting on the N-Ethylmaleimide-Sensitive Factor signaling pathway, being able in this way to decrease its plasma membrane localization (Chou et al., 2010).

GABA-A de-sensitization seems to play a role in neuroactive steroids modulation of the receptor (Zhu and Vicini, 1997). Probably, the state of the receptors determines how responsive they are to neuroactive steroids. Furthermore, neuroactive steroids, and in particular allopregnanolone, can induce changes in the GABAergic neurotransmission through synaptic and extra synaptic receptor modulation. Recently, it has been demonstrated that neuroactive steroids may increase the surface expression of specific GABA-A receptors, mainly α4

containing ones, modulating GABAergic tonic inhibition in the hippocampus (Abramian et al., 2014). Other GABA-A allosteric modulator (such as benzodiazepines) have been reported to be able to influence their diffusion and clustering at synapses (Levi et al., 2015). On the other hand, neuroactive steroids potentiating effect can be decreased by the stimulation of PKC pathway, both in physiological and pathological conditions (Brussaard et al., 2000; Brussaard and Koksma, 2003; Maguire et al., 2005; Oberlander et al., 2012; Mtchedlishvili et al., 2001; Kia et al., 2011). For example, neuroactive steroids modulatory effect is decreased in dentate granule cells from epileptic rats (Mtchedlishvili et al., 2001). At the same time, cell specific changes in GABA-A phosphorylation status may occur in several epilepsy models (Schwarzer et al., 1997; Fritschy et al., 1999; Peng et al., 2004) and in the temporal lobe of human epileptic individuals (Loup et al., 2000; Ferando and Mody, 2012). A similar correlation can be observed when GABA-A receptor phosphorylation status changes after kindling; this changes, together with the modification of subunits composition, can contribute to the reduced response to neuroactive steroids (Kia et al., 2011; Carver et al., 2014). The diminished allopregnanolone sensitivity can increase the susceptibility to seizures, as it happens in catamenial epilepsy, where increased seizures probability correlates with progesterone decreased levels before the onset of menstruations (Reddy, 2009).

Some evidence supports the presence of a cross-talk between PKCε, neuroactive steroids and the GABA-A receptor in pain perception and transmission (Figure 10). Indeed, the synthesis of neuroactive steroids is up-regulated following neuropathic pain onset (Poisbeau et al., 2005), and also PKCε levels were observed to be

modulated in pathological pain condition (Parada et al., 2003). It has been proposed that allopregnanolone could participate to a compensatory mechanism that counteracts the sustained activation of spinal cord nociception in pathological conditions (Vergnano et al., 2007).

PKCε presence and activity have been demonstrated also in the peripheral nervous system, and in particular in DRG neurons., where it may alter the permeability of N-type Ca^{++} channels and enhance nociception (Van Kolen et al., 2008).

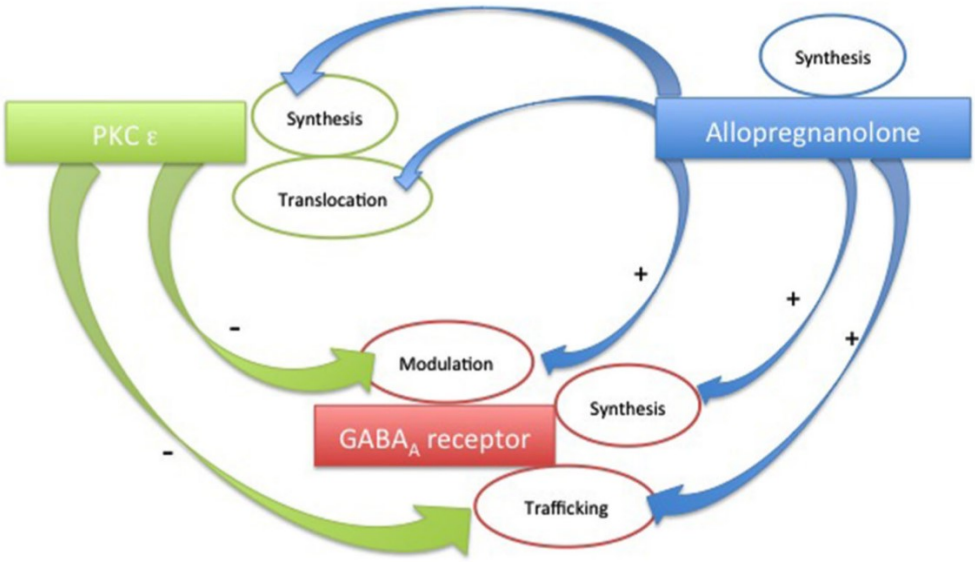


Figure 10 – cross-interaction between PKCε, the neuroactive steroid allopregnanolone and the GABA receptor. PKCε is a negative modulator of the GABA-A receptor and reduces its cell membrane expression. On the other hand, allopregnanolone is a positive modulator of the GABA-A receptor and of its trafficking towards the cell membrane. Furthermore, it increases its synthesis. A direct interaction between PKCε and allopregnanolone, even if not well documented yet, is probable. Adapted from Puia et al., 2015.

NEUROACTIVE STEROIDS

GENERAL CONCEPTS

The term “neurosteroids” was firstly introduced by Baulieu and colleagues at the end of the ‘80s, to describe hormones synthesized by the nervous system, in order to differentiate them from hormones synthesized by classical steroidogenic tissues (Baulieu, 1997). The concept was furtherly developed in the following years, leading to the concept of “neuroactive steroids”, which includes properly defined neurosteroids, synthesized in the nervous system and acting on the nervous system, but also steroid hormones synthesized outside the nervous system but acting on it.

Neurosteroids are synthesized in some cerebral area, including hypothalamus, hippocampus, cerebral cortex, cerebellum and peripheral nervous system (Mellon et al., 2001, Melcangi et al., 2001). For instance, Purkinje cells of the cerebellum and pyramidal neurons of the hippocampus are the major neuronal population producing neurosteroids in the brain (Ukena et al., 1999, Kimoto et al., 2001, Hojo et al., 2004). The first step in steroidogenesis is cholesterol conversion to pregnenolone, catalyzed by the mitochondrial cytochrome P450 cholesterol side-chain cleavage enzyme (P450 SCC). This step requires cholesterol to be transferred from the outer mitochondrial membrane to the inner membrane, where P 450 SCC is located. The steroidogenic acute regulatory protein (StAR) and the translocator protein of 18 kDA (TSPO) are involved in this process, probably forming a complex (Stoffel-Wagner et al., 2003; Korneyev et al., 1993; Jefcoate, 2002; Papadopoulos

et al., 2006). It is interesting to note that TSPO is highly expressed in every steroidogenic tissue, including brain (Papadopoulos et al., 2006).

Cholesterol is therefore converted to pregnenolone, which following different biosynthetic pathways, leads to the formation of neuroactive steroids (As shown in figure 11).

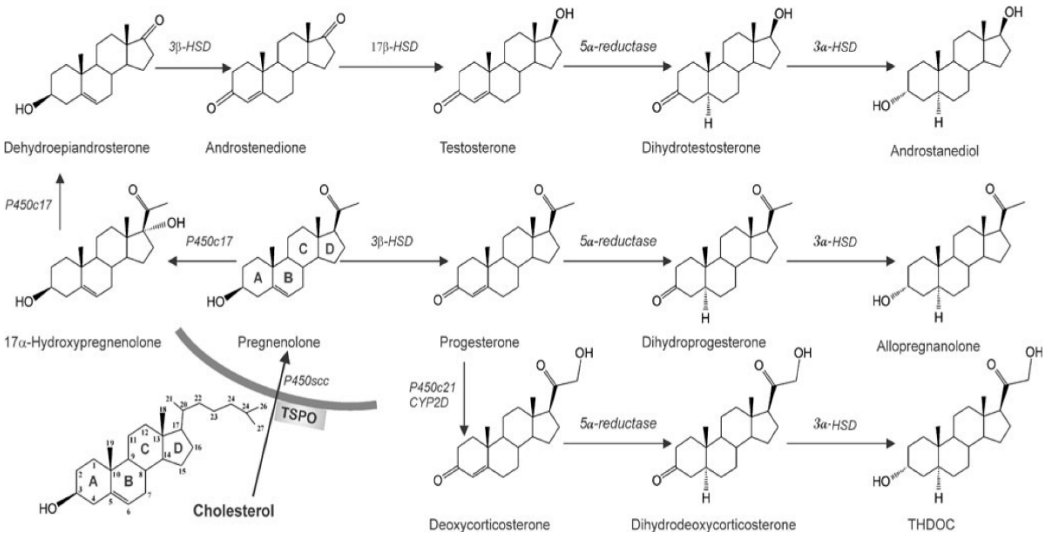


Figure 11 – Neuroactive steroids synthesis. Cholesterol is converted to pregnenolone through the action of the P450 SCC enzyme, that is the substrate for the synthesis of other neuroactive steroids. Adapted from Carver and Reddy, 2013.

Allopregnanolone and tetrahydro-deoxycorticosterone (THDOC), which are classified as pregnane steroids, are the most commonly studied endogenous neuroactive steroids (Reddy and Kulkarni, 2000; Belelli and Lambert, 2005). Pregnenolone, progesterone and deoxycorticosterone also exhibit neuroactive effects. Androstanes are another important class of neuroactive steroids. They include androstanol, androstanediol and testosterone (Reddy, 2004; Kaminski et al., 2006; Reddy, 2008).

The first studies in the neuroactive steroids field mainly focused on the central and peripheral nervous system neurons. However, different studies pointed out to the important effects exerted by these neuroactive steroids on glia (Celotti et al., 1992, Melcangi et al., 2001). Glial cells, indeed, are able to synthesize steroid hormones, expressing the enzymatic machinery necessary for their synthesis, in particular CYP450 SCC, 17α- hydroxysteroid dehydrogenase (HSD) and 3β-HSD (Mellon et al., 2001). Furthermore, they also express the enzymes required for the conversion of steroid hormones to neuroactive steroids, in particular 5α-reductase and 3α-HSD. It is interesting to notice that these two enzymes resulted to be more expressed in glial cells than in neuronal ones. The presence of these enzymes has been characterized both at central (Steckelbroeck et al., 2001) and peripheral level (Melcangi et al., 1990, 1992, 1999; Celotti et al., 1992).

MECHANISM OF ACTION

It is possible to distinguish two different mechanisms of neuroactive steroids action: the classical genomic mechanism and the non-classical mechanism mediated by neuroactive steroids binding to different nervous receptors.

The first mechanism is mediated by classical intracellular steroid hormone receptors. It involves the modulation of genic transcription and leads to slow but long-lasting effects. Different hormonal receptors have shown to be expressed by glial cells. The progesterone receptor (PR) has been shown to be present in rat Schwann cell culture and sciatic nerve (Jung-Testas et al., 1996; Jung-Testas and Baulieu, 1998; Thi et al., 1998; Magnaghi et al., 1999, 2001). The same study also

revealed the presence of the estrogen receptor (ER) in rat Schwann cell cultures (Jung-Testas and Baulieu, 1998). Also the androgen receptor (AR) was reported to be present in rat sciatic nerve (Jordan et al., 2002; Magnaghi et al., 1999).

The non-classical mechanism, as already mentioned, involves neuroactive steroids binding with neurotransmitter receptors, such as the already cited GABA, N-Methyl-d-aspartate (NMDA), 5-hydroxytryptamine type 3 (5-HT3) and opioid σ-receptors (Lambert et al.,1996; Rupprecht et al., 2001; Monnet and Maurice, 2006; Sedlacek et al., 2008), leading to rapid actions mediated by the intracellular activation of second messengers.

However, neuroactive steroid rapid actions may involve also the activation of novel putative membrane receptors for steroids (Hammes and Levin, 2007; Dressing et al., 2011; Levin, 2011; Faroni and Magnaghi 2011; Zhu et al., 2003a, b; Pang et al., 2013).

PROGESTERONE DERIVATIVES

Among other neuroactive steroids, progesterone derivatives demonstrated to be very interesting for their ability to modulate the nervous system in different physio-pathological conditions.

As shown in Figure 11, progesterone derives from pregnenolone through a reaction catalyzed by the enzyme 3β-HSD. Progesterone can be subsequently converted to 3α-dihydroprogesterone (DHP) by the action of the 5α-reductase. Both compounds are ligands of the classic PR. DHP can be further metabolized to 3α,5α-tetrahydroprogesterone (THP), more commonly known as allopregnanolone, that

does not bind the PR, but is known to exert most of is action through the binding with the GABA-A receptor (Belelli and Lambert, 2005), already described above. This interaction was studied through patch clamp studies, revealing that it can be due both to positive allosteric modulation or direct activation of the GABA-A receptor, depending on concentration. In particular, at nanomolar concentration allopregnanolone acts as an allosteric modulator, while at micromolar concentration it is able to induce the Cl^- channel opening even if GABA is not present (Callachan et al., 1987; Shu et al., 2004; Park-Chung et al., 1999; reviewed in Belelli and Lambert, 2005).

Studies conducted using a chimeric receptor model allowed the identification of two different binding sites for neuroactive steroids on the GABA-A receptor, corresponding to two discrete groups of residues in the trans-membrane domains. The first binding site, defined as the "activation site", is located at the interface between α and β subunits at the level of αThr236 and βTyr284 amino acidic residuals, and is responsible for the initiation of the direct activation of the receptor. The second binding site, instead, called "potentiation site", is located in a cavity formed by α subunit transmembrane domains, between αGln241 and αAsn407, and mediates the potentiation of GABA or neuroactive steroids binding on the other site. The direct activation of the receptor requires allopregnanolone binding in both sites (Hosie et al., 2006). Both the activation and the potentiation sites are structurally highly conserved among different α and β sub-types, and δ subunit seems to be not involved in neuroactive steroids binding to the potentiation site, suggesting it may modulate neurosteroid potentiation by a mechanism other than by affecting their initial binding (Hosie et al., 2006, 2009).

Both synaptic and extra-synaptic receptors were reported to be modulated by allopregnanolone activity. Electrophysiological studies indicate that, in most brain regions, the neuroactive steroids activity at synaptic level produces a low effect on the rise time or amplitude of GABA-gated channel opening, prolonging instead the decay time of the event (Lambert et al., 2003). In particular, allopregnanolone, such as other positive modulators of the GABA-A receptor, exerts its action on the channel increasing the channel opening probability (Puia et al., 1990; Twyman and Macdonald, 1992; Zhu and Vicini, 1997), and prolonging the decay time of inhibitory post-synaptic currents (Harrison et al., 1987; Fáncsik et al., 2000).

As previously mentioned, neuroactive steroids are importantly involved in the modulation of extra-synaptic GABA-A receptors. Indeed, receptors containing the δ subunit instead of γ ones show a stronger GABA-A modulatory effects of neuroactive steroids (Belelli et al., 2002; Brown et al., 2002; Wohlfarth et al., 2002). This has been suggested to be true in particular in specific cerebral areas, such as the cerebellar granule cells (Stell at al., 2003). However, in other areas, such as thalamo-cortical neurons, extra-synaptic receptors are relatively insensitive also to high levels of allopregnanolone (Brown et al., 2009). Other factors, such as the phosphorylation state (as previously mentioned) and the local steroid metabolism may also contribute to this selectivity (Belelli et al., 2009).

Furthermore, allopreganolone may be also retro-converted to DHP (Martini et al., 2003) and interact with the classic PR (Rupprecht et al., 1993, Finn et al., 2006). DHP has less affinity than progesterone for the PR (Schumacher et al., 2007), however it has been reported to mediate some PR mediate effects, in particular on

GFAP in astrocytes and on P0 in Schwann cells (Melcangi et al., 1996, 2001; Martini et al., 2003, detailed below).

PROGESTOGENS ACTION ON GLIAL CELLS

Progestogens, and in particular allopregnanolone, have important effects on the glial cells of the central nervous system. In oligodendrocytes, allopregnanolone proved able to modulate the expression of MBP and the 2'-3'-cyclicnucleotide-3-phospodiesterase (Verdi and Campagnoni, 1990; Jung Testas et al., 1996). For example, a study showed allopregnanolone and DHP ability to reverse age-related MBP down-regulation in old male rats (Ibanez et al., 2003). Progestogens ability to up-regulate MBP both through PR and GABA-A mediated mechanism was suggested also in organotypic cerebellum slice cultures of 7-days old mice and rats (Ghoumari et al., 2003). In these cells, neuroactive steroids and GABA signaling are also important regulators of the proliferation process and differentiation of oligodendrocyte progenitor cells. Indeed, the neuronal progenitor polysialylated-neural cell adhesion molecule positive cells (PSA-NCAM+) express different GABA-A subunits, such as α1-5, β2-3 and γ2 (Nguyen et al., 2003, Gago et al., 2004). There is evidence that allopregnanolone has a positive proliferative effect on these cells, since this effect is also exerted by GABA and blocked by bicuculline, suggesting that it is GABA- mediated (Gago et al., 2004). These data indicate that progestogens may have an important role in the maturation and development of oligodendrocytes (Ben-Ari, 2002, Gago et al., 2004), both through classic and non-classic mechanisms.

Progestogens, in particular progesterone derivatives DHP and allopregnanolone, play an important role also in astrocytes cell physiology, in particular modulating GFAP expression, which has a role in the control of astrocytes shape and motility (Laping et al., 1994). In astrocytes cell cultures GFAP expression has been reported to be down-regulated by allopregnanolone and up-regulated by DHP (Melcangi et al., 1996). Allopregnanolone action may be mediated by the GABA-A receptor, whose subunit are widely expressed in astrocytes (Bovolin et al., 1992; Hosli et al.,1997; Israel et al., 2003).

Progesterone and estrogens have been reported to exert anti-inflammatory effect in the central nervous system, mainly modulating microglial cells (Stone et al., 1997; Vegeto et al., 1999, 2001; Drew and Chavis, 2000; Bruce-Keller et al., 2001). The concentrations of allopregnanolone are remarkably high in the fetal brain and further rise in response to acute hypoxia, representing an endogenous protective mechanism in the developing brain (Hirst et al., 2006). Allopregnanolone is also reported to be a neuroprotective agent in different brain injury conditions, such as ischemic damage or oxygen glucose deprivation (Kelley et al., 2011, Ardeshiri et al., 2006). Even if these allopregnanolone effects may be linked to the regulation of anti-inflammatory cytokines and endogenous antioxidants, only little evidence supports a role for allopregnanolone in microglia modulation (Ghezzi et al., 2000, Djebaili et al., 2005).

Nowadays, a consistent number of observations support the importance of neuroactive steroids, and in particular progestogens, in the regulation of Schwann cell patho-physiology. There is evidence they are able to modulate the expression of myelin proteins P0 and PMP22 *in vivo* in young and old male rats (Melcangi et

al., 1998, 1999, 2000, Magnaghi et al., 2001). In particular, P0 expression seems to be modulated mainly through a classic PR mediated mechanism, while PMP22 expression resulted to be under allopregnanolone-GABA-A control (Magnaghi et al., 2001). Indeed, the allopregnanolone mediated up-regulation of PMP22 expression was mimicked with GABA-agonist muscimol and blocked with its antagonist bicuculline (Magnaghi et al., 2001, 2006). However, also a classic PR mediated mechanism may be involved in the modulation of the latter, since mifepristone (RU38486), a classic PR antagonist, was able to counteract PMP22 overexpression in a model of CMT-1A (Sereda et al., 2003). Progestogens proved also able to exert positive effects in sciatic nerves after aging (Azcoitia et al., 2003), cryolesion (Koenig et al., 1995), transection (Melcangi et al., 2000) and crush injury (Roglio et al., 2008).

As already mentioned, allopregnanolone is also involved in GABA receptors cross-talk in Schwann cells. In particular, it has been proposed to exert a biphasic effect on GABA-B subunits expression, up-regulating them after short (4 hours) treatment and down-regulating their expression after 24 hours (Magnaghi et al., 2006). This effect was mimicked by muscimol and GABA, while the 24 hours effect was mimicked by progesterone and DHP (probably after conversion to allopregnanolone), further suggesting an important role for allopregnanolone in the modulation of Schwann cell physiology (Magnaghi et al., 2006; Magnaghi, 2007). Allopregnanolone has been hinted to have a role also in GABA synthesis in Schwann cells, being able to modulate GAD 67 expression (Magnaghi et al., 2010). Moreover, it regulates the uptake of GABA biological precursor, glutamate, through membrane translocation of the EAAC1 glutamate transporter, in a process

that involves PKC activation (Perego et al., 2012). Through the regulation of the trafficking of this transporter, allopregnanolone has also been shown to induce Schwan cells proliferation *in vitro* (Perego et al., 2012).

NOVEL SIGNALING PATHWAYS

Aside neurotransmitter receptors, several other receptors have a role in the modulation of non-classical action of neuroactive steroids, in particular membrane progesterone receptors (mPRs: mPRα, mPRβ, mPRγ, mPRδ and mPRε), the progesterone receptor membrane component 1 (PGRMC1) and the pregnane X-receptor (PXR, Figure 12).

PXR is a promiscuous nuclear receptor, activated by different compounds, including steroids, whose expression has been detected in different parts of the brain and spinal cord (Lamba et al., 2004; Ma et al., 2008, Mellon et al., 2008). It is known to be a key modulator of xenobiotic action (Kliewer et al., 2002) Allopregnanolone has been reported to activate PXR both *in vivo* and *in vitro* and to induce PXR target genes (Lamba et al., 2004; Langmade et al., 2006). Progesterone, otherwise, has low affinity for this receptor (Cooke et al., 2013). PXR seems to mediate some neuroprotective actions of allopregnanolone, in particular following traumatic brain injury (Cooke et al., 2013) and in a murine model of Niemann-Pick C disease (Langmade et al., 2006).

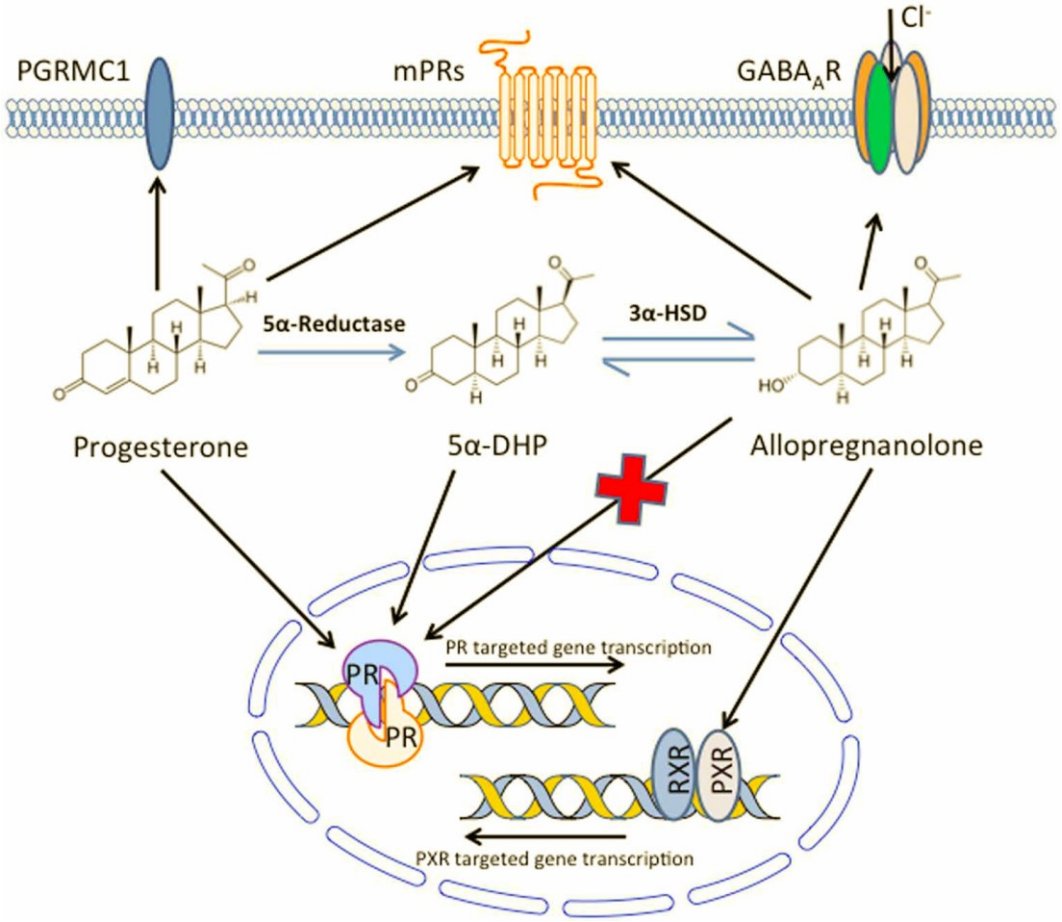


Figure 12 – Schematic representation of known target receptors of progestogen neuroactive steroids. Progesterone exerts its action through PGRMC1, mPRs and the classic PR. DHP action has been characterized only through the binding with PR. The most studied target for allopregnanolone, which does not bind PR, is the GABA-A receptor; however recent evidence suggests that also mPRs and PXR are mediators of allopregnanolone pharmacological activity. Adapted from Cooke et al., 2013.

PGRMC1

PGRMC1 is a member of a group of molecules which are characterized by the presence of highly conserved cytochrome b5/heme steroid binding domain, that include also include PGRMC2, neudesin and neuferricin (Kimura et al., 2012). PGRMC1 is the only member of this family who has been reported to bind

progesterone (Meyer et al., 1996; Peluso et al., 2008, 2009). It was originally cloned in 1996 from porcine liver (Falkenstein et al., 1996) and is widely expressed in brain and up-regulated after brain injury, both in neurons and astrocytes (Meffre et al., 2005; Guennoun et al., 2008; Intlekofer and Petersen, 2011; Petersen et al., 2013). mRNA expression data support an important expression of PGRMC1 in discrete neuroendocrine nuclei and in hippocampal, cortical and cerebellar regions (Intlekofer and Petersen, 2011). It is also expressed in regions involved in the production and osmoregulation of the cerebrospinal fluid, suggesting it may play a role in progesterone beneficial effects on the blood-brain barrier after traumatic brain injury (Meffre et al., 2005).

The mechanism by which PGRMC1 mediates neuroprotective progesterone actions is not clear, but it may involve the up-regulation of BDNF, which is reported to promote neuronal survival (Gonzalez et al., 2005). Moreover, it is reported that in glial cell lines and primary astrocytes PGRMC1 activation is able to induce BDNF increased release through a Erk5 mediated mechanism (Su et al., 2012).

PGRMC1 expression has been found also in spinal cord neurons, in particular in the dorsal horn and ependymal cells lining in the central canal (Guennoun et al., 2015). It was reported to be up-regulated in dorsal horn after injury, pointing out to a possible protective role for PGRMC1 also at this level (Labombarda et al., 2003)

Even if progesterone binds PGRMC1 in membrane fractions (Falkenstein et al., 1999), there is no consensus about the effective ability of PGRMC1 to bind progesterone and originate intracellular responses *in vitro* or *in vivo* (Cahill, 2007; Price, 2013). PGRMC1 does not appear to function as a traditional receptor, requiring a binding partner known as serpine mRNA binding protein 1 (Serbp1,

Peluso, 2006). It was also suggested that PGRMC1 may represent the σ-2 receptor binding site (Xu et al., 2011). Allopregnanolone binding with PGRMC1 has not been analyzed yet (Cooke et al., 2013).

Recent data show that PGRMC1 can function as an adaptor protein, mediating mPRα translocation to the cell membrane, and that PGRMC1 actions may be therefore mediated by the formation of membrane progesterone receptor complex formed by PGRMC1 and mPRα, with the latter being responsible for progesterone binding and intracellular signaling (Thomas et al., 2014).

mPRs

mPRs are a group of progesterone binding receptors localized on the cell membrane, belonging to the progestin and adipoQ receptor (PAQR) family (Thomas et al., 2007, Tang et al., 2005). The first member of this family, mPRα (also known as PAQR7), was originally identified in spotted seatrout ovaries and was demonstrated to be a membrane steroid receptor, with a saturable, displaceable, single binding site specific for progestogens (Zhu et al., 2003b). Shortly after the first detection of this first receptor, it became clear that mPRα was member of a receptor family, including mPRβ or PAQR8 and mPRγ or PAQR5, and that homologous to these genes were present in several vertebrates, including humans (Zhu et al., 2003a). Subsequently two other members of this family, previously characterized only in yeast recombinant expression systems (Smith et al., 2008) were identified: mPRδ or PAQR6 and mPRε or PAQR9 (Pang et al., 2013).

They are metabotropic receptors with seven trans-membrane domains, coupled to G proteins. In particular, mPRα, β and γ seem to be coupled to an inhibitory $G_{i/0}$ protein (Zhu et al., 2003a, b; Thomas et al., 2007), while mPRδ and ε are coupled with stimulatory Gs proteins (Pang et al., 2013).

Studies conducted on the human mPRα have shown clearly that it has a distinct binding profile compared to PR, and specific mPR ligands (19α-methylprogesterone or Org OD 13-0 and 10-ethenyl-19-norprogesterone or Org OD 02-0) suitable for physiological analyses have been identified (Kelder et al., 2010). In the last years, different studies evaluated mPRs expression in different areas of the central nervous system (Zhu et al., 2003b; Labombarda et al., 2010; Intlekofer and Petrsen, 2011; Zuloaga et al., 2012; Pang et al., 2013, Meffre et al., 2013). A first study evaluated by in situ hybridization the mRNA expression of mPRα and β (plus PGRMC1, 2 and Serbp1) in female rats. The results of this study suggest that mPRα and β expression is low and homogeneous in the hypothalamus, being higher in thalamic and cortical brain regions, suggesting a possible role for them in the mediation of progestogen actions in cognitive and sensory functions (Intlekofer and Petrsen, 2011). However, a later study, performed using different techniques such as saturation binding, quantitative Real Time polymerase chain reaction (qRT-PCR), Western Blot and immunohistochemistry, has shown specific mPR progesterone binding in rat cortex, hypothalamus and pre-optic area obtained from female rat brain. qRT-PCR analysis has shown that mPRβ is more expressed than mPRα in all those areas, and subsequent immunohistochemistry analysis of mPRβ localization suggested that it particularly expressed in in select nuclei of the hypothalamus (paraventricular nucleus, ventromedial hypothalamus, and arcuate

nucleus), forebrain (medial septum and horizontal diagonal band), and midbrain (oculomotor and red nuclei) and throughout many areas of the cortex and thalamus (Zuloaga et al., 2012). Another study, that analyzed mPRα expression by *in situ* hybridization and immunofluorescence in mouse and rat brain, found that it is particularly expressed in olfactory bulb, striatum, cortex, thalamus, hypothalamus, septum, hippocampus and cerebellum, with a pattern similar to 3β-HSD, the enzyme that converts pregnenolone to progesterone. Interestingly, its expression resulted to be specific to neurons in physiological conditions, being absent in oligodendrocytes and astrocytes (Meffre et al., 2013). Lastly, all five mPR isoforms expression was evaluated in human brain by qRT-PCR (Table 1), shown that mPRδ is the most abundant mPR isoform in the human brain, in particular in corpus callosum and hypothalamus, being also higher than other isoforms in neocortex lobes, the limbic system (amygdala, hippocampus, and nucleus accumbens), thalamus, and in brain regions important for memory and movement (caudate, putamen), reward (substantia nigra), and autonomic functions (medulla and pons). mPRβ expression was high in similar regions compared to mPRδ, such as the corpus callosum, hippocampus, hypothalamus, substantia nigra and cerebellum. mPRα expression was lower in many regions compared to mPRδ, mPRβ and mPRε, with highest levels being detected in temporal lobe and medulla oblongata. mPRγ levels were lower than the other mPRs, with highest relative levels being expressed in choroid plexus and pons. mPRε highest relative levels were observed in the pituitary gland and hypothalamus, while relatively high expression levels were observed also in areas where mPRδ was highly expressed, such as the limbic system, the caudate, the nucleus accumbens, the pons and the olfactory bulb.

Interestingly, mPRδ, that seems to be a neuron-specific form, resulted to be the mPR isoform with the highest affinity for allopregnanolone, that is able to bind all mPR subtypes, even if with lesser affinity than progesterone (ranging from 4.00% of progesterone affinity for mPRε to 33.64 % for mPRδ, Pang et al., 2013).

mPRs expression was evaluated also in the spinal cord. mPRα, β and γ were identified by RT-PCR in mice spinal cord, while only α and β were detected also following *in situ* hybridization. This discrepancy may be due to low mPRγ levels. mPRα expression resulted to be constitutive and widespread, with no significant differences between males and females, and not being altered by PR knockout. *In situ* hybridization analysis showed its presence in ventral horn motor neurons and dorsal horn interneurons. Specific immunostaining in both gray and white matter revealed that almost all astrocytes and oligodendrocytes, as well as an important fraction of NG^+ precursor cells expressed mPRα. The same analysis showed that mPRβ, instead, presents a much more restricted distribution, being absent in the dorsal horn and glial cells (Labombarda et al., 2010). qRT-PCR studies in human spinal cord revealed that the most expressed isoforms are mPRδ and mPRβ, followed by mPRγ, mPRα and mPRε (Pang et al., 2013).

Several lines of evidence suggest that mPRs may be involved in the modulation of several progestogen neuroprotective actions in the nervous system. For example, a study conducted using the GT1-7 neuronal cell line model, which express mPRα and β (Sleiter et al., 2009) and very low level of mPRδ, has shown that both progesterone and allopregnanolone at 20 and 100 nM concentration were able to significantly reduce apoptosis in a serum-starvation cell death induced experiment (Thomas and Pang., 2012). A similar effect was observed in mPRδ transfected cells

and in hippocampal cell line, suggesting that mPRδ could contribute to this anti-apoptotic effect (Pang et al., 2013). Another study revealed that, even if mPRα is not expressed in rat brain by central glial cells in physiological conditions, its expression is induced after traumatic brain injury in oligodendrocytes, astrocytes and microglia, both in the lesion core and peri-lesioned area, suggesting it may have a role in glia-mediated neuroprotective action of progesterone (Meffre et al., 2013).

Aims

Nowadays, therapeutic approaches to peripheral neuropathies are, in the majority of cases, still aimed to symptomatic relief, with no specific drugs able to lead to full nerve regeneration. This is due to the fact that in most of the cases, the etiology and cellular mechanism of this pathologies are unknown. Therefore, the identification of new specific drugs or alternative approaches, relying on the deep knowledge of the mechanisms regulating the peripheral nervous system, represents a strong and actual medical need.

Among the many different mediators which have been reported to have a role in the development and, possibly, on the cure of peripheral neuropathies, the laboratory in which I carried out my PhD studies has always focused on the role of the GABAergic system and neuroactive steroids.

Therefore, the aim of my PhD project was to deepen the study on the GABAergic system and neuroactive steroids, in particular progestogens, in order to unravel their role in peripheral neuropathies and neuropathic pain. Moreover, our studies were aimed at the identification of new specific drugs for the treatment of neuropathies.

The studies conducted can be divided into four research lines:

- Study of the role of the GABAergic system, in particular through the evaluation of the effects of specific GABA-B ligands, on nerve regeneration in an experimental model of nerve injury.
- Study of the cross-correlations between the GABA-A and GABA-B receptors in a conditional knockout mice model.
- Study of the interaction among allopregnanolone, GABA-A receptor and PKCε in Schwann cell and primary sensory neuronal cultures.

- Characterization of the expression of a novel class of membrane progesterone receptors (mPRs and PGRMC1) in Schwann cells and evaluation of their potential role in the modulation of their physiology.

Materials and Methods

ANIMALS

For the experiments described in the first part of chapter 1 and in chapter 2 of the results section, male Sprague-Dawley rats were used. They were purchased from an authorized breeder (Charles River, Calco, Italy) at the age of 3-4 months old and used for experimental procedures shortly after.

For the experiments described in the second part of chapter 1, GABA-B1$^{fl/fl}$ mice (Haller et al., 2004) were crossed with P0-CRE deleter mice (Feltri et al., 1999) to obtain P0-CRE_GABA-B1$^{fl/wt}$ mice, which were then back-crossed with GABA-B1$^{fl/fl}$ mice. The resulting P0-CRE_GABA-B1$^{fl/fl}$ mice, with specific deletion of GABA-B1 in Schwann cells, were chosen as experimental group, and were compared with GABA-B1$^{fl/fl}$ mice chosen as control (Faroni et al., 2014). Experiments were conducted on 3 months old animals.

All animals were bred in the animal facility of the Department of Pharmacological and Biomolecular Sciences of the University of Milan. They had access to water and food *ad libitum* and were maintained in a 12 hours of light/12 hours of dark daily cycle. All the experiments were performed according to the guidelines of the Animal Research Committee of our Institution and in compliance with the policy on the use of animals approved by the European Community Council Directive (2010/63/EC) and the Italian law on the use of animals for scientific studies (D.Lgs. 26/2014). The study was approved by the Animal Care and Use Committee of the University of Milan.

GENOTYPING

For the experiments described in the second part of chapter 1, animals genotype was determined by PCR analysis on genomic DNA, extracted from tails, sciatic nerves and liver using microLYSIS®–Plus (Microzone Limited, Haywards Heath, UK) following manufacturer's protocol. Used primers are reported in Table 1. PCR reaction with CRE-1 and CRE-2 primers on mice tails extracts allowed to determine which mice expressed the CRE recombinase; reactions with P5 and P6 on mice tails extracts allowed to determine which mice presented inserted sequences in the GABA-B1 gene; lastly, *post-mortem* reactions with P7 and P8 on sciatic nerve and liver extracts allowed to verify the GABA-B1 exon VII and VIII specific deletion in Schwann cells (Faroni et al., 2014). PCR was performed in a standard reaction mix under the following conditions (30 cycles): 94°C for 30 s; 56°C for 1 min; 72°C for 1 min. A final extension step at 72°C for 10 min was performed. The amplified products were analyzed on 1.5% agarose gels.

CELL CULTURES

For the experiments described in chapter 1 and 2, Schwann cells and DRG neurons primary cultures were used.

Schwann cell cultures were obtained from sciatic nerves of rats and mice. Non-nervous tissue was removed, then the nerve was cut in fragments (2–3 mm each), and placed in a 35 mm petri dish with the culture medium: DMEM (Dulbecco's

modified Eagle's medium) plus 10% fetal bovine serum (FBS; Life Technologies Italy, Monza, Italy) and 2 μM forskolin (Sigma-Aldrich Italy, Milan, Italy).

Primer name	5'-3' sequence
CRE-1	CCACCACCTCTCCATTGCAC
CRE-2	GCTGGCCCAAATGTTCGTGG
P5	TGGGGTGTGTCCTACATGCAGCGGACGG
P6	GCTCTTCACCTTTCAACCCAGCCTCAGGCAGGC
P7	ATCTCTTCCTTGGGCT GGGTCTTTGCTTCGCTCG
P8	GGGTTATTTGAATATGATCGGAATTCCTCGACT

Table 1 – Primer sequences used for genotyping.

For mice cultures, 2% insulin growth factor (Sigma-Aldrich Italy) was added. After two weeks, the sciatic nerves were digested with 0.0625% (w/v) collagenase Type IV (Worthington Biochemical, Lakewood, NJ) and dispase (0.5 mg/mL; Life Technologies Italy), then mechanically dissociated, filtered through 100 μm filters (BD Biosciences, San José, CA) and centrifuged 5 min at 1100 rpm. Pellets were re-suspended in the culture medium, and then the cell suspension was seeded on 60 mm petri dishes coated with Poly-L-lysine and laminin (Sigma-Aldrich Italy) and grown in presence of 2 μM forskolin at 37°C, 5% CO2, and 95% humidity.

DRG neuronal cultures were obtained from the spinal cord of mice and rats. The DRG were dissociated by 40 minutes incubation in Ham's F12 medium (Life Technologies Italy) containing 0.125% (w/v) collagenase Type IV (Worthington Biochemical), then treated for 30 min with 0.25% (w/v) trypsin (Worthington Biochemical). The dissociated DRG neurons were filtered through a 100 μm membrane (BD Biosciences) and centrifuged 5 min at 500 rpm. Cells were then re-

suspended in Ham's F12 and purified on a gradient of 15% (w/v) bovine serum albumin (BSA; Sigma-Aldrich Italy). Dissociated neurons were re-suspended in modified Bottenstein and Sato's medium (BSM; F12 medium plus 100 μM putrescine, 30 nM sodium selenite, 20 nM progesterone, 1 mg/mL BSA, 0.1 mg/mL transferrin, and 10 pM insulin, all Sigma-Aldrich Italy). A cell suspension of DRG neurons was seeded on 35 mm petri dishes coated with poly-L-lysine and laminin (Sigma-Aldrich Italy). After 2 h, NGF 50 ng/mL was added and the cells grown at 37°C, 5% CO2, and 95% humidity.

For the experiments described in chapter 3, a Schwann cell line model was used. S42 (ATCC® CRL2942™) Schwann cells (ATCC, Manassas, VA) were thawed and re-suspended in the culture medium: DMEM (Sigma-Aldrich, St. Louis, MO) plus 10% FBS (Thermo Fisher Scientific, Waltham, MA) and seeded in T75 cell culture flasks. Before experimental procedures, cells where differentiated for 1-2 days by adding 10 μM forskolin (Sigma-Aldrich) to the culture medium.

SURGICAL PROCEDURE

For the experiments described in the first part of chapter 1, rats were anesthetized by an intraperitoneal injection of a mixed solution of ketamine (80mg/Kg) and xylazine (5mg/Kg). After that, animals underwent surgical procedure following the procedure of the partial sciatic ligation (PSL) model, as previously described (Seltzer et al., 1990). An incision was made through skin and muscle of the right hind limb in order to expose the nerve. For every group, except the sham operated one, the nerve was ligated with a node made using a suture (Vicryl 3-0 2) which

crossed the nerve at half or 1/3 of its diameter, 1 cm proximally to the forking of the sciatic nerve into the peroneal and tibial branches.

IN VIVO PHARMACOLOGICAL TREATMENTS

In each experiment described in the first part of chapter 1, 4 rats were used as sham operated animals (control group), while 16 rats underwent PSL. PSL rats were divided into 4 experimental groups for treatments: (1) PSL + vehicle (saline solution); (2) PSL + baclofen (Bac in graphics, 10mg/kg); (3) PSL + CGP56433 (CGP in graphics, 3mg/kg); (4) PSL + baclofen + CGP56433. The pharmacological treatments started the day of PSL surgery (day 0) and lasted 7 days, consisting in drugs intraperitoneal injection every 12 hours. All drugs were kindly provided by Dr. Klemens Kaupmann, Novartis Pharma AG, Basel, Switzerland.

IN VITRO PHARMACOLOGICAL TREATMENTS

For the experiments described in chapter 2, cells were incubated overnight in serum-free medium (DMEM only for Schwann cells, F12 only for DRG neurons), and were then treated with allopregnanolone (Sigma-Aldrich Italy) for 24 hours at a final concentration of 1 μM, diluted in the same serum-free medium. For the conditioned medium experiments, Schwann cell primary cultures where incubated overnight in serum-free medium and then treated with allopregnanolone for 24

hours. Their culture medium was therefore aspirated and transferred onto DRG neurons, that were previously incubated overnight in serum-free medium.

For the experiments described in chapter 3, S42 Schwann cells were incubated overnight in serum-free medium and then treated with the following drugs: 10-ethenyl-19-norprogesterone (O2, Organon, Oss, The Netherlands), progesterone (P4 in graphics, Steraloids, Newpot, RI) and promegestone (R5020, GE Healthcare, Piscataway, NJ), all at the final concentration of 20 nM.

WALKING TEST

For the experiments described in chapter 1, the walking footprint test was performed on all rats of each experimental group, at the end of pharmacological treatments. Rats with their hind feet stained with black ink were placed on a 100 cm long gangway, which yielded at least 10 footprints per rat. Rats did not undergo any training before the test. Footprints were acquired and analyzed with the Footprint 1.22 software (Klapdor et al., 1997). The parameters analyzed were the area touched by a single step (in cm^2), the toe 1–5 and 2–4 spreads (in cm), the length of the foot step (in cm), the stride width and the stride length (in cm). Data of each parameter are mean of 8–10 footprints of each animal.

RNASE PROTECTION ASSAY (RPA)

For the experiments described in chapter 1, total RNA was extracted from sciatic nerves by phenol-chloroform extraction and quantified using the Nanodrop

(Thermo-Fisher Scientific). 5 μg of each sample were dissolved in 20 μL of hybridization solution containing [^{32}P]-labeled cRNA probes, 250,000 counts per minute (CPM) for P0 and PMP22 and 50,000 CPM for 18S, and incubated at 45°C overnight. The following day, the samples were incubated 30min at 30°C with 200µL of digestion buffer containing 1 µg/µL RNase A and 20 U/µL RNase T1 (Sigma-Aldrich, Milan, Italy) then treated for 15 min at 37°C with 10µg of proteinase K and sodium dodecyl sulfate (SDS) 20%. After phenol-chloroform extraction, the samples were separated on a 5% polyacrylamide gel, under denaturing conditions (7 M urea). The protected fragments were visualized by autoradiography. The levels of P0 and PMP22 and 18S rRNA were calculated by measuring the peak densitometric area with Image J software (NIH, Bethesda, MD) and data were normalized on 18S rRNA. In order to ensure that the auto radiographic bands were in the linear range of intensity, different exposure times were used. The mean values of the controls from different experiments were within 10% of each other.

qRT-PCR AND RETRO TRANSCRIPTION-PCR (RT-PCR)

For all the qRT-PCR experiments described, total RNA was extracted from samples using TRI reagent (Sigma-Aldrich), following manufacturer's instructions, and quantified using the Nanodrop. Samples were treated with the TURBO DNA-free™ kit (Thermo-Fisher Scientific), in order to remove any DNA residual. RNA was retro-transcribed to cDNA using iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA). 10 ng of cDNA for each sample were then used

for Real Time PCR. Every sample was run in triplicate. For the experiments described in chapter 1 and 2, Real Time PCR was performed using a CFX96 touch thermal cycler (Bio-Rad); for the ones described in chapter 3, instead, it was performed with a Mastercycler® RealPlex 2 (Eppendorf North America, Hauppauge, NY). In both cases, SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) was used. Data analysis was performed according to the Pfaffl method (Pfaffl, 2001), and results are expressed as relative expression normalized on the geometric mean of a set of housekeeper genes (Table 2). Primers for qRT-PCR were designed using the PrimerBlast software (NIH, Ye et al., 2012), with the exception of the ones for PKCε, who were obtained from literature (Li et al., 2006, Table 2). For the RT-PCR experiments described in chapter 3, total RNA extracted from cells as described above for qRT-PCR was reverse transcribed into cDNA using SuperScript III (Thermo-Fisher Scientific), following mafucaturer's protocol. The obtained cDNA was used as a template for PCR, which was performed in a standard reaction mix. Used primers are reported in Table 2. The cycling profile was the following: 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min for 30 cycles, followed by a 72°C, 10 min extension step. The amplified products were analyzed on 1.5% agarose gels.

Primer name (Accession number)	5'-3' sequence
TARGET GENES	
PKCε-F (NM017171.1)	CCCCTTGTGACCAGGAACTA
PKCε-R	AGCTGGCCATCAGTAGACGA
RAα2-F (NM008066.3)	AGACCAGGACTGGGAGACAGTA
RAα2-R	AGACAGGGCCAAAACTGGTCA
RAα3-F (NM008067.4)	GCTTAGCCTCCAACTTGTTTCTCC
RAα3-R	GAAGAGACCTGTGAGATCGAGTGT
RAα4-F (NM010251.2)	TCCTGGATTTGGGGGTCCTGTTA
RAα4-R	TCAACATCAGAAACGGGCCCAA

RAα5-F (NM176942.4)	CCAGTCACTTTGGCTTTTCGCA
RAα5-R	AGGCCGCAGTCTGTTGTCAT
RAβ1-F (NM008069.4)	TTGGGGCTTCTCTCTTTTCCCG
RAβ1-R	TGCTGGGTTCATTGGAGCTGT
RAβ2-F (NM008070.3)	TGCTGGGTTCATTGGAGCTGT
RAβ2-R	CGCACGGCGTACCAAAACAT
RAβ3-F (NM008071.3)	TGTCACTGGCGTGGAAAGGA
RAβ3-R	ATAGGCACCTGTGGCGAAGA
RAγ2-F (NM177408.6)	CATGGAGCATTGGAAGCTCAGTC
RAγ2-R	TGTGAAGCCTGGGTAGAGCGAT
Raδ-F (NM008072.2)	TGCCCACTTCAATGCCGACT
Raδ-R	CATCCATCTCTGCCCTTGGCTT
mPRα-F (NM001034081.1)	GTGCACCGCATCATAGTGTC
mPRα-R	TGATAGTCCAGCGTCACAGC
mPRβ-F (NM001014099.1)	CTGCAGCCTCTTGGCCCACC
mPRβ-R	CAGCCGCCGGCAGGAAGAAA
mPRγ-F (NM001014092.1)	TACATTGGCCACAGTCACCAGC
mPRγ-R	AGCCAGCACCACATCAACGA
mPRδ-F (NM001191077.1)	GCAAACAGGTCAACGTGGAGGTA
mPRδ-R	ACCAAGTGGGCAGGAAGTGA
mPRε-F (NM198414.2)	TGGGCACAAAGACGCAAACC
mPRε-R	TGACACTCACAGGCCTTCACA
PGRMC1-F (NM021766.1)	TGACCAAAGGCCGCAAGTTCT
PGRMC1-R	CCACGTGATGGTACTTGAAGGTGA
P0-F (NM017027.2)	CCTGCTCTTCTCTTCTTTGGTGCT
P0-R	GGTTGACCCTTGGCATAGTGGA
MAG-F (NM017190.4)	GCCAGACCATCCAACCTTCTGT
MAG-R	AAGTCGAAACGGCAGGGGAT
GFAP-F (NM017009.2)	ACGTTAAGCTAGCCCTGGACAT
GFAP-R	ACTCCTTAATGACCTCGCCATCC
PMP22-F (NM017037.1)	CCCTGGCTCTCGATTGCAAAGAA
PMP22-R	CTGAAGCCATTCGCTCACAGATG
HOUSEKEEPER GENES	
α-tubulin-F (m+r) (NM_022298.1; NM011653.2)	TCGCGCTGTAAGAAGCAACACC
α-tubulin-R (r)	GGAGATACACTCACGCATGGTTGC
α-tubulin-R (m)	ATGGAGATGCACTCACGCATGG
β-Actin-F (r) (NM031144.3)	ACAGCTGAGAGGGAAATCGTGC

β-Actin-R (r)	CCACAGGATTCCATACCCAGGAAG
β2-microglobulin-F (m) (NM009735.3)	CCCTGGTCTTTCTGGTGCTTGT
β2-microglobulin-R (m)	ATGTTCGGCTTCCCATTCTCCG
β2-microglobulin-F (r) (NM012512.2)	GCTTGCCATTCAGAAAACTCCCC
β2-microglobulin-R (r)	TGACGGTTTTGGGCTCCTTCA

Table 2 – Primer sequences used for qRT-PCR and RT-PCR (F: forward; R: reverse; RA: GABA-A receptor subunit; m: mouse; r: rat). Accession numbers are reported alongside forward primers.

PROTEIN EXTRACTION AND PLASMA MEMBRANE PREPARATION

For some the experiments described in chapter 3, cells were collected in ice-cold HAED buffer (25 mM HEPES, 10 mM NaCl, 1 mM dithioerythritol, and 1 mM EDTA) at pH 7.6, containing 1% protease inhibitor cocktail (Pierce, Rockford, IL). Cells were then homogenized using a sonicator, and the homogenate was centrifuged for 7 min at 1000 x g. The supernatant was collected as protein sample. For some experiments, this step was followed by centrifugation of the supernatant at 20,000 x g for 20 min to pellet plasma membranes, that were then re-suspended in HAED buffer. For some studies, the plasma membrane was furtherly purified by centrifuging it with a sucrose pad (1.2 M sucrose) at 6,900 x g for 45 min. In this case, the membrane layer on the sucrose pad was collected and centrifuged again at 20,000 x g for 20 minutes.

WESTERN BLOT

For the experiments described in chapter 2 and 3, protein samples were heated for 20 minutes at 55°C or for 1 min at 100°C to denaturate their secondary structure, then 10-15 μg were loaded onto a SDS-PAGE gel and run at 200 V for 40 min in

running buffer. Gels were then electro-blotted to Hybond nitrocellulose membrane (GE Healthcare). Membranes were blocked with 5% not-fat dry milk (Bio-Rad) in PBS before incubation with the primary antibody diluted in the blocking solution. Primary antibodies used were rabbit anti PKCε (1: 200, Abcam, Cambridge, UK), rabbit anti-mPRα (1: 1,000, Sigma-Genosys, Woodlands, TX), rabbit anti-mPRβ (1: 1,000, Sigma-Genosys), rabbit anti-mPRγ (1: 1,000, Sigma-Genosys), rabbit anti-mPRδ (1:200, Sigma-Aldrich), rabbit anti-mPRε (1:1,000, Sigma-Genosys), goat anti-PGRMC1 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-MAG (1: 100, EMD Millipore, Billerica, MA) and mouse anti-Actin (1:1,000, Abcam) as loading control. In some cases, membranes were incubated with appropriated HRP-conjugated secondary antibodies (diluted 1:8,000-1:10,000, Millipore, Temecula, CA), and immune-complexes were revealed by enhanced chemiluminescence (ECL; Thermo-Fisher Scientific) and detected with photographic film (Nikon, Tokyo, Japan) or the ChemiDoc™ XRS^{+} system (Bio-Rad). In other cases, specific secondary antibodies compatible with the Odyssey® CLx system (LI-COR, Lincoln, NE) were used, and their IR emission was detected.

MORPHOMETRIC ANALYSIS

For the experiments described in chapter 1, rats were perfused transcardially, under deep anesthesia, with 2% PFA and 2% glutaraldehyde solution in 0.1 M sodium cacodylate buffer (all Sigma-Aldrich Italy) at pH 7.3. After fixation, sciatic nerves were removed and immersed in the same fixative solution overnight at 4°C. The specimens were then post-fixed in 2% OsO_4 (Sigma-Aldrich Italy), dehydrated, and

embedded in Epon-Araldite (Sigma-Aldrich Italy). Semithin (0.5 μm) transverse sections were stained with toluidine blue and analyzed with an Axioskop 200 microscope (Zeiss, Gottingen, Germany), at final 1500x magnification. Morphological alterations of the peripheral nervous system were assessed qualitatively and quantitatively. 5 sections for each animal's nerve were analyzed, and at least 25 fields for each section, corresponding to 25% of the total nerve area, were acquired (Mayhew and Sharma, 1984). Then number of myelinated fiber per fields was then counted.

ELECTRON MICROSCOPY

For the experiments described in chapter 1, ultra-thin sections (60–90 nm) for ultrastructural observations were obtained from each sciatic nerve used for light microscopy. Sections were collected on formvar film coated grids with a single hole, counterstained with lead citrate, and examined with a EM10 electron microscope (Zeiss).

IMMUNOFLUORESCENCE

For the experiments described in chapter 1, sciatic nerves were fixed in a 4 % paraformaldehyde (PFA) solution in phosphate buffer saline (PBS, all Sigma-Aldrich Italy). Fixed nerves were then dehydrated through subsequent incubation in sucrose solution (7.5%, 15% and 30% m/v) and included in O.C.T. (Tissue-Tek, Torrance, CA). 10 μm thick transverse frozen sections were cut with a cryostat

microtome. Nerve sections were incubated 30 min at room temperature in presence of Fluoromyelin red (Life Technologies Italy, Monza, Italy), diluted 1:200 in a buffer constituted by 0.25% m/v bovine serum albumin (BSA), 0.1% m/v Triton-X-100 in PBS. Slides were then incubated overnight at 4°C with an anti-CD68 primary antibody produced in mouse (Abcam) diluted 1:200 in the same buffer. The following day, slides were washed three times in PBS and incubated 2 h with the specific goat anti-mouse FITC-antibody (Sigma-Aldrich Italy), diluted 1:200 in the buffer described above. After washing, slides were mounted using Vectashield^{TM} (Vector Laboratories, Burlingame, CA), which contains 4',6-diamidino-2-phenylindole (DAPI). Controls for specificity included a lack of primary antibody. Confocal microscopy was carried out using a LSM 510 microscope (Zeiss).

For the experiments described in chapter 2 and 3, cells were grown on poly-L-lysine coated coverslips, washed with PBS and then fixed with 4% PFA. They were blocked for 1 hour using a 0.25% m/v BSA solution in PBS and then incubated overnight with the following primary antibodies, all produced in rabbit, diluted at the following concentrations in a BSA 0.1% m/v solution in PBS: pPKCε (Abcam), mPRα (Sigma-Genosys) and mPRβ (Sigma-Genosys) were diluted 1:200, mPRδ (Sigma-Aldrich) was diluted 1:100. Coverslips were washed three times in PBS and cells were incubated with the specific secondary antibody goat anti-rabbit Alexa 488 (Life Technologies, Carlsband CA) diluted 1:500. After washing, coverslips were mounted using Vectashield^{TM}. Controls for specificity included a lack of primary antibody. For the experiments described in chapter 2, the same confocal microscope mentioned above was used. For the fluorescence microscopy

experiments described in chapter 3, an Eclipse TE2000-U (Nikon) light microscope was used.

BINDING ASSAY

For the experiments describe in chapter 3, specific mPR binding to plasma membranes was assessed. Two-point competitive binding assays were conducted in triplicate, with plasma membranes (prepared as described above) being incubated for 30 min at 4° with cold progesterone, O2 and R5020 (at 10 μM and 1 μM concentrations), and 4 nM [^{3}H]-progesterone. At the same time, they were also incubated with 4 nM [^{3}H]-progesterone and vehicle (ethanol) to determine total binding. Bound [^{3}H]-progesterone was separated from free by rapid filtration over Whatman GF/B filters using a 36-well cell harvester (Brandel, Gaithersburg, MD) and radioactivity bound to the filters counted in a liquid scintillation counter. Data were expressed as displacement, that is the percentage of the total binding observed in samples with cold compounds.

IN VITRO WOUND HEALING ASSAY

For the experiments described in chapter 3, the wound healing assay (Liang et al., 2007) was performed. Cells were plated on 12-wells multiwell plates and cultured overnight in serum-free condition as described above. The following day, the wound was created making a scratch on the bottom of the multiwell plate with 200 μL pipette tip and cells were treated with drugs as described above. Cells were then

photographed in phase contrast with the Eclipse TE2000-U microscope (Nikon) at different time points: 2, 4, 6, 8 and 24 hours after the scratch. The distances between cell fronts were measured with the Image J software (NIH), considering at least nine measurements from the top to the bottom of the well. Data are expressed as migrated distance, that is the difference between the initial gap between cell fronts and the same distance at a given time point.

DATA ANALYSIS AND STATISTICS

All the described experiments were repeated at least three times.

Data were statistically evaluated using Prism 4.0 (GraphPad, San Diego, CA) and Systat 12 (Systat Inc., Chicago, IL) softwares. Significance was determined, depending on the experimental design, by two-tailed unpaired t-test, one-way ANOVA or two-way ANOVA. When one-way ANOVA was used, single conditions were then compared among them with Tukey's post-hoc test. When two-way ANOVA was used, single conditions were compared among them with Bonferroni's post-hoc test. P values < 0.05 were considered significant. All results are expressed as mean ± standard error of the mean (SEM).

Results and Discussion

CHAPTER 1

Modulation of the GABAergic system as a strategy to promote nerve regeneration and cross-modulation of GABA receptors in the peripheral nervous system

PART I - GABA-B LIGANDS AS POTENTIAL PHARMACOLOGICAL APPROACH FOR NERVE REGENERATION

In order to study the effects of GABA-B ligands as a pharmacological tool to promote nerve regeneration after peripheral nerve lesion, we used the experimental model of PSL. This is a consolidated model of neuropathic pain. Indeed, the model mimics many typical signs (such as the rapid onset of allodynia) of the complex regional pain syndrome type II (CRPS-II) in humans, that is a chronic pain condition associated with nerve injuries (Seltzer et al., 1990). Another interesting advantage of the model is that it induces a denervation process that seems to affect in the same way axons of all sizes (Bennett et al., 2003). The morphological consequences of PSL have never been studied in detail in previous studies.

The sciatic nerve was exposed and then ligated with a suture that crossed the nerve approximately at 1/3 of its diameter. Morphological evaluation performed after seven days revealed that sham operated animals had a normal nerve morphology,

while PSL animals presented typical signs of myelin disruption and axonal atrophy (Figure 13).

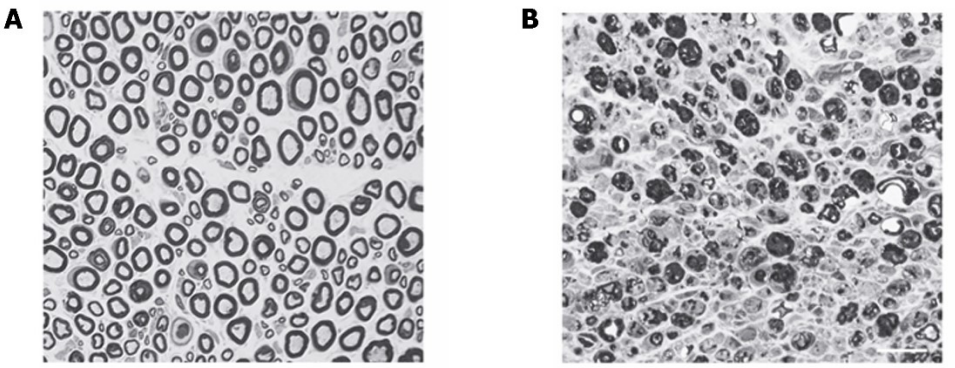


Figure 13 – Morphological evaluation of sham and PSL animals' sciatic nerves coronal sections 7 days after surgery. While sham animals (A) present normal nerve morphology, PSL animals (B) show clear signs of axonal and myelin degeneration.

Then, we evaluated the effects of pharmacological treatments on PSL animals morphology. We counted the number of myelinated fibers per field, as an index of nerve integrity. As shown in figure 14, 7-day treatment with the specific GABA-B agonist baclofen had no effect on the number of myelinated fibers. GABA-B specific antagonist CGP56433 was able to significantly up-regulate myelinated fibers per field. Surprisingly, the co-administration of the two drugs had an additive effect, increasing myelinated fibers number more CGP56433 alone (Figure 14). The positive effect of the co-treatment on nerve morphology was confirmed also by electron microscopy analysis, which revealed the presence of normal myelinated large fibers and small thinly myelinated regenerating ones (Figure 15).

In order to understand if the morphological changes observed could be ascribable to a direct effect on Schwann cells, we also analyzed the gene expression of important myelin markers.

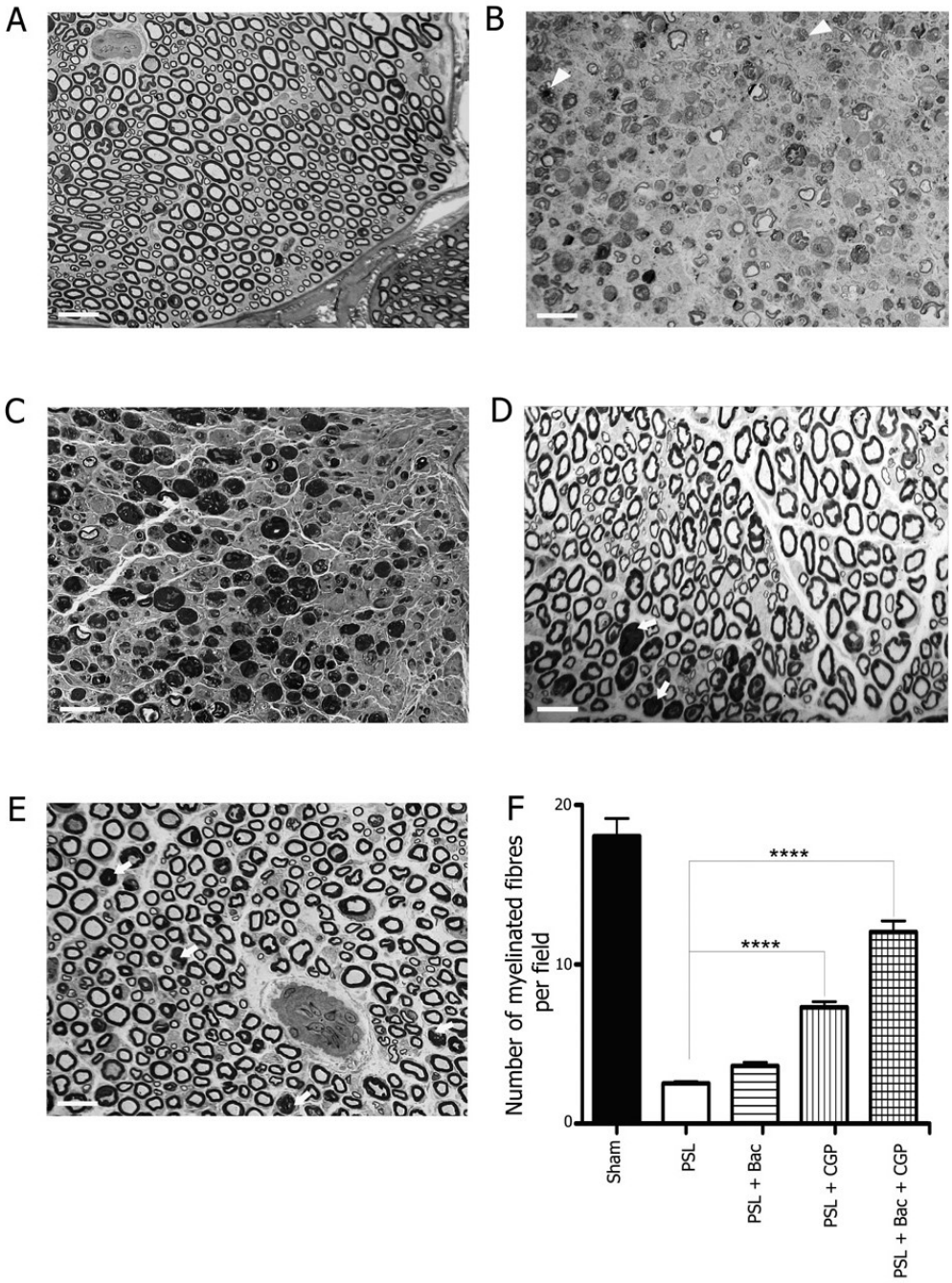


Figure 14 – Morphological evaluation of sciatic nerves coronal sections after 7 days of treatment. Macrophages are evidenced by arrowheads. A: sham; B: PSL; C: PSL + Bac; D: PSL + CGP56433; E: PSL + Bac + CGP56433; F: number of myelinated fibers per field in each condition. PSL induces a dramatic reduction of the number of myelinated fibers, and baclofen alone does not affect their number. CGP56433 alone significantly increase the number of myelinated fibers, and the co-treatment show an additive effect. Scale bar: 20 μm. ****: $p < 0.0001$.

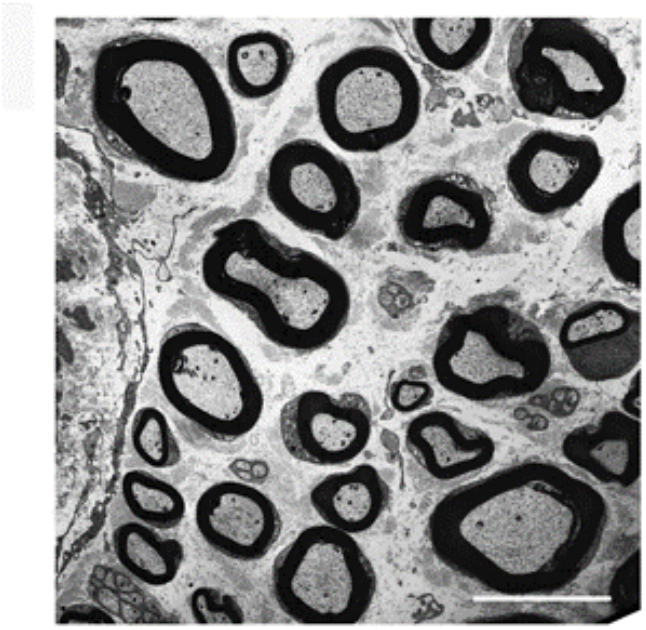


Figure 15 – Electron microscopy image of a PSL + Bac + CGP sciatic nerve coronal section. Normal myelinated large fibers and small thinly myelinated regenerating fibers are shown. Scale bar: 10 μm.

In particular, we assessed the expression levels of P0 and PMP22 glycoproteins by RPA. Both glycoproteins are important constituents of the myelin sheath, required in particular for the stabilization of compact myelin, whose quantitative changes may alter Schwann cell development, growth, and differentiation (Suter and Scherer, 2003; Salzer, 2015). Expression of both proteins was significantly down-regulated in PSL-operated rats. Baclofen treatment alone did not prove able to lead to any change in their expression. Interestingly, CGP56433 treatment increased P0 and PMP22 expression to a level similar to Sham operated animals, while the co-treatment showed only a partial effect, with expression levels that are intermediate between CGP56433 treatment and PSL (Figure 16).

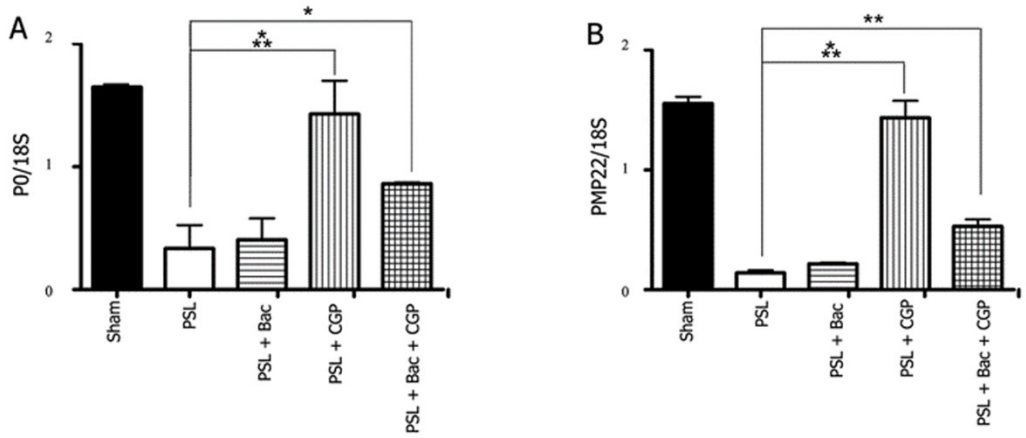


Figure 16 – RPA evaluation of P0 (A) and PMP22 (B) mRNA expression. In both cases, CGP56433 induces a significant up-regulation of both myelin proteins RNA. Baclofen has no effect, and the co-treatment shows only a partial effect. *: $p < 0.05$; **: $p < 0.01$; ***: $p< 0.001$.

We also evaluated motor functionality using the gait test, in order to understand if the observed improvements in nerve morphology could correlate with positive functional outcomes. Rats were allowed to walk in a corridor leaving a trail of footprints. The following parameters were analyzed: stride length and width, step length and area, distance between 1–5 and 2–4 toes. As shown in table 3, most parameters resulted to be significantly altered in PSL animals compared to Sham ones, confirming that the partial ligation led to a deficit of the motor function. Baclofen treatment alone showed different effects on different parameters, showing no effect on some and worsening (such as the 2-4 toes distance) or ameliorating (such as stride width) others. Treatment with CGP56433, alone or in co-treatment with baclofen, proved able to reverse most of PSL-induced changes. In particular, the co-administration of the two drugs seems to be the most effective pharmacological treatment, being able to induce functional recovery, especially for

1-5 and 2-4 toes distance, leading to values similar to Sham operated animals (Figure 17).

	Distance 1-5 toe		Distance 2-4 toe		Length foot step		Stride width		Stride length		Area touched	
Sham	1,95	± 0,03	1,06	± 0,03	3,21	± 0,09	3,64	± 0,06	11,46	± 0,49	1,79	± 0,08
PSL	0,76	± 0,04	0,5	± 0,03	4,39	± 0,13	4,59	± 0,11	13,32	± 0,54	1,9	± 0,17
	p =0.000116		p =0.000131		p =0.000116		p =0.000119		p =0.404063		p =0.968606	
PSL + Baclofen	0,7	± 0,05	0,35	± 0,03	4,1	± 0,18	3,85	± 0,15	9,6	± 0,71	1,06	± 0,14
	p =0.921960		p =0.547398		p =0.648819		p =0.002619		p =0.018476		p =0.000914	
PSL + CGP56433	1,42	± 0,05	0,86	± 0,03	2,61	± 0,12	3,28	± 0,11	9,29	± 0,64	1,13	± 0,20
	p =0.000116		p =0.000135		p =0.000113		p =0.000117		p =0.018430		p =0.005125	
PSL + Baclofen + CGP56433	1,58	± 0,09	0,98	± 0,04	3,51	± 0,12	3,63	± 0,15	10,16	± 0,93	1,51	± 0,11
	p =0.000116		p =0.000131		p =0.000466		p =0.000129		p =0.056044		p =0.289398	

Table 3 – PSL and pharmacological treatments effects on walking parameters. PSL was able to alter different parameters. Pharmacological treatments were able to change most of them. The co-treatment, in particular, among other pharmacological treatments seems to restore different parameters to sham level.

Lastly, we evaluated the neuro-inflammatory response in the experimental model, assessing the presence of immunopositivity for CD68 as a marker of activated macrophages. Observing myelin structure, stained in red using Fluoromyelin, it is possible to see how PSL animals show altered myelination, that is recovered in animals treated with CGP56433 and with the co-administration of the two drugs, furtherly confirming morphological data. Observing CD68 immunopositivity in green, instead, it is clear how in sham animals there is no clear sign of macrophage activation in the nerve (Figure 18).

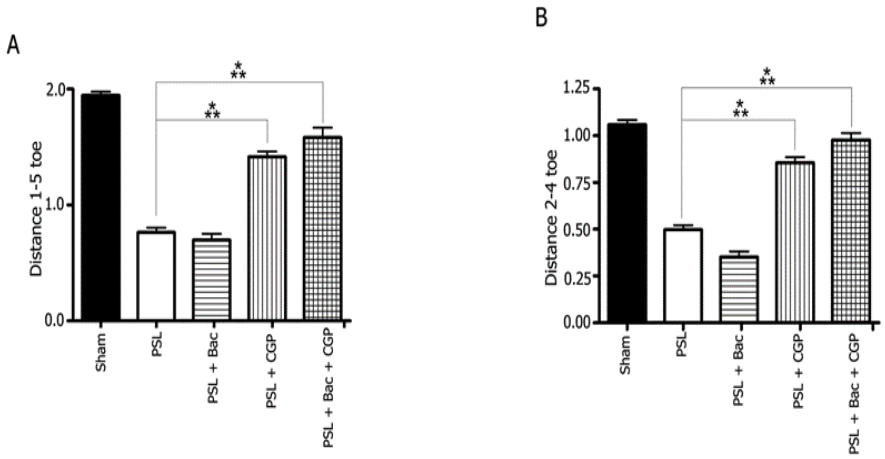


Figure 17 – 1-5 (A) and 2-4 (B) toe distance. PSL significantly reduces both parameters, indicating motor impairment. Baclofen alone has no effect, while CGP56433 alone proved able to restore both parameters to a level closer to the sham group. Again, the co-treatment showed a stronger recovery than CGP56433 alone, suggesting an additive effect. ***: $p < 0.001$.

In PSL and baclofen treated animals, instead, clear signs of macrophage infiltration are present, with merged images showing infiltrated macrophages inside the myelin structure, suggesting a general increase in neuro-inflammation. CD68 immunopositivity resulted to not be present in CGP56433 animals, both in single treated and co-treated ones, suggesting that only the GABA-B antagonist may be able to reduce neuro-inflammation following peripheral nerve injury (Figure 18).

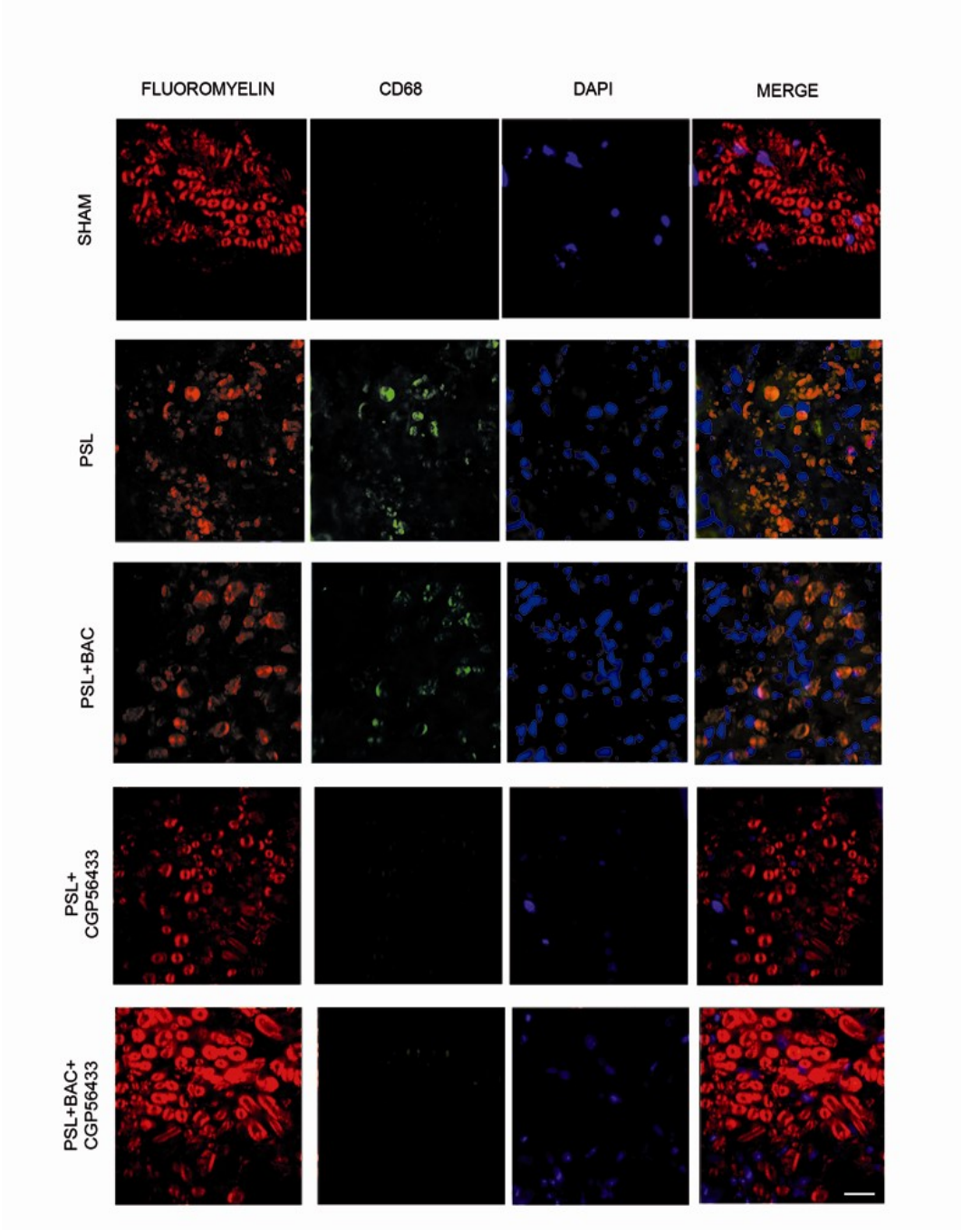


Figure 18 - Immunofluorescence images of sciatic nerves coronal sections. Myelin structures are evidenced in red with Fluoromyelin. Macrophages specific marker CD68 is in green. Nuclei are stained in blue with DAPI. PSL causes an inflammatory response, as evidenced by CD68 activation. Baclofen has no effect on it, while CGP56433 proved able to counteract it. Scale bar: 20 µm.

PART II - GABA-A SUBUNITS MODULATION IN GABA-B1 KNOCKOUT MICE PERIPHERAL NERVOUS SYSTEM

Different lines of evidence suggest that GABA-A and GABA-B receptors may be involved in an important functional cross-talk in the peripheral nervous system, and in particular in Schwann cells (recently reviewed in Faroni and Magnaghi, 2011). GABA-A activation, for example, proved able to exert an effect on GABA-B expression and responsiveness (Magnaghi et al., 2006, 2010). On the other hand, GABA-B specific deletion in Schwann cells had an important effect in conditional knockout (P0-CRE_GABA-B1$^{fl/fl}$) animals, inducing biochemical, functional and morphological modifications, also with alterations in pain sensitivity (Faroni et al., 2014).

Therefore, in order to better characterize the experimental model, and to deepen our knowledge on the interactions between the two receptors in the peripheral nervous system and on non-autonomous effects exerted by GABA-B deletion in Schwann cells, we analyzed (by qRT-PCR) GABA-A subunits mRNA expression in the DRG total tissue harvested from P0-CRE_GABA-B1$^{fl/fl}$ mice and in DRG neurons primary cultures obtained from the same animals.

The first step was the evaluation of the basal expression of selected GABA-A subunits in control mice, analyzing also sciatic nerves and Schwann cell primary cultures. In particular, we focused our attention on subunits typically present in extra-synaptic receptors, about whom interest rose in recent years (Belelli et al., 2009; Fritschy and Panzanelli, 2014) and were not previously characterized in the peripheral nervous system. As shown in table 4, δ, α4 and α5 subunits were found

to be present in DRG, sciatic nerves, DRG neurons and Schwann cells. Looking at the relative expression level of considered subunits, it can be noticed that synaptic subunits (α2 and γ2) appear to be more expressed in DRG total tissue and neurons, while extra synaptic ones (α4, α5 and δ) seem to be in general more expressed in sciatic nerves and, partially, in Schwann cells.

	α2	β1	γ2	δ	α4	α5
DRG tot	+++	++	+++	+	++	+++
DRG neurons	+++	+++	+++	++	+++	+++
Sciatic Nerves	+	++++	++	+++++	++++	+++
SCs	+	++	+	++	+	+

Table 4- GABA-A subunits relative expression in DRG tissue (DRG tot), DRG neurons, sciatic nerves and Schwann cells (SCs). Synaptic subunits resulted to be more expressed in DRG tissue and neurons, extra-synaptic ones in sciatic nerves and Schwann cells. Relative expression level (R.e.l.): +: R.e.l. < 5; ++: 5 < R.e.l. < 50; +++: 50 < R.e.l. < 500; ++++: 500 < R.e.l. <1000; +++++: R.e.l. > 1000.

We then evaluated the expression levels of 9 different GABA-A subunits (α2, α3, α4, α5, β1, β2, β3, γ2, δ) in DRG neurons and tissue extracts of experimental and control animals. Data show a clear tendency to down-regulation of several subunits both in tissue extracts and neuronal cultures (Figure 19), suggesting a general reduced number of GABA-A receptors in conditional knockout animals DRG. In both cases, α3 and β1 subunits resulted to be significantly down-regulated. β2 and β3 were significantly down-regulated in tissue extracts, but showed only a tendency to down-regulation in primary cultures. α2 and γ2 subunits showed a tendency to down-regulation in both cases, but this difference was not statistically significant,

although in tissue extracts the difference for γ2 is at the very limit of statistical significance (p = 0.05). Some differences can be noted for extra synaptic subunits; indeed, while in tissue extracts α4, α5 and δ subunits did not result to be modulated (Figure 19A), in neuronal primary cultures α4 and δ resulted to be significantly down-modulated, suggesting this may be a neuron-specific change (Figure 19B).

Discussion

The identification of new treatments for nerve regeneration and chronic neuropathic pain is currently a challenge for clinical neurologists, and possible improvements in therapy mostly rely on the comprehension of the mechanisms underlying these pathologies.

As mentioned above, PSL is an established model for the study of neuropathic pain. However, the morphological consequences of PSL treatment and its impact on Schwann cell molecular parameters, such as the expression of the two main myelin proteins, P0 and PMP22 (Quarles, 2002; Salzer, 2015), were poorly understood. A severe degeneration of the nerve structure was observed, with loss of large myelinated fibers, nerve de-myelination, and phagocytosis. P0 and PMP22 expression was strongly decreased, confirming a massive degenerative process affecting peripheral nerves myelin forming compartment. According to the literature (Bennett et al., 2003), the nerve degeneration was expected to be equal for axons of all sizes, affecting either myelinated (type Aβ/Aδ) and unmyelinated (type C, mostly nociceptors) fibers.

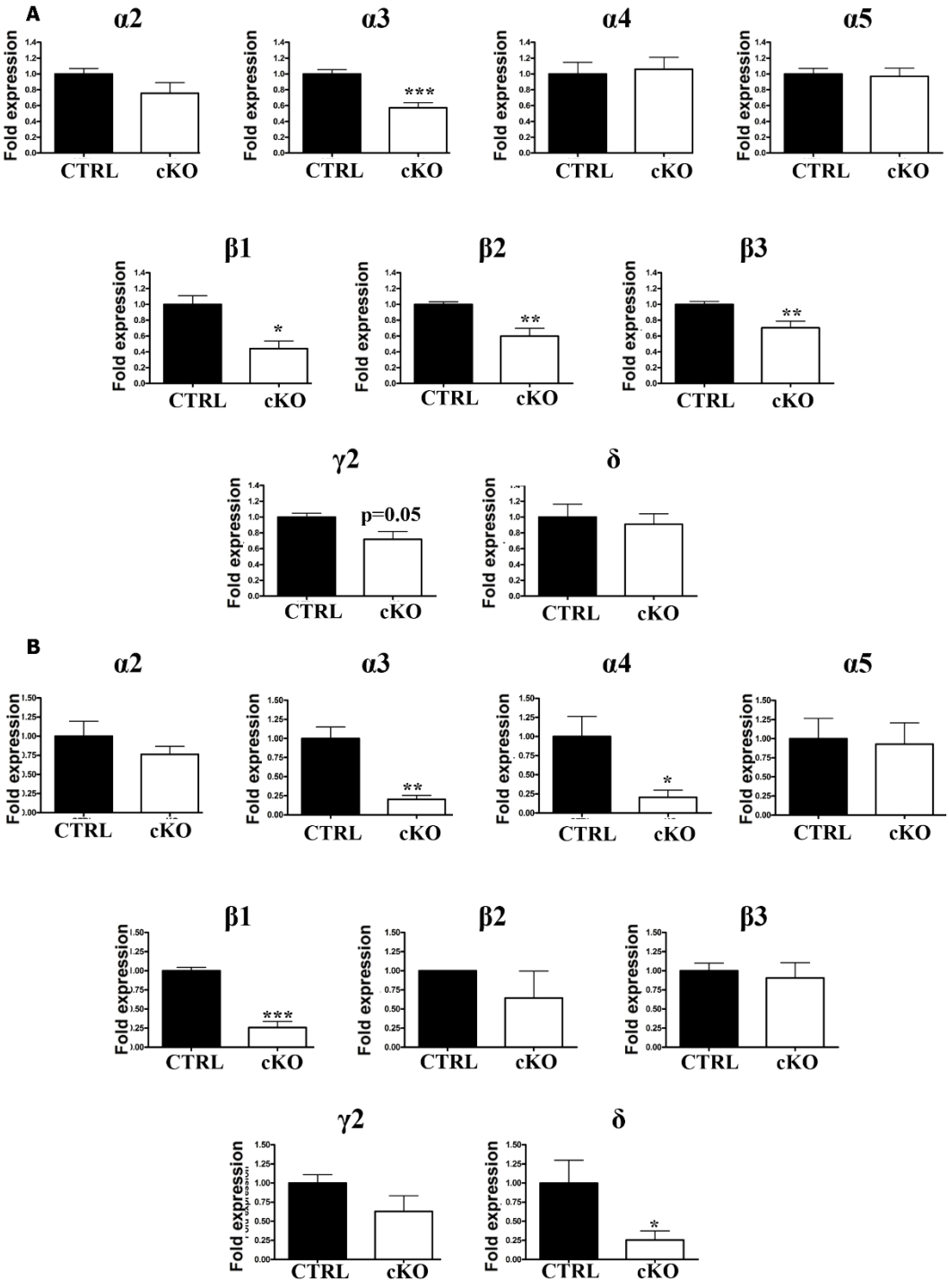


Figure 19 – GABA-A subunits mRNA expression in DRG tissue extract (A) and neuronal cultures (B). A clear tendency to down-regulation can be observed in conditional knockout (cKO) mice. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

The putative therapeutic effect of two GABA-B ligands on the altered behavioral, biochemical, and morphological parameters of the PSL model has been investigated. The choice to use baclofen as a GABA-B ligand relies on previous findings demonstrating the involvement of GABA-B receptors in the control of Schwann cell biology, being involved in their differentiation, in myelination and in nociception (Magnaghi et al., 2004, 2008; Procacci et al., 2012; Faroni et al., 2013). Moreover, baclofen showed anti-allodynic and anti-nociceptive actions in chronic pain models in rats (Cui et al., 1998; Patel et al., 2001). Baclofen was also used in a limited number of clinical trials to treat some types of neuropathic pain, including the use of spinally administered baclofen to enhance spinal stimulation effects (Fromm, 1989; Hering-Hanit, 1999; Lind et al., 2004). However, its use was limited because of its sedative properties and the rapid development of tolerance.

CGP56433 is a high affinity antagonist of the GABA-B receptor, active at nanomolar concentration in numerous *in vitro* and *in vivo* studies (de Groote et al., 1999; Froestl, 2010). Present results are the first to show CGP56433 effects linked to peripheral nerve regeneration.

Surprisingly, the agonist baclofen and the antagonist CGP56433 exerted an additive effect. This is not a common phenomenon in the nervous system. However, a GABA-B ligands additive effect on brain stimulation rewards has been previously described. Indeed, the GABA-B agonist CGP44532 and the antagonist CGP56433 induced a reward decrement when administered separately, while their co-administration induced an additive effect on threshold, rather than blocking the agonist-induced threshold elevations (Macey et al., 2001).

In the present model, part of the effects achieved with CGP56433 treatment alone may result from the blocking of GABA-B receptor activation by endogenous GABA, that, as previously mentioned, can be synthesized and released by Schwann cells (Magnaghi et al., 2010). Schwann cells, indeed, resulted to be particularly responsive to CGP56433, revealing a specific effect mediated by the GABA-B receptors present on their cell surfaces. The GABA-B antagonist significantly up-regulated P0 and PMP22 mRNA level, in line with previous findings indicating that GABA-B activation leads to myelin proteins decreased expression (Magnaghi et al., 2004). Present findings suggest that the additive effects exerted by baclofen and CGP56433 may be the result of a double simultaneous action; indeed, CGP56433 may act through the myelin-forming component, while baclofen likely acts through the neuronal (central and/or peripheral) compartment. Given the additive effects on locomotor parameters, the activation of mixed central and peripheral targets should not be excluded. Interestingly, CGP56433 alone partially recovered the 1–5 and 2–4 toes distances, suggesting that these effects may be ascribed to the antagonism of the GABA-B mediated tonic control of motor coordination.

Macrophages are committed to neuroimmune surveillance in the peripheral nervous system, and intervene during the Wallerian degeneration following axonal injury (Faroni et al., 2015). Macrophages concur to the onset of pain hyperactivity, therefore a reduction in their recruitment is beneficial (Scholz and Woolf, 2007). The reduced recruitment observed following CGP56433 and CGP56433-baclofen treatments is in agreement with a protection against neuroimmune-induced neuropathic pain, revealing that only the GABA-B antagonist is active in this

context. This effect is likely due to a direct effect on macrophage cells, which express the GABA-B receptor (Tamura et al., 2007).

Even if most of the effects described derive from a direct action of drugs on peripheral components, the involvement of central pathways should not be excluded. It is indeed reported that central mechanisms of GABA ligands, administered intrathecally, equally affect the behavioral signs of neuropathy (Hwang and Yaksh, 1998; Malan et al., 2002). The possibility to administer GABA-B ligands *in situ*, close to the nerve injury site, is therefore an opportunity which deserves further investigation.

Different studies suggest that GABA-A and GABA-B receptor are involved in a complex cross-interaction in the peripheral nervous system, often exerting opposite effects on Schwann cells (reviewed in Faroni and Magnaghi, 2011).

The basal expression level of some GABA-A subunits has been previously investigated in rat sciatic nerves and Schwann cells, suggesting for example that α1 and α6 subunits may be not importantly expressed in peripheral nerves (Magnaghi et al., 2006). However, extra-synaptic subunits expression was never assessed, therefore this is the first evidence about the expression of these subunits in peripheral glial cells. GABA-subunits expression in mature oligodendrocytes has recently been analyzed (Arellano et al., 2016). In this study, the subunits more importantly expressed resulted to be α3, β2, β3, γ1 and γ3, with the δ subunit being expressed at very low levels, if not at all. This suggests a different subunits composition between the myelinating cells of the peripheral and the central nervous system. In particular, the most important difference seems to be the fact that Schwann cells express extra-synaptic subunits, and in particular the δ subunit,

important for neuroactive steroids affinity (Belelli and Lambert, 2005); Conversely, oligodendrocytes express almost only synaptic subunits. Therefore, taken together these data suggest that Schwann cells and oligodendrocytes express GABA-A receptors with different subunits composition, thus displaying a different pharmacological profile.

Present data suggest that synaptic receptors may be more importantly expressed in DRG, while extra-synaptic ones seem to be predominant in sciatic nerves and, partially, in Schwann cells. This is in line with data previously reported for DRG, where the most common receptor composition was found to be α2β3γ2 (Ma et al, 1993; Liu et al., 2004). Another interesting point is that subunits expression seems to be almost overlapping between DRG tissue and neurons, and deeply different between Schwann cells and sciatic nerves, suggesting that in both cases GABA-A receptors may be more expressed in the neuronal compartment. However, for what regards the discrepancy between sciatic nerves and Schwann cells, the possibility that part of the observed difference may be due to the different Schwann cell differentiation state cannot be ruled out; Schwann cells in sciatic nerves are still in contact with their axons, and therefore may be mostly myelinating, while cultured Schwann cells are not in contact with the neuronal compartment, and may show mostly a non-myelinating phenotype.

Even if some studies reported the GABA-A- mediated modulation of GABA-B expression (Magnaghi et al., 2006), little is known about GABA-A modulation by GABA-B activation/de-activation. Moreover, the analysis of GABA-A receptor modulation in the P0-CRE_GABA-B1$^{fl/fl}$ model may help to understand some of the variations observed (Faroni et al., 2014).

Observing GABA-A subunit expression in DRG total tissue extracts and neurons, it is evident how these animals show a general GABA-A down-regulation. This evidence suggests a reduced GABAergic inhibitory tone, which may contribute to the hyperalgesic and allodynic state observed (Faroni and Magnaghi, 2014). Indeed, increased DRG neurons excitability was reported to play a role in the initiation and maintenance of central sensitization, that is an important contributor to the development of neuropathic pain (Julius and Basbaum, 2001). It was also reported that the neuropathy following peripheral nerve injury involves GABA-A reduced expression in sensory neurons (Obata et al., 2003). Moreover, α2 subunit silencing in rat DRG proved able to worsen mechanical and thermal hypersensitivity in crush-injured rats, as well as endogenous GABA up-regulation alleviated neuropathic pain symptoms (Obradovic et al., 2015).

Analyzing with more attention which subunits are involved in this modulation, it seems clear that GABA-B conditional knockout in Schwann cells has a greater effect on synaptic receptors in DRG total extracts, while extra-synaptic ones do not seem to be modulated. Therefore, the hypothetical reduced GABAergic tone in DRG may regard mainly a reduction in phasic synaptic currents. However, the strong specific reduction of α4 and δ subunits (typical constituents of the most common extra-synaptic receptor sub-type, Fritscy and Panzanelli, 2014) in DRG neurons, but not in the total tissue extract, may suggest a specific down regulation of δ containing extra-synaptic receptors, while α5-contaning seem to be not modulated. These different sub-types of extra-synaptic receptors have different characteristics; in particular, δ-containing GABA-A receptors have high affinity for GABA and neuroactive steroids, being the most important target of

allopregnanolone action (Belelli et al., 2009, Carver and Reddy, 2013). Alteration of their expression levels have been correlated with nociception in spinal neurons (Bonin et al., 2011), but their role in DRG has not been elucidated yet. Altogether, these data shed some light on GABA-A receptors composition in the peripheral nervous system, suggesting for the first time the presence of extra-synaptic subunits in Schwann cells, giving a possible explanation for the altered nociception observed in P0-CRE_GABA-B1$^{fl/fl}$ mice.

Some of the results presented in this chapter are published in the following article:

Magnaghi V*, **Castelnovo LF*,** Faroni A, Cavalli E, Caffino L, Colciago A, Procacci P, Pajardi G (2014) **Nerve regenerative effects of GABA-B ligands in a model of neuropathic pain** *BioMed Res Int 2014:368678.*

***equally contributing**

Other results will be submitted for publication in an article currently in preparation.

CHAPTER 2

Study of PKCε modulation by allopregnanolone in the peripheral nervous system as a possible strategy for neuropathic pain treatment

Strong evidence supports the hypothesis that PKCε is importantly involved in the modulation of neuropathic pain, given its ability to phosphorylate both excitatory (such as TRPV1 and N-type Ca^{++}channels) and inhibitory (such as GABA-A and opioid μ) receptors. (Olah et al., 2002; Morenilla-Palao, 2004; Narita et al., 1997; Zhang et al., 2004, Van Kolen et al., 2008). Moreover, some data suggest it may be involved in a cross-interaction with allopregnanolone and the GABA-A receptor (Puia et al., 2015), which has not been clearly elucidated yet.

The first step of our study was the qRT-PCR evaluation of PKCε relative expression in Schwann cell and DRG neuron primary cultures. As expected, PKCε resulted to be more expressed in neurons, but it resulted to be significantly expressed also by Schwann cells (Figure 20).

Then, we analyzed how PKCε expression was modulated by allopregnanolone treatment. In particular, we treated Schwann cell and DRG neuron primary cultures for 24 h with allopregnanolone at 1 μM concentration. We also performed conditioned medium experiments, in which Schwann cells were treated for 24 h with allopregnanolone, and then their medium was used to treat DRG neurons for 24 h.

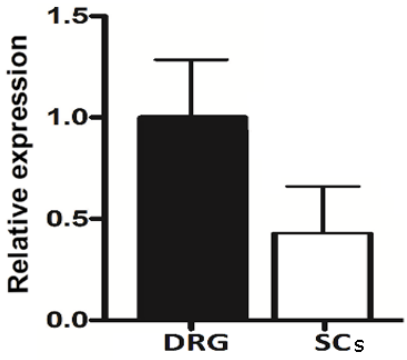
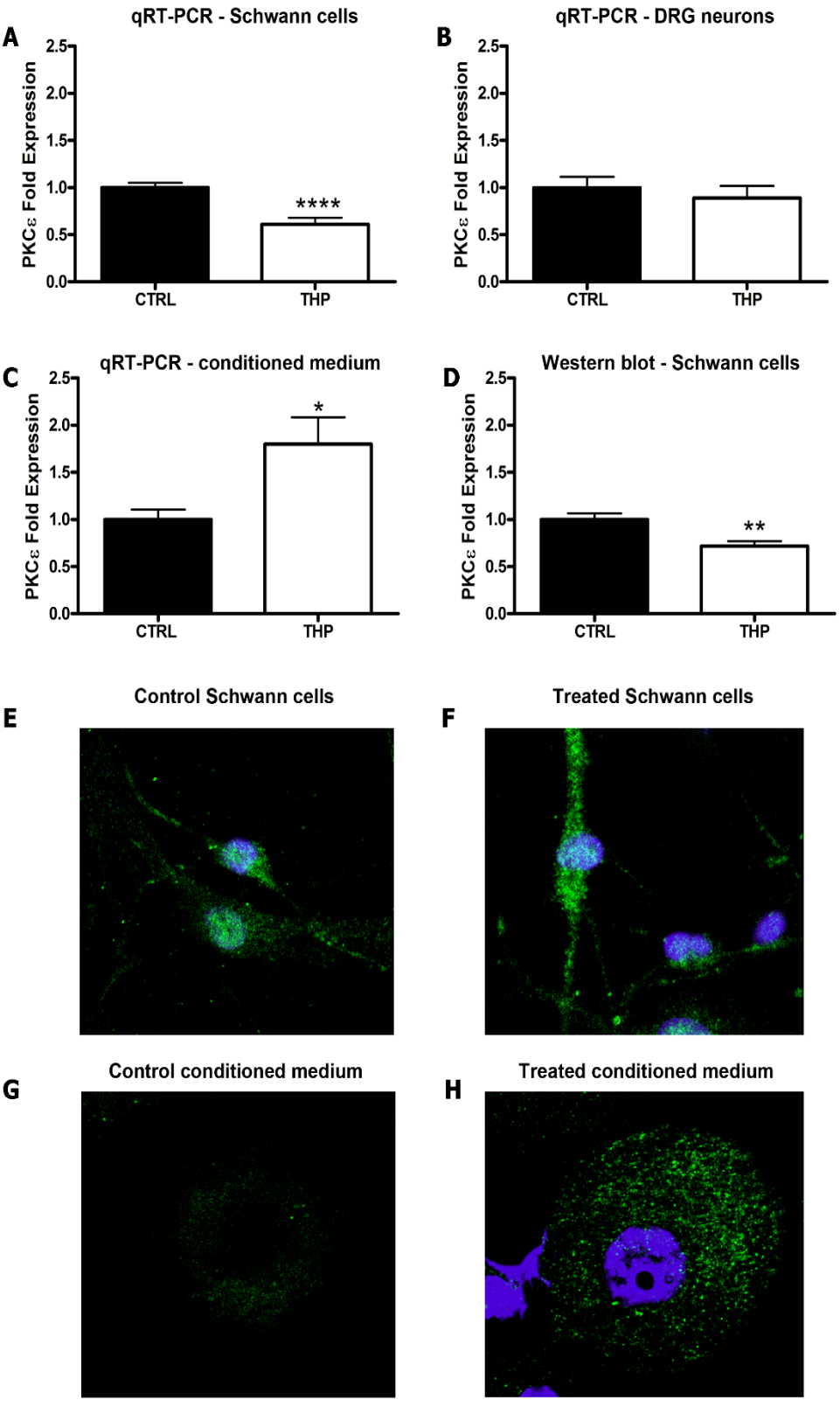


Figure 20 – PKCε mRNA relative levels in DRG neurons (DRG) and Schwann cell (SCs) primary cultures. Even if the expression is higher in DRG neurons, also Schwann cells express PKCε.

Allopregnanolone treatment proved able to induce a significant down-regulation of PKCε expression in Schwann cells (Figure 21A), while it exerted no effect on DRG neurons (Figure 21B). Interestingly, in neurons treated with Schwann cells conditioned medium, PKCε expression was significantly increased (Figure 21C), suggesting that allopregnanolone may modulate PKCε expression in DRG neurons through a Schwann cell-mediated mechanism. PKCε modulation in Schwann cells and conditioned medium-treated DRG neurons was confirmed also at the protein level, with Western blot in Schwann cells and immunofluorescence in Schwann cells and neurons. In particular, in Schwann cells, the Western blot confirms PKCε expression down-regulation (Figure 21D), while the immunofluorescence analysis, targeting the active PKCε phosphorylated form (pPKCε) suggested a possible increase in its phosphorylation (Figure 21E-F). Immunofluorescence analysis of conditioned medium experiments, instead, showed an increased immunopositivity for pPKCε, suggesting that the up-regulation observed in the mRNA levels leads also to higher levels of the active form on the cell membrane (Figure 21G-H).



(caption in the next page)

Figure 21 *(previous page)* – Results of allopregnanolone (THP) treatments on Schwann cells, DRG neurons and conditioned medium experiments. Allopregnanolone was able to induce a significant down-regulation of PKCε gene expression in Schwann cells (A), but was not effective on neurons (B). Schwann cells conditioned medium, however, induced a significant up-regulation of PKCε mRNA levels in DRG neurons (C). PKCε down-regulation in Schwann cells was confirmed also by Western blot analysis (D); however, pPKCε signal appears to be stronger in allopregnanolone-treated Schwann cells (F), compared to control (E). The treatment with Schwann cells conditioned medium was also able to induce a stronger immunopositivity for pPKCε (G: control; H: allopregnanolone treatment). *: $p < 0.05$; **: $p < 0.01$; ****: $p < 0.0001$;

Discussion

The peripheral mechanisms regulating neuropathic pain, although under extensive study, are not yet completely clear and need further investigation. Some evidence suggest that PKCε may be involved in this process. Indeed, it is present in DRG neurons., where it was reported to alter the permeability of N-type Ca^{++} channels and enhance nociception (Van Kolen et al., 2008). In the present study, its expression in DRG neurons has been confirmed, and moreover it was found to be significantly expressed also by Schwann cells, even if at a lower level compared to DRG neurons. PKCε basal expression in Schwann cells was previously hinted in some studies (Borghini et al., 1994; Kawano et al., 1997), but was never properly characterized in physiological conditions.

Pharmacological treatments revealed that allopregnanolone was able to reduce PKCε mRNA and protein levels in Schwann cells; however, immunofluorescence analysis suggests an up-regulation of its phosphorylation and trafficking to the cell

membrane. The physiological importance of this effect is still not clear. There is evidence in literature that the infection by the causative organism of leprosy, *Mycobacterium leprae*, is able to induce the activation of the Erk 1-2 pathway through a PKCε-dependent mechanism, leading to increased Schwann cell proliferation (Tapinos and Rambukkana, 2005). Allopregnanolone has been reported to modulate Schwann cell proliferation through a mechanism involving GABA-A binding and EAAC1 glutamate transporter trafficking (Perego et al., 2012). Therefore, the possibility that this effect is partially regulated by PKCε modulation deserves further investigation.

Allopregnanolone treatment did not change PKCε expression in DRG neurons, but neurons treated with Schwann cell conditioned medium showed an increased PKCε expression and phosphorylation. This evidence may appear in contrast with a consistent number of paper, that suggested a possible role for allopregnanolone in neuropathic pain reduction (reviewed in Patte-Mensah et al., 2014). However, it is important to notice that some *in vitro* data suggest that the activation of GABA-A receptors in DRG may be excitatory, because of a higher Cl^- concentration in the intracellular compartment, and hence potentially responsible for painful behavior (Aptel et al., 2007). There is also some electro-physiological evidence suggesting that, in a subset of human unmyelinated sensitive fibers, GABA may lead to depolarization (Carr et al., 2010). Therefore, increased PKCε activity, considering its ability to de-sensibilize GABA-A (McMahon and Koltzenburg, 1990; Si et al., 2004; Yamada et al, 1996; Ma et al., 2006), may indeed lead to hyper-polarization of at least a subset of sensitive fibers, possibly mediating an anti-nociceptive effect. However, as mentioned above, the GABA-A receptor has also been reported to be

down-regulated after nerve damage (Obata et al., 2003), and its down-regulation in DRG neurons might exacerbate hyperalgesic reactions after crush injury in rats (Obradovic et al., 2015). Therefore, the effective role of GABA-A activation in DRG during neuropathic pain is far from being elucidated, and will require further investigation, probably involving different actions, which might be exerted by different GABA-A receptor sub-types (Obradovic et al., 2015; Bravo-Hernandez et al., 2014, 2016).

Taken together, these findings support the hypothesis of a GABA-A/PKCε/allopregnanolone cross-correlation in the peripheral nervous system, strengthening the role of neuron/glia cross talk in the modulation of neuropathic pain.

Some of the results presented in this chapter are published in the following article:

Puia G, Ravazzini F, **Castelnovo LF**, Magnaghi V (2015) **PKCε and allopregnanolone: functional cross-talk at the GABAA receptor level** *Front Cell Neurosci 9:83.*

Other results will be submitted for publication in an article currently in preparation.

CHAPTER 3

Characterization of the expression in Schwann cells of novel non-classical membrane progesterone receptors (mPRs and PGRMC1) and evaluation of their potential role in the modulation of Schwann cell physiology

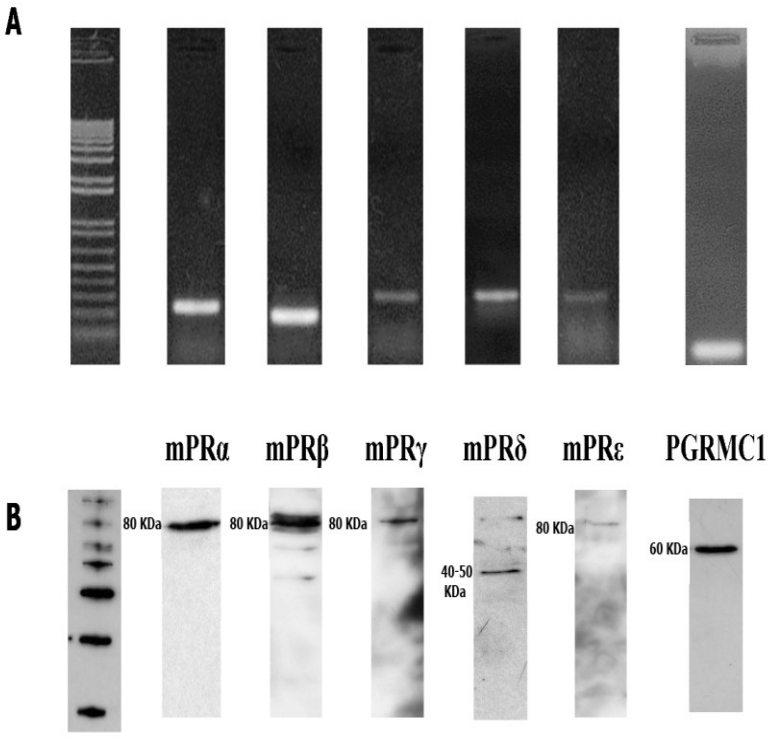
In the last decades, an increasing amount of data in literature dealt with the important role of progestogens in the nervous system. It is well known that progesterone and DHP bind the classical PR, while allopregnanolone exerts its action through the GABA-A receptor (Belelli and Lambert, 2005). However, these two mechanisms of action are not sufficient to explain all the actions exerted by progestogens in the nervous system.

A new class of membrane progestogen receptors, mPRs, was originally identified in seatrout ovaries by Thomas and colleagues (Zhu et al., 2003a, b), and was subsequently shown to be functionally present in the central nervous system (reviewed in Guennoun et al., 2015). The presence and role of these receptors, however, has never been deeply investigated in the peripheral nervous system, and in particular in Schwann cells.

For this line of experiments, we used a commercially available Schwann cell line (S42), directly derived from rat primary Schwann cell cultures (Toda et al., 1994). We decided to use these cells instead of primary Schwann cells for both ethical and practical reasons; we preferred to use this model because we thought it was suitable

for a first characterization of mPRs expression in Schwann cells, reducing the number of animals used in our research, as well as the economic cost.

The first goal of our research was to demonstrate the mRNA expression of the five mPR sub-types (mPRα, β, γ, δ and ε) and PGRMC1 in the S42 Schwann cell line, using RT-PCR analysis. As shown in figure 22A, all receptors were significantly expressed. mPRα, β, δ and PGRMC1 were highly expressed, while mPRγ and ε were lowly expressed. The presence of the proteins encoded by these genes has been confirmed by Western blot on membrane enriched protein extracts, suggesting that the receptors are not only expressed, but also localized on the cell membrane (figure 22B) All the receptors, with the exception of mPRδ, were detected at a molecular weight around 60-80 Kda, almost double than expected, as previously reported in rat brain (Zuloaga et al., 2012).



(Caption in the next page)

Figure 22 (previous page) – Characterization of mPRs and PGRMC1 expression in S42 Schwann cells. All receptors resulted to be expressed both at the mRNA (A) and protein (B) level. In particular, RT-PCR suggests that mPRα, β, δ and PGRMC1 are more expressed than mPRγ and ε. The fact that all receptors were detected in Western blot in membrane enriched fractions suggests their functional presence on the cell membrane.

We then measured the basal expression levels of different mPR isoforms and PGRMC1 through qRT-PCR. mPRβ, PGRMC1 and mPRδ resulted to be the most expressed receptors; mPRα was expressed at lower levels, while the expression levles of mPRγ and mPRε are almost neglectable (Figure 23).

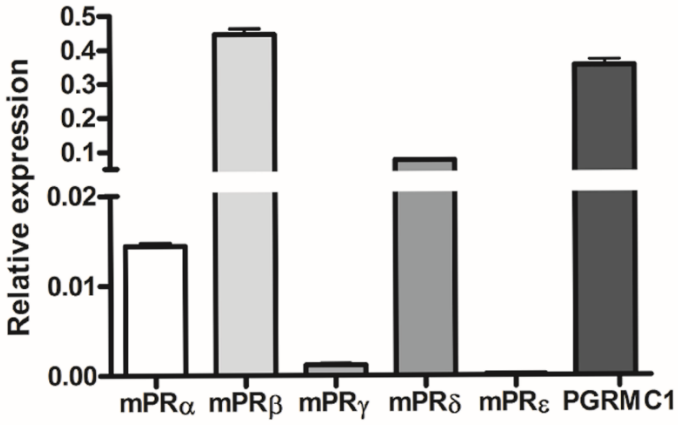


Figure 23 – qRT-PCR analysis of different mPR isoforms and PGRMC1 in S42 Schwann cells. mPRβ, mPRδ and PGRMC1 are higly expressed, while mPRγ and mPRε are present at extremely low levels, and mPRα is present at an intermediate level

We then performed an immunocytochemical evaluation of the cell localization of the highly expressed mPR isoforms (α, β and δ). All the receptors shown clear immunopositivity, but they appeared to be expressed both on the plasma membrane and in the cytoplasm (Figure 24).

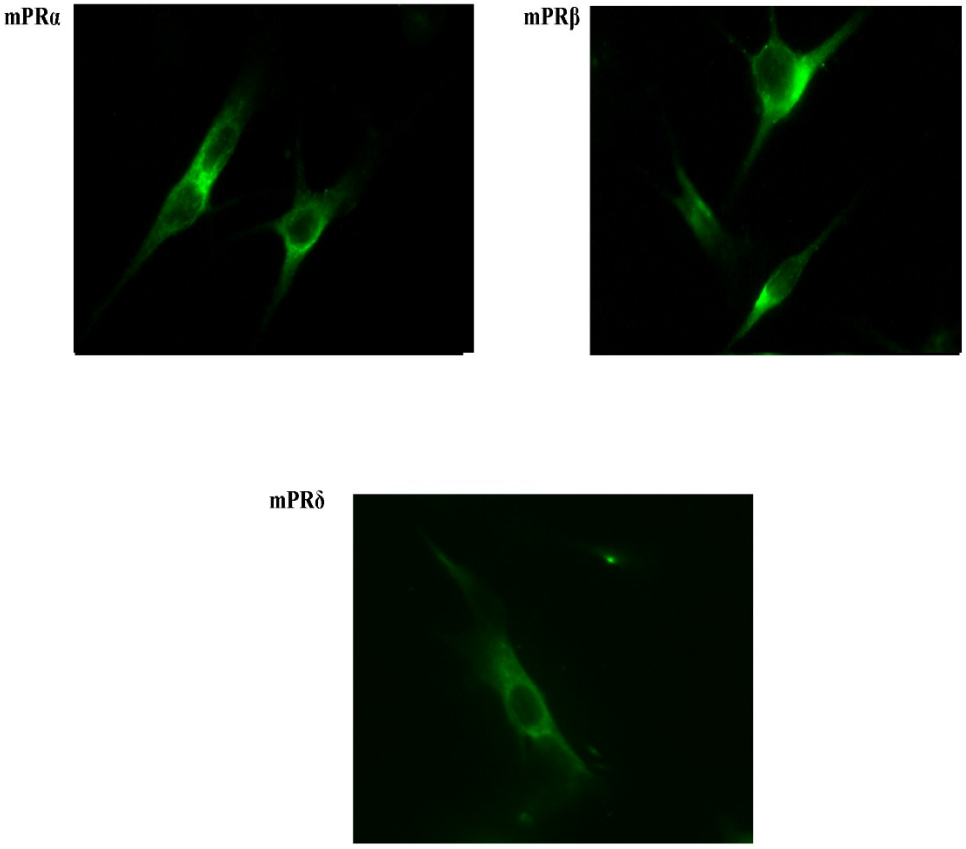


Figure 24 – Immunocytochemical evaluation of mPRα, β and δ expression in S42 Schwann cells. The three isoforms seem to be present both on the cell membrane and in the cytoplasm.

To confirm specific membrane localization, necessary for the functionality of the receptors, we repeated Western blot experiments including a glucose pad centrifugation step in the membrane preparation, in order to verify the effective expression of mPRs and PGRMC1 on the cell membrane of the S42 Schwann cell line model (Figure 25).

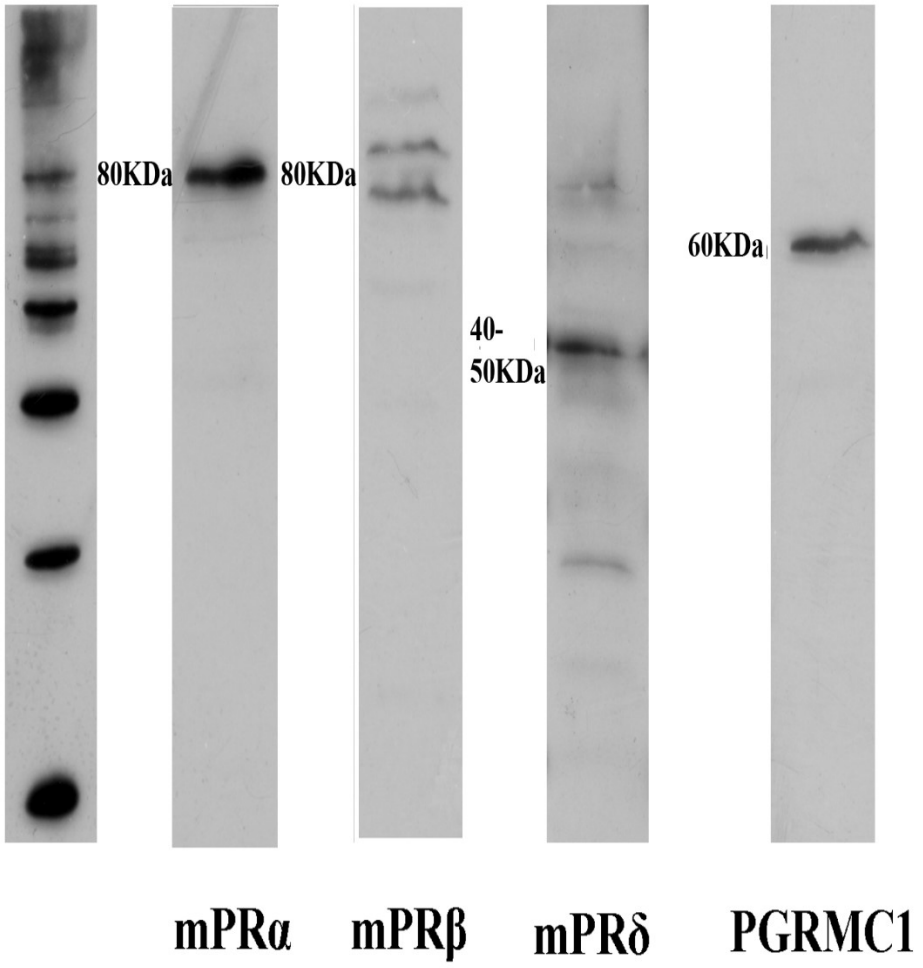


Figure 25 – Western blot analysis of mPRs expression in S42 Schwann cells after extraction including a centrifuge step with sucrose pad. The presence of specific bands in membrane extract after the further purification steps corroborates the hypothesis of their membrane expression.

In order to furtherly demonstrate the effective expression of functional mPRs on the cell membrane, we performed a two-point competition binding assay, analyzing the ability of progesterone, the specific mPR ligand O2 and the PR specific ligand R5020 to displace radioactive progesterone (^{3}H-progesteorne) from membrane protein extracts. The results confirm the presence of functional mPRs on the cell

membrane of S42 cells, since both progesterone and O2, but not R5020, resulted able to displace ^{3}H-progesterone (Figure 26).

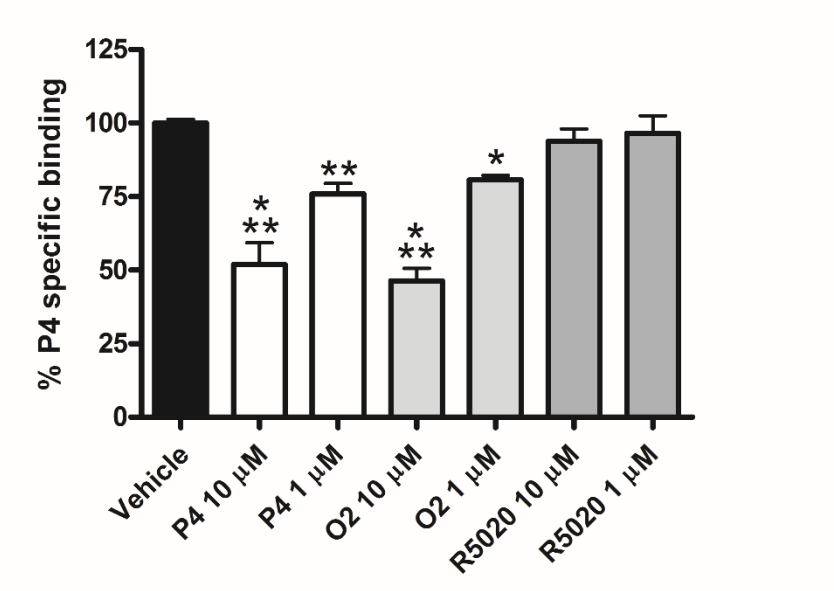


Figure 26 – Binding assay. Both cold progesterone (P4) and O2, but not R5020, are able to displace ^{3}H-progesterone, indicating that the binding is specific to mPRs and does not involve PR. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, compared to vehicle.

Once confirmed mPRs functional presence on the plasma membrane, we analyzed mPR proteins levels on the cell membrane after treatment with their specific agonist. Therefore, we treated S42 Schwann cells with O2 for 12 hours, and then we incubated cells in serum-free condition before membrane preparation; otherwise, receptors may have been internalized, preventing a reliable identification of their level (Foster et al., 2010). We evaluated mPRα, β and δ expression levels by Western blot. We did not observe significant changes in the protein level of any receptor (Figure 27).

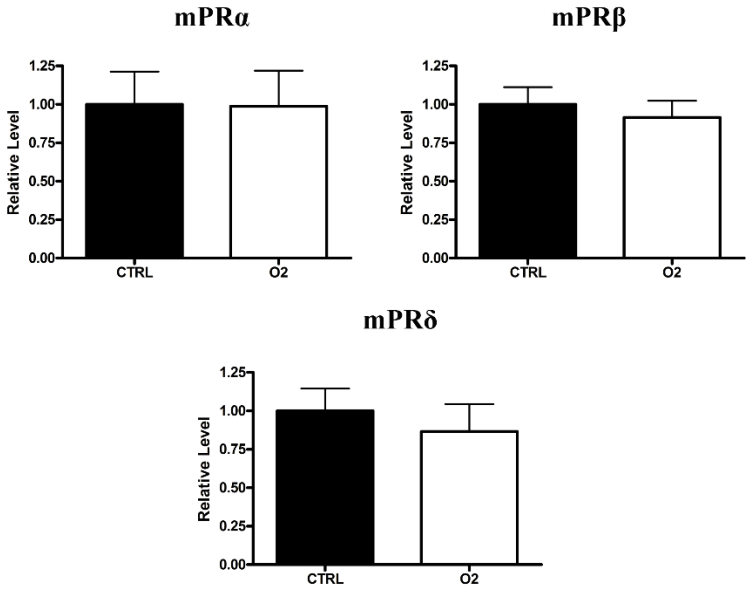
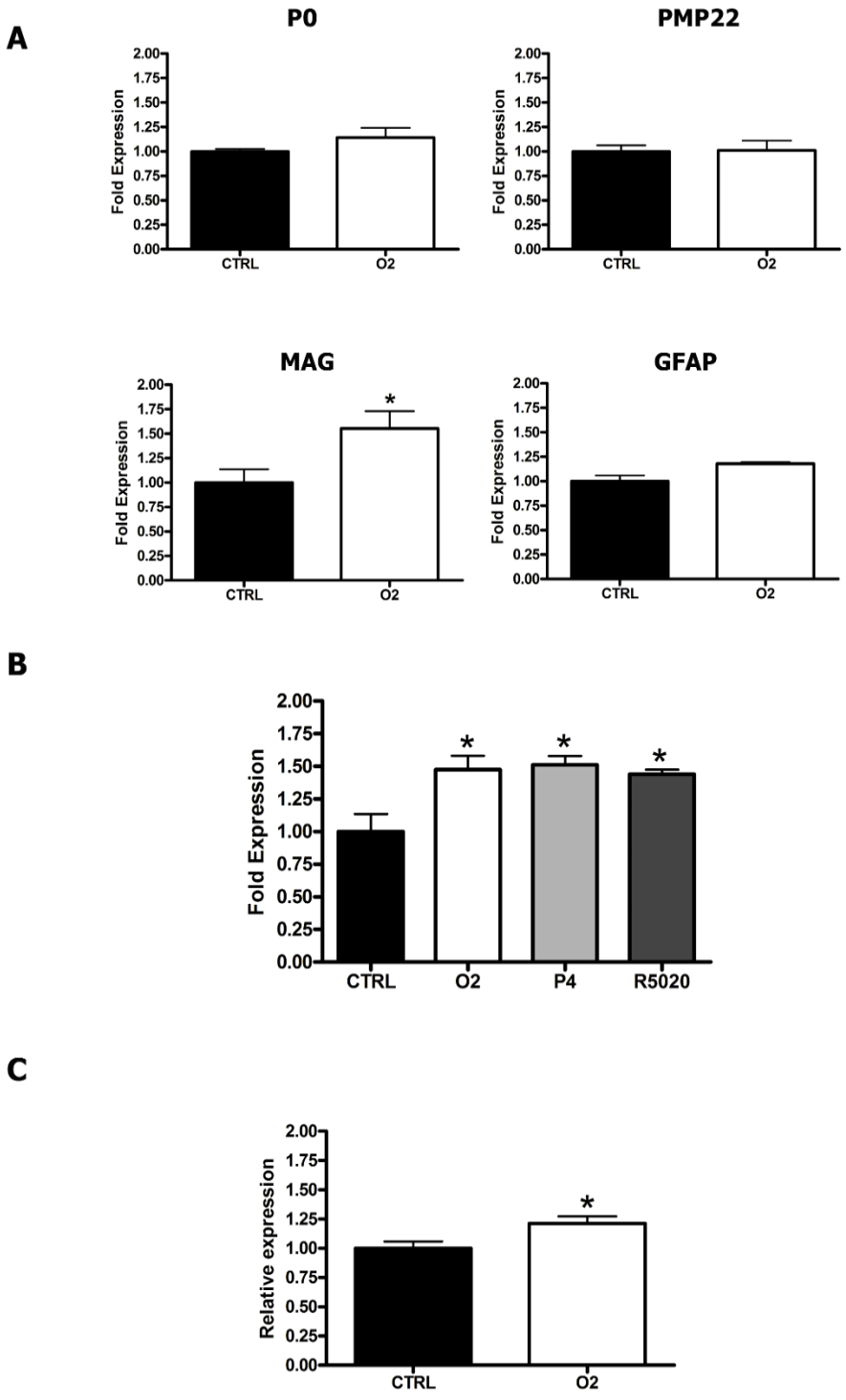


Figure 27 – mPR proteins level on the plasma membrane after O2 20 nM treatment. None of the receptors resulted to be modulated by the treatment with the specific agonist.

We then analyzed the possible involvement of mPRs in progestogens-mediated modulation of Schwann cell physiology, starting with the analysis of specific Schwann cell protein expression modulation. Therefore, we treated cells for 24 hours with the specific mPR agonist O2 and we analyzed possible alterations in the mRNA expression levels of P0, PMP22, MAG (markers of the myelinating phenotype) and GFAP (marker of the non-myelinating phenotype) by qRT-PCR. MAG resulted to be significantly upregulated, while other proteins where not modulated (Figure 28A).

We expanded our analysis, treating cells with O2, progesterone and R5020 for 24 hours and evaluating MAG expression. O2 confirmed its ability to up-regulate MAG gene expression, and this effect was mimicked both by progesterone and

R5020 (Figure 28B). O2-mediated MAG up-regulation has also been confirmed by Western blot analysis on total cell lysate after 36 hours of treatment (Figure 28C).



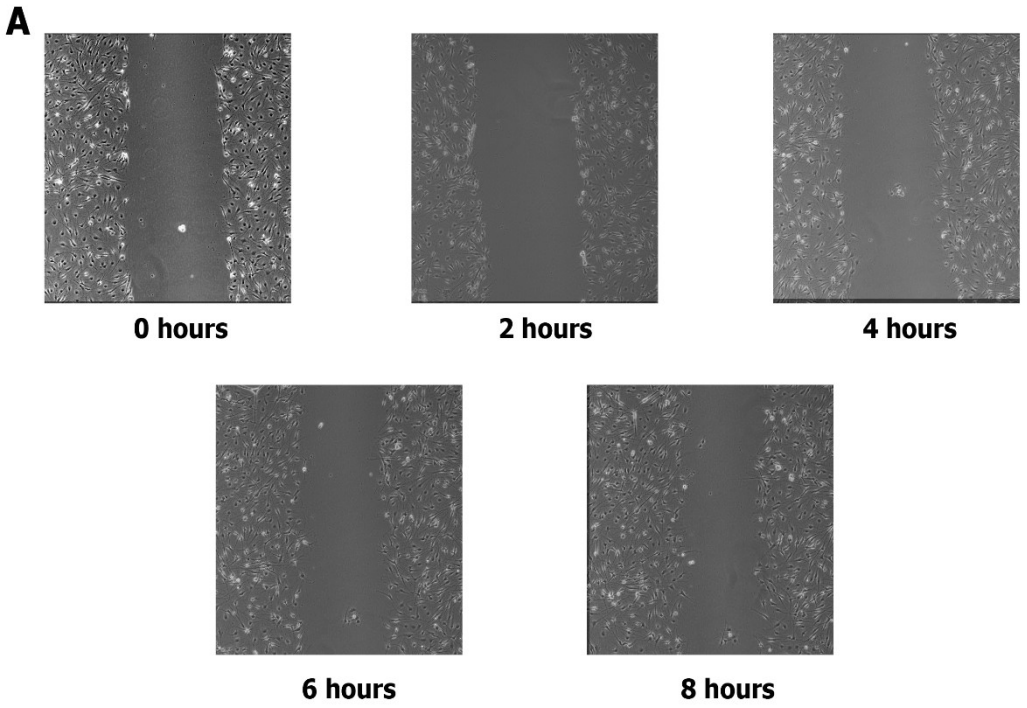
(Caption in the next page)

Figure 28 ***(Previous page)*** **–** Evaluation of mPR activation with O2 20 nM treatment on Schwann cell proteins expression. qRT-PCR analysis revealed that, among considered targets, only MAG was significantly modulated (A). further analysis with progesterone (P4) 20 nM and R5020 20 nM suggest that this effect may be not specifically mPR-mediated (B). MAG up-regulation was confirmed by Western blot (C). *: $p < 0.05$.

Lastly, in order to analyze possible effects of mPRs on S42 Schwann cell motility, we performed an *in vitro* wound healing assay, scratching the bottom of the culture vessel where cells were plated and observing how pharmacological treatments may modulate their migration into the scratch (Figure 29A). In a first preliminary experiment, the effect of O2 treatment was evaluated at 2, 4, 6, 8 and 24 hours after the scratch (Figure 29B). 24 hours data were not considered since in different wells the gap was closed in many points, making the analysis not reliable. Since this first analysis suggested that O2 treatment may exert its action mainly after 2 and 4 hours, we repeated the test considering only this two time points, including also progesterone and R5020 treatments. The results show that the effect may be mPR specific, since it is mimicked by progesterone, but not by R5020 (Figure 29C).

Discussion

In the last two decades, several studies pointed out to an important role for progestogens, and in particular allopregnanolone, in the modulation of Schwann cell physiology, modulating several biochemical and functional parameters.



(Caption in the next page)

Figure 29 (*previous page)* – Assessment of mPR role on S42 Schwann cell migration. After a scratch is made on the bottom of their culture vessel, S42 Schwann cells progressively migrate in order to close the gap (A). A preliminary experiment, performed using only O2 20 nM as pharmacological treatment, suggested that mPR effect on S42 Schwann cell migration may be rapid, being evident 2 and 4 hours after the scratch (B). Further analysis of this two time points, which included also pharmacological treatment with progesterone (P4) and R5020 (both 20nM), suggested that this effect may be mPR specific, since it is mimicked by progesterone, but only partially by R5020 (C).

In particular, it has been reported that progestogens may control Schwann cell proliferation, myelin proteins expression, GABA synthesis, GABA-B receptor modulation and glutamate trafficking (Magnaghi et al., 2001, 2006, 2009, 2010; Perego et al., 2012; Faroni and Magnaghi, 2011). Most of these action (regulation of cell proliferation, PMP22 expression, GABA synthesis, GABA-B receptor expression levels and glutamate trafficking) resulted to be mediated by allopregnanolone interaction with the GABA-A receptor, others (for example the modulation of P0 expression) were mediated by DHP binding with the classic PR. However, the exact mechanism underlying progestogens modulation of Schwann cell physiology is not completely clear, and other mechanisms may be involved.

After their initial identification in seatrout ovaries (Zhu et al., 2003b), mPRs have been demonstrated to be present in several tissues, and in particular in the central nervous system, both in the brain and in the spinal cord (Zuloaga et al., 2012; Meffre et al., 2013; Pang et al., 2013; Labombarda et al., 2010). In physiological conditions, mPRs were found to be not expressd by glial cells in the brain (Meffre et al., 2013), and only mPRα was expressed by oligodendrocytes and astrocytes in the spinal cord (Labombarda et al., 2010). However, it was also reported that mPRα

expression is induced after traumatic brain injury in oligodendrocytes, astrocytes and microglia, both in the lesion core and peri-lesioned area, suggesting it may have a role in glia-mediated neuroprotective action of progesterone (Meffre et al., 2013). Taken together, these findings suggested the hypothesis that mPRs may have a role in the modulation of progestogens action in Schwann cells.

In order to perform this study, we decided to use a Schwann cell line model, directly derived from primary rat Schwann cell cultures after many generations in culture, the S42 Schwann cells. These cells have been reported to resemble, under some aspects, Schwann cells at an early stage in their preparation for myelination, and to express some typical markers of Schwann cells, such as MAG, which is expressed at high levels, and P0, that is instead expressed at lower levels than in sciatic nerves (Toda et al., 1994). Compared to similar cell lines (named S16 and S16Y), S42 Schwann cells expressed higher amounts of MAG and were characterized by slower proliferation (Toda et al., 1994).

RT-PCR identification of mPRs and PGRMC1 mRNA, and the subsequent Western blot analysis of their presence on membrane enriched fraction, represent the first time mPRs have been identified in peripheral glial cells. All the receptors in Western blot appear to have a higher molecular weight than expected, being almost double. This phenomenon has been reported before in rat brain and human umbilical vein endothelial cells, and the authors suggested it could be linked to dimer formation (Zuloaga et al., 2012, Pang et al., 2015). Another possible explanation may be a strong glycosylation. The following qRT-PCR analysis showed that mPRβ and mPRδ are the more expressed mPR isoforms in S42 Schwann cells, while mPRα is present at lower but still significant levels. These

findings are in line with previously reported data, identifying mPRβ as the main isoform in the rat brain (Zuloaga et al., 2012) and mPRδ as a specific isoform of the nervous system (Pang et al., 2013).

Immunofluorescence analysis revealed that mPRα, β and δ immunopositivity is present on the cell membrane, but is also clearly present in the cytoplasm, particularly in the perinuclear area, while it is weak in cellular processes. Even if membrane localization was not evident, the effective presence of the receptors on the cell membrane was confirmed by Western blot and binding studies. Indeed, Western blot analysis was repeated including a sucrose pad centrifugation step to assure more reliable membrane fraction separation (Thomas et al., 2005; Pang et al., 2008), therefore confirming membrane localization of mPRα, β, δ and PGRMC1. This evidence was further confirmed with two-point binding assay, that confirmed the presence on S42 Schwann cell membranes of functional receptors able to bind progesterone and the specific mPR agonist O2, but not the specific PR agonist R5020 (Kelder et al.,2010).

For the experiment involving pharmacological treatments, progesterone, O2 and R5020 were used. O2 is a specific mPR agonist; indeed, it was reported to bind human mPRα with higher affinity than progesterone, and even if it can bind PR, it has no agonist activity on it (Kelder et al., 2010). On the other hand, R5020 is a specific PR agonist, having little or no affinity for mPRs (Kelder et al., 2010). Therefore, the cell treatment with progesterone and these two compounds allows to discriminate between mPR and PR mediated effect.

In line with published data, mPRα, β and δ levels resulted to be not modulated by the treatment with their agonist. Indeed, progesterone treatment does not modulate mPRα protein levels in male mouse brain (Meffre et al., 2013).

Schwann cell treatment with the specific mPR agonist O2 was not able to modulate the mRNA expression levels of P0, PMP22 (that are the main protein components of compact myelin, Quarles, 2002; Salzer, 2015) and GFAP (that is a marker of non-myelinating Schwann cells, Procacci et al., 2012). However, it proved able to up-regulate MAG mRNA and protein level. As mentioned above, MAG is a glycoprotein mainly expressed on the adaxonal membrane and in other specialized structures in peripheral nervous system myelin, such as Schmidt-Lanterman incisures (Quarles, 2002; Salzer, 2015). It is involved in myelin-axon interactions, having a role in the bi-directional signaling between myelin-forming glia and axons (Quarles, 2007). Different studies with knockout models suggested that MAG is not necessary for the myelination process (Quarles, 2007), however it is deputed to stabilize myelinated axons (Fruttiger et al. 1995; Pan et al. 2005; Nguyen et al. 2009; Lopez et al., 2011). A recent report suggests that MAG protects neurons from excitotoxicity (Lopez et al., 2011).

O2 proved able to induce MAG mRNA and protein levels up-regulation, but this effect does not seem to be specifically mPR mediated, being mimicked by progesterone and R5020. Therefore, progestogens may regulate MAG level both through classical and non-classical mechanisms.

After nerve injury, myelinating Schwan cells change their differentiation status, assuming a phenotype that promotes nerve regeneration (Faroni et al., 2015), proliferating and migrating (Mantuano et al., 2015). Our data suggest that mPR

mediated progestogen action on Schwann cells may be important in the early stages of this process. Indeed, using a consolidated migration assay (Liang et al., 2007), we observed that O2 was able to significantly increase Schwann cell migration 2 and 4 hours after treatment, but this effect was lost at longer time points. Interestingly, progesterone treatment has the same pro-migration effect, while R5020 does not significantly affect S42 Schwann cell migration.

In conclusion, this line of evidence constitutes the first demonstration of mPRs functional expression in Schwann cells, also indicating some effects of this receptors activation in peripheral glial cells. The study of contribution of different isoforms to this effects, as well as the analysis of the intracellular pathways involved, both deserve further investigation.

Most of the results presented in this chapter are currently under consideration for publication:

Castelnovo LF, Magnaghi V, Thomas P (n.d.) **Expression of membrane progesterone receptors (mPRs) in rat peripheral glial cell membranes and their potential role in the modulation of cell migration and protein expression** *Submitted to Steroids.*

Conclusions

The different lines of research pursued during my PhD program and described in this thesis investigated different aspects of the GABAergic system and neuroactive steroids role in the patho-physiology of the peripheral nervous system.

In particular, the first research line regarding the nerve regenerative effects of GABA-B ligands and the GABA-A subunits modulation in GABA-B conditional knockout mice suggested that GABA-B modulation in the peripheral nervous system may be a promising tool for the development of new pharmacological approaches for nerve regeneration. Moreover, these outcomes strengthened the hypothesis that the two GABA receptors are cross-modulated in the peripheral nervous system, contributing to the modulation of GABA effects in this compartment. For what concerns GABA-B ligands as a tool for nerve regeneration, future experiments will be aimed at a better comprension of the mechanisms underlying the observed additive effect; these studies will include both experiments of *in situ* drug delivery, to better distinguish between central and peripheral effects, and experiments performed using cell culture approaches, in order to better discriminate the contribution of neurons and Schwann cells to the observed effects. For what regards GABA-A subunits expression in conditional knockout mice, the study will proceed including sciatic nerves and Schwann cell primary cultures in the analysis, then evaluating also GABA-A subunits protein levels by Western blot analysis and immunolocalization through immunofluorescence. These experiments will be performed in order to better characterize the model and to understand the contribution of different GABA-A subtypes.

The second research line, regarding allopregnanolone effect on PKCε expression in Schwann cells and DRG neurons, indicates that allopregnanolone-mediated

modulation of this protein kinase may indeed have a role in the control of neuropathic pain peripheral mechanisms. These outcomes further confirmed the importance of neuron-glia interaction in the modulation of neuropathic pain processes. Future experiments will regard the identification of the mediator responsible for Schwann-cell mediated allopregnanolone action on PKCε expression levels and localization. Moreover, the effective role of PKCε in pain transmission in the peripheral nervous system will be investigated with behavioural and/or electrophysiological approaches.

Lastly, the analysis of mPRs presence and activity in Schwann cells deepens our knowledge about progestogen modulation of peripheral glial cell physiology, adding new players in this already complicated context. These findings will open a completely new line of research on the role of progestogens in the peripheral nervous system. The first studies will regard the identification of the molecular mechanism underlying the short-term effect on cell migration and the long-term effect on protein expression. Following studies will investifigate the role of mPR activation on all the parameters that were previously shown to be modulated by progestogens, in order to determine the putative mPRs contribution to these effects. Taken together, these results shed some light on different important mechanisms involved in peripheral nervous system modulation. Indeed, our findings open new intriguing lines of research that may hopefully contribute to a better knowledge of mechanisms underlying inherited and acquired peripheral neuropathies, paving the way for the identification of more effective clinical treatments.

References

Abramian AM, Comenencia-Ortiz E, Modgil A, Vien TN, Nakamura Y, Moore YE, Maguire JL, Terunuma M, Davies PA, Moss SJ (2014) **Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic GABAA receptors** *Proc Natl Acad Sci USA 111(19):7132-7.*

Abramian AM, Comenencia-Ortiz E, Vithlani M, Tretter EV, Sieghart W, Davies PA, Moss SJ (2010) **Protein kinase C phosphorylation regulates membrane insertion of GABAA receptor subtypes that mediate tonic inhibition** *J Biol Chem 285(53):41795-805.*

Aley KO, Messing RO, Mochly-Rosen D, Levine JD (2000) **Chronic hypersensitivity for inflammatory nociceptor sensitization mediated by the epsilon isozyme of protein kinase C** *J Neurosci 20(12):4680-5.*

Amir R, Kocsis JD, Devor M (2005) **Multiple interacting sites of ectopic spike electrogenesis in primary sensory neurons** *J Neurosci 25(10):2576-85.*

Aptel H, Hilaire C, Pieraut S, Boukhaddaoui H, Mallié S, Valmier J, Scamps F (2007) **The Cav3.2/alpha1H T-type Ca2+ current is a molecular determinant of excitatory effects of GABA in adult sensory neurons** *Mol Cell Neurosci 36(2):293-303.*

Ardeshiri A, Kelley MH, Korner IP, Hurn PD, Herson PS (2006) **Mechanism of progesterone neuroprotection of rat cerebellar Purkinje cells following oxygen-glucose deprivation** *Eur J Neurosci 24(9):2567-74.*

Arellano RO, Sánchez-Gómez MV, Alberdi E, Canedo-Antelo M, Chara JC, Palomino A, Pérez-Samartín A, Matute C (2016) **Axon-to-Glia Interaction Regulates GABAA Receptor Expression in Oligodendrocytes** *Mol Pharmacol 89(1):63-74.*

Arthur-Farraj P, Wanek K, Hantke J, Davis CM, Jayakar A, Parkinson DB, Mirsky R, Jessen KR (2011) **Mouse schwann cells need both NRG1 and cyclic AMP to myelinate** *Glia 59(5):720-33.*

Atanasoski S, Scherer SS, Sirkowski E, Leone D, Garratt AN, Birchmeier C, Suter U (2006) **ErbB2 signaling in Schwann cells is mostly dispensable for maintenance of myelinated peripheral nerves and proliferation of adult Schwann cells after injury** *J Neurosci 26(7):2124-31.*

Azcoitia I, Leonelli E, Magnaghi V, Veiga S, Garcia-Segura LM, Melcangi RC (2003) **Progesterone and its derivatives dihydroprogesterone and tetrahydroprogesterone reduce myelin fiber morphological abnormalities and myelin fiber loss in the sciatic nerve of aged rats** *Neurobiol Aging 24(6):853-60.*

Balasubramanian S, Fam SR, Hall RA (2007) **GABAB receptor association with the PDZ scaffold Mupp1 alters receptor stability and function** *J Biol Chem 282(6):4162-71.*

Balic E, Rudolph U, Fritschy JM, Mohler H, Benke D (2009) **The alpha5(H105R) mutation impairs alpha5 selective binding properties by altered positioning of the alpha5 subunit in GABAA receptors containing two distinct types of alpha subunits** *J Neurochem 110(1):244-54.*

Balice-Gordon RJ, Bone LJ, Scherer SS (1998) **Functional gap junctions in the schwann cell myelin sheath** *J Cell Biol 142(4):1095-104.*

Basbaum AI, Bautista DM, Scherrer G, Julius D (2009) **Cellular and molecular mechanisms of pain** *Cell 139(2):267-84.*

Basbaum AI, Jessell T (2000) **The perception of pain**. *In Principles of Neuroscience, E.R. Kandel, J. Schwartz, and T. Jessell, eds. (New York: Appleton and Lange), pp. 472–491.*

Baulieu EE (1997) **Neurosteroids: of the nervous system, by the nervous system, for the nervous system** *Recent Prog Horm Res 52:1-32.*

Belelli D, Casula A, Ling A, Lambert JJ (2002) **The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors** *Neuropharmacology 43(4):651-61.*

Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW (2009) **Extrasynaptic GABAA receptors: form, pharmacology, and function** *J Neurosci 29(41):12757-63.*

Belelli D, Lambert JJ (2005) **Neurosteroids: endogenous regulators of the GABA(A) receptor** *Nat Rev Neurosci 6(7):565-75.*

Ben-Ari Y (2002) **Excitatory actions of gaba during development: the nature of the nurture** *Nat Rev Neurosci 3(9):728-39.*

Bennett GJ, Chung JM, Honore M, Seltzer Z (2003) **Models of neuropathic pain in the rat** *Curr Protoc Neurosci Chapter 9:Unit 9.14.*

Berger P, Niemann A, Suter U (2006) **Schwann cells and the pathogenesis of inherited motor and sensory neuropathies (Charcot-Marie-Tooth disease)** *Glia 54(4):243-57.*

Berger T, Walz W, Schnitzer J, Kettenmann H (1992) **GABA- and glutamate-activated currents in glial cells of the mouse corpus callosum slice** *J Neurosci Res 31(1):21-7.*

Bettler B, Tiao JY (2006) **Molecular diversity, trafficking and subcellular localization of GABAB receptors** *Pharmacol Ther 110(3):533-43.*

Blein S, Ginham R, Uhrin D, Smith BO, Soares DC, Veltel S, McIlhinney RA, White JH, Barlow PN (2004) **Structural analysis of the complement control protein (CCP) modules of GABA(B) receptor 1a: only one of the two CCP modules is compactly folded** *J Biol Chem 279(46):48292-306.*

Boileau AJ, Pearce RA, Czajkowski C (2005) **Tandem subunits effectively constrain GABAA receptor stoichiometry and recapitulate receptor kinetics but are insensitive to GABAA receptor-associated protein** *J Neurosci 25(49):11219-30.*

Bonin RP, Labrakakis C, Eng DG, Whissell PD, De Koninck Y, Orser BA (2011) **Pharmacological enhancement of δ-subunit-containing GABA(A) receptors that generate a tonic inhibitory conductance in spinal neurons attenuates acute nociception in mice** *Pain 152(6):1317-26.*

Borghini I, Ania-Lahuerta A, Regazzi R, Ferrari G, Gjinovci A, Wollheim CB, Pralong WF (1994) **Alpha, beta I, beta II, delta, and epsilon protein kinase C isoforms and compound activity in the sciatic nerve of normal and diabetic rats** *J Neurochem 62(2):686-96.*

Bostock H, Campero M, Serra J, Ochoa JL **Temperature-dependent double spikes in C-nociceptors of neuropathic pain patients** *Brain 128(Pt 9):2154-63.*

Bourque PR, Chardon JW, Massie R (2015) **Autoimmune peripheral neuropathies** *Clin Chim Acta 449:37-42.*

Bovolin P, Santi MR, Puia G, Costa E, Grayson D (1992) **Expression patterns of gamma-aminobutyric acid type A receptor subunit mRNAs in primary cultures of granule neurons and astrocytes from neonatal rat cerebella** *Proc Natl Acad Sci USA 89(19):9344-8.*

Bowery NG (1993) **GABAB receptor pharmacology** *Annu Rev Pharmacol Toxicol 33:109-47.*

Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, Turnbull M (1980) **(-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor** *Nature 283(5742):92-4.*

Boyd JG, Gordon T (2003) **Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury** *Mol Neurobiol 27(3):277-324.*

Bozzali M, Wrabetz L (2004) **Axonal signals and oligodendrocyte differentiation** *Neurochem Res 29(5):979-88.*

Braathen GJ, Sand JC, Lobato A, Høyer H, Russell MB (2011) **Genetic epidemiology of Charcot-Marie-Tooth in the general population** *Eur J Neurol 18(1):39-48.*

Bravo-Hernández M, Corleto JA, Barragán-Iglesias P, González-Ramírez R, Pineda-Farias JB, Felix R, Calcutt NA, Delgado-Lezama R, Marsala M, Granados-Soto V (2016) **The α5 subunit containing GABAA receptors contribute to chronic pain** *Pain 157(3):613-26.*

Bravo-Hernández M, Feria-Morales LA, Torres-López JE, Cervantes-Durán C, Delgado-Lezama R, Granados-Soto V, Rocha-González HI (2014) **Evidence for the participation of peripheral α5 subunit-containing GABAA receptors in GABAA agonists-induced nociception in rats** *Eur J Pharmacol 734:91-7.*

Bremer J, O'Connor T, Tiberi C, Rehrauer H, Weis J, Aguzzi A (2010) **Ablation of Dicer from murine Schwann cells increases their proliferation while blocking myelination** *PLoS One 5(8):e12450.*

Brown AR, Herd MB, Belelli D, Lambert JJ (2009) **The actions of neurosteroids and THIP upon thalamic inhibitory transmission: the role of extrasynaptic α4β2δ GABAA receptors** *Trabajos del Instituto Cajal International Meeting: Steroids and nervous system (Panzica GC, Gotti S, eds), pp 182. Madrid: Instituto Cajal.*

Brown DA, Adams PR, Higgins AJ, Marsh S (1979**) Distribution of gaba-receptors and gaba-carriers in the mammalian nervous system** *J Physiol (Paris) 75(6):667-71.*

Brown DA, Marsh S (1978) **Axonal GABA-receptors in mammalian peripheral nerve trunks** *Brain Res 156(1):187-91.*

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (2002) **Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors** *Br J Pharmacol 136(7):965-74.*

Bruce-Keller AJ, Barger SW, Moss NI, Pham JT, Keller JN, Nath A (2001) **Pro-inflammatory and pro-oxidant properties of the HIV protein Tat in a microglial cell line: attenuation by 17 beta-estradiol** *J Neurochem 78(6):1315-24.*

Brushart TM (2011) **Nerve repair** *Oxford University Press, New York; Oxford*

Brussaard AB, Koksma JJ (2003) **Conditional regulation of neurosteroid sensitivity of GABAA receptors** *Ann NY Acad Sci 1007:29-36.*

Brussaard AB, Wossink J, Lodder JC, Kits KS (2000) **Progesterone-metabolite prevents protein kinase C-dependent modulation of gamma-aminobutyric acid type A receptors in oxytocin neurons** *Proc Natl Acad Sci USA 97(7):3625-30.*

Burgess GM, Mullaney I, McNeill M, Dunn PM, Rang HP (1989) **Second messengers involved in the mechanism of action of bradykinin in sensory neurons in culture** *J Neurosci 3314-25.*

Burstyn-Cohen T, Frumkin A, Xu YT, Scherer SS, Klar A (1998) **Accumulation of F-spondin in injured peripheral nerve promotes the outgrowth of sensory axons** *J Neurosci 18(21):8875-85.*

Cahill MA (2007) **Progesterone receptor membrane component 1: an integrative review** *J Steroid Biochem Mol Biol 105(1-5):16-36.*

Callachan H, Cottrell GA, Hather NY, Lambert JJ, Nooney JM, Peters JA (1987) **Modulation of the GABAA receptor by progesterone metabolites** *Proc R Soc Lond B Biol Sci 231(1264):359-69.*

Camargo N, Smit AB, Verheijen MH (2009) **SREBPs: SREBP function in glia-neuron interactions** *FEBS J 276(3):628-36.*

Campbell JN, Raja SN, Meyer RA, Mackinnon SE (1988) **Myelinated afferents signal the hyperalgesia associated with nerve injury** *Pain 32(1):89-94.*

Campbell V, Berrow N, Dolphin AC (1993) **GABAB receptor modulation of Ca2+ currents in rat sensory neurones by the G protein G(0): antisense oligonucleotide studies** *J Physiol 470:1-11.*

Carr RW, Sittl R, Fleckenstein J, Grafe P (2010) **GABA increases electrical excitability in a subset of human unmyelinated peripheral axons** *PLoS One 5(1):e8780.*

Carver CM, Reddy DS (2013) **Neurosteroid interactions with synaptic and extrasynaptic GABA(A) receptors: regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability** *Psychopharmacology (Berl) 230(2):151-88.*

Carver CM, Wu X, Gangisetty O, Reddy DS (2014) **Perimenstrual-like hormonal regulation of extrasynaptic δ-containing GABAA receptors mediating tonic inhibition and neurosteroid sensitivity** *J Neurosci 34(43):14181-97.*

Cesare P, McNaughton P (1996) **A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin** *Proc Natl Acad Sci USA 93(26):15435-9.*

Celotti F, Melcangi RC, Martini L (1992) **The 5 alpha-reductase in the brain: molecular aspects and relation to brain function** *Front Neuroendocrinol 13(2):163-215.*

Chan JR, Cosgaya JM, Wu YJ, Shooter EM (2001) **Neurotrophins are key mediators of the myelination program in the peripheral nervous system** *Proc Natl Acad Sci USA 98(25):14661-8.*

Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE (2006) **GABAA receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol** *Proc Natl Acad Sci USA 103(41):15230-5.*

Charles KJ, Deuchars J, Davies CH, Pangalos MN (2003) **GABA B receptor subunit expression in glia** *Mol Cell Neurosci 24(1):214-23.*

Charles KJ, Evans ML, Robbins MJ, Calver AR, Leslie RA, Pangalos MN (2001) **Comparative immunohistochemical localisation of GABA(B1a), GABA(B1b) and GABA(B2) subunits in rat brain, spinal cord and dorsal root ganglion** *Neuroscience 106(3):447-67.*

Chen G, Masana MI, Manji HK (2000) **Lithium regulates PKC-mediated intracellular cross-talk and gene expression in the CNS in vivo** *Bipolar Disord 2(3 Pt 2):217-36.*

Chen ZL, Yu WM, Strickland S (2007) **Peripheral regeneration** *Annu Rev Neurosci 30:209-33.*

Cheng HL, Russell JW, Feldman EL (1999) **IGF-I promotes peripheral nervous system myelination** *Ann NY Acad Sci 883:124-30.*

Chernousov MA, Yu WM, Chen ZL, Carey DJ, Strickland S (2008) **Regulation of Schwann cell function by the extracellular matrix** *Glia 56(14):1498-507.*

Chou WH, Wang D, McMahon T, Qi ZH, Song M, Zhang C, Shokat KM, Messing RO (2010) **GABAA receptor trafficking is regulated by protein kinase C(epsilon) and the N-ethylmaleimide-sensitive factor** *J Neurosci 30(42):13955-65.*

Connelly WM, Errington AC, Di Giovanni G, Crunelli V (2013) **Metabotropic regulation of extrasynaptic GABAA receptors** *Front Neural Circuits 7:171.*

Cooke PS, Nanjappa MK, Yang Z, Wang KK (2013) **Therapeutic effects of progesterone and its metabolites in traumatic brain injury may involve non-classical signaling mechanisms** *Front Neurosci 7:108.*

Costigan M, Scholz J, Woolf CJ (2009) **Neuropathic pain: a maladaptive response of the nervous system to damage** *Annu Rev Neurosci 32:1-32.*

Couve A, Thomas P, Calver AR, Hirst WD, Pangalos MN, Walsh FS, Smart TG, Moss SJ (2002) **Cyclic AMP-dependent protein kinase phosphorylation facilitates GABA(B) receptor-effector coupling** *Nat Neurosci 5(5):415-24.*

Crandall M, Kwash J, Yu W, White G (2002) **Activation of protein kinase C sensitizes human VR1 to capsaicin and to moderate decreases in pH at physiological temperatures in Xenopus oocytes** *Pain 98(1-2):109-17.*

Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Blüthmann H, Möhler H, Rudolph U (2002) **Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors** *Proc Natl Acad Sci USA 99(13):8980-5.*

Cui JG, Meyerson BA, Sollevi A, Linderoth B (1998) **Effect of spinal cord stimulation on tactile hypersensitivity in mononeuropathic rats is potentiated by simultaneous GABA(B) and adenosine receptor activation** *Neurosci Lett 247(2-3):183-6.*

D'Amour ML, Butterworth RF (1994) **Pathogenesis of alcoholic peripheral neuropathy: direct effect of ethanol or nutritional deficit?** *Metab Brain Dis 9(2):133-42.*

Debby-Brafman A, Burstyn-Cohen T, Klar A, Kalcheim C (1999) **F-Spondin, expressed in somite regions avoided by neural crest cells, mediates inhibition of distinct somite domains to neural crest migration** *Neuron 22(3):475-88.*

Decker L, Desmarquet-Trin-Dinh C, Taillebourg E, Ghislain J, Vallat JM, Charnay P (2006) **Peripheral myelin maintenance is a dynamic process requiring constant Krox20 expression** *J Neurosci 26(38):9771-9.*

de Groote C, Wüllner U, Löchmann PA, Luiten PG, Klockgether T (1999) **Functional characterization and expression of thalamic GABA(B) receptors in a rodent model of Parkinson's disease** *Neuropharmacology 38(11):1683-9.*

de la Monte SM, Kril JJ (2014) **Human alcohol-related neuropathology** *Acta Neuropathol 127(1):71-90.*

Devor A, Fritschy JM, Yarom Y (2001) **Spatial distribution and subunit composition of GABA(A) receptors in the inferior olivary nucleus** *J Neurophysiol 85(4):1686-96.*

Diversé-Pierluissi M, Remmers AE, Neubig RR, Dunlap K (1997) **Novel form of crosstalk between G protein and tyrosine kinase pathways** *Proc Natl Acad Sci USA 94(10):5417-21.*

Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG (2005) **The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats** *J Neurotrauma 22(1):106-18.*

Dolphin AC, Scott RH (1986) **Inhibition of calcium currents in cultured rat dorsal root ganglion neurones by (-)-baclofen** *Br J Pharmacol 88(1):213-20.*

Dong Z, Brennan A, Liu N, Yarden Y, Lefkowitz G, Mirsky R, Jessen KR (1995) **Neu differentiation factor is a neuron-glia signal and regulates survival, proliferation, and maturation of rat Schwann cell precursors** *Neuron 15(3):585-96.*

Dray A, Bettaney J, Forster P, Perkins MN (1988) **Bradykinin-induced stimulation of afferent fibres is mediated through protein kinase C** *Neurosci Lett 91(3):301-7.*

Dressing GE, Goldberg JE, Charles NJ, Schwertfeger KL, Lange CA (2011) **Membrane progesterone receptor expression in mammalian tissues: a review of regulation and physiological implications** *Steroids 76(1-2):11-7.*

Drew PD, Chavis JA (2000) **Female sex steroids: effects upon microglial cell activation** *J Neuroimmunol 111(1-2):77-85.*

Dworkin RH, Backonja M, Rowbotham MC, Allen RR, Argoff CR, Bennett GJ, Bushnell MC, Farrar JT, Galer BS, Haythornthwaite JA, Hewitt DJ, Loeser JD, Max MB, Saltarelli M, Schmader KE, Stein C, Thompson D, Turk DC, Wallace MS, Watkins LR, Weinstein SM (2003) **Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations** *Arch Neurol 60(11):1524-34.*

Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacey BR, Treede RD, Turk DC, Wallace MS (2007) **Pharmacologic management of neuropathic pain: evidence-based recommendations** *Pain 132(3):237-51.*

Dyck PJ, Chance P, Lebo R, Carney JA (1993) **Peripheral Neuropathy** *Eds Dyck PJ et al. 1094-1136.*

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ (1991) **Two genes encode distinct glutamate decarboxylases** *Neuron 7(1):91-100.*

Falkenstein E, Heck M, Gerdes D, Grube D, Christ M, Weigel M, Buddhikot M, Meizel S, Wehling M (1999) **Specific progesterone binding to a membrane protein and related nongenomic effects on Ca2+-fluxes in sperm** *Endocrinology 140(12):5999-6002.*

Falkenstein E, Meyer C, Eisen C, Scriba PC, Wehling M (1996) **Full-length cDNA sequence of a progesterone membrane-binding protein from porcine vascular smooth muscle cells** *Biochem Biophys Res Commun 229(1):86-9.*

Fáncsik A, Linn DM, Tasker JG (2000) **Neurosteroid modulation of GABA IPSCs is phosphorylation dependent** *J Neurosci 20(9):3067-75.*

Faroni A, Calabrese F, Riva MA, Terenghi G, Magnaghi V (2013) **Baclofen modulates the expression and release of neurotrophins in Schwann-like adipose stem cells** *J Mol Neurosci 49(2):233-43.*

Faroni A, Castelnovo LF, Procacci P, Caffino L, Fumagalli F, Melfi S, Gambarotta G, Bettler B, Wrabetz L, Magnaghi V (2014) **Deletion of GABA-B receptor in Schwann cells regulates remak bundles and small nociceptive C-fibers** *Glia 62(4):548-65.*

Faroni A, Magnaghi V (2011) **The neurosteroid allopregnanolone modulates specific functions in central and peripheral glial cells** *Front Endocrinol (Lausanne) 2:103.*

Faroni A, Mobasseri SA, Kingham PJ, Reid AJ (2015) **Peripheral nerve regeneration: experimental strategies and future perspectives** *Adv Drug Deliv Rev 82-83:160-7.*

Feltri ML, D'Antonio M, Previtali S, Fasolini M, Messing A, Wrabetz L (1999) **P0-Cre transgenic mice for inactivation of adhesion molecules in Schwann cells** *Ann NY Acad Sci 883:116-23.*

Feltri ML, Poitelon Y, Previtali SC (2016) **How Schwann Cells Sort Axons: New Concepts** *Neuroscientist 22(3):252-65.*

Fenrich K, Gordon T (2004) **Canadian Association of Neuroscience review: axonal regeneration in the peripheral and central nervous systems--current issues and advances** *Can J Neurol Sci 31(2):142-56.*

Ferguson C, Hardy SL, Werner DF, Hileman SM, Delorey TM, Homanics GE (2007) **New insight into the role of the beta3 subunit of the GABAA-R in development, behavior, body weight regulation, and anesthesia revealed by conditional gene knockout** *BMC Neurosci 8:85.*

Ferando I, Mody I (2012) **GABAA receptor modulation by neurosteroids in models of temporal lobe epilepsies** *Epilepsia 53 Suppl 9:89-101.*

Fields RD, Burnstock G (2006) **Purinergic signalling in neuron-glia interactions** *Nat Rev Neurosci 7(6):423-36.*

Filbin MT, Walsh FS, Trapp BD, Pizzey JA, Tennekoon GI (1990) **Role of myelin P0 protein as a homophilic adhesion molecule** *Nature 344(6269):871-2.*

Finn DA, Beadles-Bohling AS, Beckley EH, Ford MM, Gililland KR, Gorin-Meyer RE, Wiren KM (2006) **A new look at the 5alpha-reductase inhibitor finasteride** *CNS Drug Rev 12(1):53-76.*

Fontana X, Hristova M, Da Costa C, Patodia S, Thei L, Makwana M, Spencer-Dene B, Latouche M, Mirsky R, Jessen KR, Klein R, Raivich G, Behrens A (2012) **c-Jun in Schwann cells promotes axonal regeneration and motoneuron survival via paracrine signaling** *J Cell Biol 198(1):127-41.*

Foster H, Reynolds A, Stenbeck G, Dong J, Thomas P, Karteris E (2010) **Internalisation of membrane progesterone receptor-α after treatment with progesterone: Potential involvement of a clathrin-dependent pathway** *Mol Med Rep 3(1):27-35.*

Fricker FR, Lago N, Balarajah S, Tsantoulas C, Tanna S, Zhu N, Fageiry SK, Jenkins M, Garratt AN, Birchmeier C, Bennett DL (2011) **Axonally derived neuregulin-1 is required for remyelination and regeneration after nerve injury in adulthood** *J Neurosci 31(9):3225-33.*

Fritschy JM, Kiener T, Bouilleret V, Loup F (1999) **GABAergic neurons and GABA(A)-receptors in temporal lobe epilepsy** *Neurochem Int 34(5):435-45.*

Fritschy JM, Mohler H (1995) **GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits** *J Comp Neurol 359(1):154-94.*

Fritschy JM, Panzanelli P (2014) **GABAA receptors and plasticity of inhibitory neurotransmission in the central nervous system** *Eur J Neurosci 39(11):1845-65.*

Froestl W (2010) **Chemistry and pharmacology of GABAB receptor ligands** *Adv Pharmacol 58:19-62.*

Fromm GH (1989) **The pharmacology of trigeminal neuralgia** *Clin Neuropharmacol 12(3):185-94.*

Fruttiger M, Montag D, Schachner M, Martini R (1995) **Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity** *Eur J Neurosci 7(3):511-5.*

Fu SY, Gordon T (1997) **The cellular and molecular basis of peripheral nerve regeneration** *Mol Neurobiol 14(1-2):67-116.*

Gago N, El-Etr M, Sananès N, Cadepond F, Samuel D, Avellana-Adalid V, Baron-Van Evercooren A, Schumacher M (2004) **3alpha,5alpha-Tetrahydroprogesterone (allopregnanolone) and gamma-aminobutyric acid: autocrine/paracrine interactions in the control of neonatal PSA-NCAM+ progenitor proliferation** *J Neurosci Res 78(6):770-83.*

Gangadharan V, Agarwal N, Brugger S, Tegeder I, Bettler B, Kuner R, Kurejova M (2009) **Conditional gene deletion reveals functional redundancy of GABAB receptors in peripheral nociceptors in vivo** *Mol Pain 5:68.*

Gassmann M, Bettler B (2012) **Regulation of neuronal GABA(B) receptor functions by subunit composition** *Nat Rev Neurosci 13(6):380-94.*

Gaudet AD, Popovich PG, Ramer MS (2011) **Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury** *J Neuroinflammation 8:110.*

Ghezzi P, Santo ED, Sacco S, Foddi C, Barbaccia ML, Mennini T (2000) **Neurosteroid levels are increased in vivo after LPS treatment and negatively regulate LPS-induced TNF production** *Eur Cytokine Netw 11(3):464-9.*

Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, Schumacher M (2003) **Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum** *J Neurochem 86(4):848-59.*

Glenn TD, Talbot WS (2013a) **Analysis of Gpr126 function defines distinct mechanisms controlling the initiation and maturation of myelin** *Development 140(15):3167-75.*

Glenn TD, Talbot WS (2013b) **Signals regulating myelination in peripheral nerves and the Schwann cell response to injury** *Curr Opin Neurobiol 23(6):1041-8.*

Goebbels S, Oltrogge JH, Kemper R, Heilmann I, Bormuth I, Wolfer S, Wichert SP, Möbius W, Liu X, Lappe-Siefke C, Rossner MJ, Groszer M, Suter U, Frahm J, Boretius S, Nave KA (2010) **Elevated phosphatidylinositol 3,4,5-trisphosphate in glia triggers cell-autonomous membrane wrapping and myelination** *J Neurosci 30(26):8953-64.*

Goebbels S, Oltrogge JH, Wolfer S, Wieser GL, Nientiedt T, Pieper A, Ruhwedel T, Groszer M, Sereda MW, Nave KA (2012) **Genetic disruption of Pten in a novel mouse model of tomaculous neuropathy** *EMBO Mol Med 4(6):486-99.*

Gonzalez SL, Labombarda F, Gonzalez Deniselle MC, Mougel A, Guennoun R, Schumacher M, De Nicola AF (2005) **Progesterone neuroprotection in spinal cord trauma involves up-regulation of brain-derived neurotrophic factor in motoneurons** *J Steroid Biochem Mol Biol 94(1-3):143-9.*

Gordon T, English AW (2015) **Strategies to promote peripheral nerve regeneration: electrical stimulation and/or exercise** *Eur J Neurosci 43(3):336-50.*

Gordon T, Tyreman N, Raji MA (2011) **The basis for diminished functional recovery after delayed peripheral nerve repair** *J Neurosci 31(14):5325-34.*

Grasshoff C, Jurd R, Rudolph U, Antkowiak B (2007) **Modulation of presynaptic beta3-containing GABAA receptors limits the immobilizing actions of GABAergic anesthetics** *Mol Pharmacol 72(3):780-7.*

Gregg EW, Cadwell BL, Cheng YJ, Cowie CC, Williams DE, Geiss L, Engelgau MM, Vinicor F (2004) **Trends in the prevalence and ratio of diagnosed to undiagnosed diabetes according to obesity levels in the U.S.** *Diabetes Care 27 (12):2806-12.*

Grossmann KS, Wende H, Paul FE, Cheret C, Garratt AN, Zurborg S, Feinberg K, Besser D, Schulz H, Peles E, Selbach M, Birchmeier W, Birchmeier C (2009) **The tyrosine phosphatase Shp2 (PTPN11) directs Neuregulin-1/ErbB signaling throughout Schwann cell development** *Proc Natl Acad Sci USA 106(39):16704-9.*

Guennoun R, Labombarda F, Gonzalez Deniselle MC, Liere P, De Nicola AF, Schumacher M (2015) **Progesterone and allopregnanolone in the central nervous system: response to injury and implication for neuroprotection** *J Steroid Biochem Mol Biol 146:48-61.*

Guennoun R, Meffre D, Labombarda F, Gonzalez SL, Gonzalez Deniselle MC, Stein DG, De Nicola AF, Schumacher M (2008) **The membrane-associated progesterone-binding protein 25-Dx: expression, cellular localization and up-regulation after brain and spinal cord injuries** *Brain Res Rev 57(2):493-505.*

Gutiérrez R (2002**) Activity-dependent expression of simultaneous glutamatergic and GABAergic neurotransmission from the mossy fibers in vitro** *J Neurophysiol 87(5), 2562-70.*

Hablitz JJ, Mathew SS, Pozzo-Miller L (2010) **GABA vesicles at synapses: are there 2 distinct pools?** *Neuroscientist 15(3):218-24.*

Haller C, Casanova E, Müller M, Vacher CM, Vigot R, Doll T, Barbieri S, Gassmann M, Bettler B (2004) **Floxed allele for conditional inactivation of the GABAB(1) gene** *Genesis 40(3):125-30.*

Hammes SR, Levin ER (2007) **Extranuclear steroid receptors: nature and actions** *Endocr Rev 28(7):726-41.*

Hanani M (2005) **Satellite glial cells in sensory ganglia: from form to function** *Brain Res Brain Res Rev 48(3):457-76.*

Harrison NL, Vicini S, Barker JL (1987) **A steroid anesthetic prolongs inhibitory postsynaptic currents in cultured rat hippocampal neurons** *J Neurosci 7(2):604-9.*

He Y, Kim JY, Dupree J, Tewari A, Melendez-Vasquez C, Svaren J, Casaccia P (2010) **Yy1 as a molecular link between neuregulin and transcriptional modulation of peripheral myelination** *Nat Neurosci 13(12):1472-80.*

Heller BA, Ghidinelli M, Voelkl J, Einheber S, Smith R, Grund E, Morahan G, Chandler D, Kalaydjieva L, Giancotti F, King RH, Fejes-Toth AN, Fejes-Toth G, Feltri ML, Lang F, Salzer JL (2014) **Functionally distinct PI 3-kinase pathways regulate myelination in the peripheral nervous system** *J Cell Biol 204(7):1219-36.*

Hering-Hanit R (1999**) Baclofen for prevention of migraine** *Cephalalgia 19(6):589-91.*

Hering-Hanit R, Gadoth N (2001) **The use of baclofen in cluster headache** *Curr Pain Headache Rep 5(1):79-82.*

Hirst JJ, Yawno T, Nguyen P, Walker DW (2006) **Stress in pregnancy activates neurosteroid production in the fetal brain** *Neuroendocrinology 84(4):264-74.*

Hodge CW, Mehmert KK, Kelley SP, McMahon T, Haywood A, Olive MF, Wang D, Sanchez-Perez AM, Messing RO (1999) **Supersensitivity to allosteric GABA(A) receptor modulators and alcohol in mice lacking PKCepsilon** *Nat Neurosci 2(11):997-1002.*

Hodge CW, Raber J, McMahon T, Walter H, Sanchez-Perez AM, Olive MF, Mehmert K, Morrow AL, Messing RO (2002) **Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase Cepsilon** *J Clin Invest 110(7):1003-10.*

Hojo Y, Hattori TA, Enami T, Furukawa A, Suzuki K, Ishii HT, Mukai H, Morrison JH, Janssen WG, Kominami S, Harada N, Kimoto T, Kawato S (2004) **Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons** *Proc Natl Acad Sci USA 101(3):865-70.*

Höke A, Ho T, Crawford TO, LeBel C, Hilt D, Griffin JW (2003) **Glial cell line-derived neurotrophic factor alters axon schwann cell units and promotes myelination in unmyelinated nerve fibers** *J Neurosci 23(2):561-7.*

Höke A, Redett R, Hameed H, Jari R, Zhou C, Li ZB, Griffin JW, Brushart TM (2006) **Schwann cells express motor and sensory phenotypes that regulate axon regeneration** *J Neurosci 26(38):9646-55.*

Hoppe D, Kettenmann H (1989) **GABA triggers a Cl- efflux from cultured mouse oligodendrocytes** *Neurosci Lett 97(3):334-9.*

Hörtnagl H, Tasan RO, Wieselthaler A, Kirchmair E, Sieghart W, Sperk G (2013) **Patterns of mRNA and protein expression for 12 GABAA receptor subunits in the mouse brain** *Neuroscience 236:345-72.*

Hosie AM, Clarke L, da Silva H, Smart TG (2009) **Conserved site for neurosteroid modulation of GABA A receptors** *Neuropharmacology 56(1):149-54.*

Hosie AM, Wilkins ME, da Silva HM, Smart TG (2006) **Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites** *Nature 444(7118):486-9.*

Hösli E, Otten U, Hösli L (1997) **Expression of GABA(A) receptors by reactive astrocytes in explant and primary cultures of rat CNS** *Int J Dev Neurosci 15(8):949-60.*

Hwang JH, Yaksh TL (1997) **The effect of spinal GABA receptor agonists on tactile allodynia in a surgically-induced neuropathic pain model in the rat** *Pain 70(1):15-22.*

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U (2000) **Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances** *Proc Natl Acad Sci USA 97(11):6155-60.*

Ibanez C, Shields SA, El-Etr M, Leonelli E, Magnaghi V, Li WW, Sim FJ, Baulieu EE, Melcangi RC, Schumacher M, Franklin RJ (2003) **Steroids and the reversal of age-associated changes in myelination and remyelination** *Prog Neurobiol 71(1):49-56.*

Imreová H, Pura M (2005) **Differential diagnosis of peripheral neuropathy** *Cas Lek Cesk 144(9):628-33; discussion 633-5.*

Intlekofer KA, Petersen SL (2011) **Distribution of mRNAs encoding classical progestin receptor, progesterone membrane components 1 and 2, serpine mRNA binding protein 1, and progestin and ADIPOQ receptor family members 7 and 8 in rat forebrain** *Neuroscience 172:55-65.*

Isomoto S, Kaibara M, Sakurai-Yamashita Y, Nagayama Y, Uezono Y, Yano K, Taniyama K (1998) **Cloning and tissue distribution of novel splice variants of the rat GABAB receptor** *Biochem Biophys Res Commun 253(1):10-5.*

Israel JM, Schipke CG, Ohlemeyer C, Theodosis DT, Kettenmann H (2003) **GABAA receptor-expressing astrocytes in the supraoptic nucleus lack glutamate uptake and receptor currents** *Glia 44(2):102-10.*

Jacob TC, Moss SJ, Jurd R (2008) **GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition** *Nat Rev Neurosci 9(5):331-43.*

Jaegle M, Ghazvini M, Mandemakers W, Piirsoo M, Driegen S, Levavasseur F, Raghoenath S, Grosveld F, Meijer D (2003) **The POU proteins Brn-2 and Oct-6 share important functions in Schwann cell development** *Genes Dev 17(11):1380-91.*

Jefcoate C (2002) **High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex** *J Clin Invest 110(7):881-90.*

Jessen KR, Mirsky R (2005) **The origin and development of glial cells in peripheral nerves** *Nat Rev Neurosci 6(9):671-82.*

Jessen KR, Mirsky R, Lloyd AC (2015) **Schwann Cells: Development and Role in Nerve Repair** *Cold Spring Harb Perspect Biol 7(7):a020487.*

Jessen KR, Mirsky R, Salzer J (2008) **Introduction. Schwann cell biology** *Glia 56(14):1479-80.*

Jivan S, Kumar N, Wiberg M, Kay S (2009) **The influence of pre-surgical delay on functional outcome after reconstruction of brachial plexus injuries** *J Plast Reconstr Aesthet Surg 62(4):472-9.*

Jones BL, Henderson LP (2007) **Trafficking and potential assembly patterns of epsilon-containing GABAA receptors** *J Neurochem 103(3):1258-71.*

Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, Tang C, Shen Q, Salon JA, Morse K, Laz T, Smith KE, Nagarathnam D, Noble SA, Branchek TA, Gerald C (1998) **GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2** *Nature 396(6712):674-9.*

Jordan CL, Price RH Jr, Handa RJ (2002) **Androgen receptor messenger RNA and protein in adult rat sciatic nerve: implications for site of androgen action** *J Neurosci Res 69(4):509-18.*

Julius D, Basbaum AI (2001) **Molecular mechanisms of nociception** *Nature 413(6852):203-10.*

Jung-Testas I, Baulieu EE (1998) **Steroid hormone receptors and steroid action in rat glial cells of the central and peripheral nervous system** *J Steroid Biochem Mol Biol 65(1-6):243-51.*

Jung-Testas I, Schumacher M, Robel P, Baulieu EE (1996) **Demonstration of progesterone receptors in rat Schwann cells** *J Steroid Biochem Mol Biol 58(1):77-82.*

Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U (2003) **General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit** *FASEB J 17(2):250-2.*

Kaminski RM, Marini H, Ortinski PI, Vicini S, Rogawski MA (2006) **The pheromone androstenol (5 alpha-androst-16-en-3 alpha-ol) is a neurosteroid positive modulator of GABAA receptors** *J Pharmacol Exp Ther 317(2):694-703.*

Kanaide M, Uezono Y, Matsumoto M, Hojo M, Ando Y, Sudo Y, Sumikawa K, Taniyama K (2007) **Desensitization of GABA(B) receptor signaling by formation of protein complexes of GABA(B2) subunit with GRK4 or GRK5** *J Cell Physiol 210(1):237-45.*

Kao SC, Wu H, Xie J, Chang CP, Ranish JA, Graef IA, Crabtree GR (2009) **Calcineurin/NFAT signaling is required for neuregulin-regulated Schwann cell differentiation** *Science 323(5914):651-4.*

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, Bettler B (1997) **Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors** *Nature 386(6622):239-46.*

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B (1998) **GABA(B)-receptor subtypes assemble into functional heteromeric complexes** *Nature 396(6712):683-7.*

Kawano S, Okajima S, Mizoguchi A, Tamai K, Hirasawa Y, Ide C (1997) **Immunocytochemical distribution of Ca(2+)-independent protein kinase C subtypes (delta, epsilon, and zeta) in regenerating axonal growth cones of rat peripheral nerve** *Neuroscience 81(1):263-73.*

Kegel L, Aunin E, Meijer D, Bermingham JR (2013) **LGI proteins in the nervous system** *ASN Neuro 5(3):167-81.*

Kelder J, Azevedo R, Pang Y, de Vlieg J, Dong J, Thomas P (2010) **Comparison between steroid binding to membrane progesterone receptor alpha (mPRalpha) and to nuclear progesterone receptor: correlation with physicochemical properties assessed by comparative molecular field analysis and identification of mPRalpha-specific agonists** *Steroids 75(4-5):314-22.*

Kelley MH, Kuroiwa M, Taguchi N, Herson PS (2011**) Sex difference in sensitivity to allopregnanolone neuroprotection in mice correlates with effect on spontaneous inhibitory post synaptic currents** *Neuropharmacology 61(4):724-9.*

Kettenmann H, Backus KH, Schachner M (1987) **gamma-Aminobutyric acid opens Cl-channels in cultured astrocytes** *Brain Res 404(1-2):1-9.*

Khasar SG, Lin YH, Martin A, Dadgar J, McMahon T, Wang D, Hundle B, Aley KO, Isenberg W, McCarter G, Green PG, Hodge CW, Levine JD, Messing RO (1999) **A novel nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice** *Neuron 24(1):253-60.*

Kia A, Ribeiro F, Nelson R, Gavrilovici C, Ferguson SS, Poulter MO (2011) **Kindling alters neurosteroid-induced modulation of phasic and tonic GABAA receptor-mediated currents: role of phosphorylation** *J Neurochem 116(6):1043-56.*

Kimoto T, Tsurugizawa T, Ohta Y, Makino J, Tamura Ho, Hojo Y, Takata N, Kawato S (2001) **Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: N-methyl-D-aspartate and calcium-dependent synthesis** *Endocrinology 142(8):3578-89.*

Kimura I, Nakayama Y, Konishi M, Terasawa K, Ohta M, Itoh N, Fujimoto M (2012) **Functions of MAPR (membrane-associated progesterone receptor) family members as heme/steroid-binding proteins** *Curr Protein Pept Sci 13(7):687-96.*

Kipanyula MJ, Woodhoo A, Rahman M, Payne D, Jessen KR, Mirsky R (2013) **Calcineurin-nuclear factor of activated T cells regulation of Krox-20 expression in Schwann cells requires elevation of intracellular cyclic AMP** *J Neurosci Res 91(1):105-15.*

Klapdor K, Dulfer BG, Hammann A, Van der Staay FJ (1997**) A low-cost method to analyse footprint patterns** *J Neurosci Methods 75(1):49-54.*

Kliewer SA, Goodwin B, Willson TM (2002) **The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism** *Endocr Rev 23(5):687-702.*

Koenig HL, Schumacher M, Ferzaz B, Thi AN, Ressouches A, Guennoun R, Jung-Testas I, Robel P, Akwa Y, Baulieu EE (1995) **Progesterone synthesis and myelin formation by Schwann cells** *Science 268(5216):1500-3.*

Kornau HC (2006) **GABA(B) receptors and synaptic modulation** *Cell Tissue Res 326(2):517-33.*

Korneyev A, Pan BS, Polo A, Romeo E, Guidotti A, Costa E (1993) **Stimulation of brain pregnenolone synthesis by mitochondrial diazepam binding inhibitor receptor ligands in vivo** *J Neurochem 61(4):1515-24.*

Kozlov AS, Angulo MC, Audinat E, Charpak S (2006) **Target cell-specific modulation of neuronal activity by astrocytes** *Proc Natl Acad Sci USA 103(26):10058-63.*

Kralic JE, Sidler C, Parpan F, Homanics GE, Morrow AL, Fritschy JM (2006) **Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha1 subunit knockout mice** *J Comp Neurol 495(4):408-21.*

Kuhn SA, van Landeghem FK, Zacharias R, Färber K, Rappert A, Pavlovic S, Hoffmann A, Nolte C, Kettenmann H (2004) **Microglia express GABA(B) receptors to modulate interleukin release** *Mol Cell Neurosci 25(2):312-22.*

Kulik A, Vida I, Fukazawa Y, Guetg N, Kasugai Y, Marker CL, Rigato F, Bettler B, Wickman K, Frotscher M, Shigemoto R (2006) **Compartment-dependent colocalization of Kir3.2-containing K+ channels and GABAB receptors in hippocampal pyramidal cells** *J Neurosci 26(16):4289-97.*

Kuner R, Köhr G, Grünewald S, Eisenhardt G, Bach A, Kornau HC (1999) **Role of heteromer formation in GABAB receptor function** *Science 283(5398):74-7.*

Kuramoto N, Wilkins ME, Fairfax BP, Revilla-Sanchez R, Terunuma M, Tamaki K, Iemata M, Warren N, Couve A, Calver A, Horvath Z, Freeman K, Carling D, Huang L, Gonzales C, Cooper E, Smart TG, Pangalos MN, Moss SJ (2007) **Phospho-dependent functional modulation of GABA(B) receptors by the metabolic sensor AMP-dependent protein kinase** *Neuron 53(2):233-47.*

Labombarda F, Gonzalez SL, Gonzalez Deniselle MC, Vinson GP, Schumacher M, De Nicola AF, Guennoun R (2003) **Effects of injury and progesterone treatment on progesterone receptor and progesterone binding protein 25-Dx expression in the rat spinal cord** *J Neurochem 87(4):902-13.*

Labombarda F, Meffre D, Delespierre B, Krivokapic-Blondiaux S, Chastre A, Thomas P, Pang Y, Lydon JP, Gonzalez SL, De Nicola AF, Schumacher M, Guennoun R (2010) **Membrane progesterone receptors localization in the mouse spinal cord** *Neuroscience 166(1):94-106.*

Lacey CJ, Boyes J, Gerlach O, Chen L, Magill PJ, Bolam JP (2005) **GABA(B) receptors at glutamatergic synapses in the rat striatum** *Neuroscience 136(4):1083-95.*

Lamba V, Yasuda K, Lamba JK, Assem M, Davila J, Strom S, Schuetz EG (2004) **PXR (NR1I2): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators** *Toxicol Appl Pharmacol 199(3):251-65.*

Lambert JJ, Belelli D, Hill-Venning C, Callachan H, Peters JA (1996) **Neurosteroid modulation of native and recombinant GABAA receptors** *Cell Mol Neurobiol 16(2):155-74.*

Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA (2003) **Neurosteroid modulation of GABAA receptors** *Prog Neurobiol 71(1):67-80.*

Langmade SJ, Gale SE, Frolov A, Mohri I, Suzuki K, Mellon SH, Walkley SU, Covey DF, Schaffer JE, Ory DS (2006) **Pregnane X receptor (PXR) activation: a mechanism for neuroprotection in a mouse model of Niemann-Pick C disease** *Proc Natl Acad Sci USA 103(37):13807-12.*

Laping NJ, Teter B, Nichols NR, Rozovsky I, Finch CE (1994) **Glial fibrillary acidic protein: regulation by hormones, cytokines, and growth factors** *Brain Pathol 4(3):259-75.*

Laplante M, Sabatini DM (2013) **Regulation of mTORC1 and its impact on gene expression at a glance** *J Cell Sci 126(Pt 8):1713-9.*

Laurie DJ, Seeburg PH, Wisden W (1992) **The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum** *J Neurosci 12(3):1063-76.*

Lazarevic V, Zullo AJ, Schweitzer MN, Staton TL, Gallo EM, Crabtree GR, Glimcher LH (2009) **The gene encoding early growth response 2, a target of the transcription factor NFAT, is required for the development and maturation of natural killer T cells** *Nat Immunol 10(3):306-13.*

Le N, Nagarajan R, Wang JY, Araki T, Schmidt RE, Milbrandt J (2005a) **Analysis of congenital hypomyelinating Egr2Lo/Lo nerves identifies Sox2 as an inhibitor of Schwann cell differentiation and myelination** *Proc Natl Acad Sci USA 102(7):2596-601.*

Le N, Nagarajan R, Wang JY, Svaren J, LaPash C, Araki T, Schmidt RE, Milbrandt J (2005b) **Nab proteins are essential for peripheral nervous system myelination** *Nat Neurosci 8(7):932-40.*

Le Douarin N, Dulac C, Dupin E, Cameron-Curry P (1991) **Glial cell lineages in the neural crest** *Glia 4(2):175-84.*

Lévi S, Le Roux N, Eugène E, Poncer JC (2015) **Benzodiazepine ligands rapidly influence GABAA receptor diffusion and clustering at hippocampal inhibitory synapses** *Neuropharmacology 88:199-208.*

Levin ER (2011) **Minireview: Extranuclear steroid receptors: roles in modulation of cell functions** *Mol Endocrinol 25(3):377-84.*

Li Y, Urban JM, Cayer ML, Plummer HK 3rd, Heckman CA (2006) **Actin-based features negatively regulated by protein kinase C-epsilon** *Am J Physiol Cell Physiol 291(5):C1002-13.*

Liang CC, Park AY, Guan JL (2007) **In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro** *Nat Protoc 2(2):329-33.*

Lieberman AR (1971) **The axon reaction: a review of the principal features of perikaryal responses to axon injury** *Int Rev Neurobiol 14:49-124.*

Lind G, Meyerson BA, Winter J, Linderoth B (2004) **Intrathecal baclofen as adjuvant therapy to enhance the effect of spinal cord stimulation in neuropathic pain: a pilot study** *Eur J Pain 8(4):377-83.*

Little AA, Albers JW (2015) **Clinical description of toxic neuropathies** *Handb Clin Neurol 131:253-96.*

Liu J, Wolfe D, Hao S, Huang S, Glorioso JC, Mata M, Fink DJ (2004) **Peripherally delivered glutamic acid decarboxylase gene therapy for spinal cord injury pain** *Mol Ther 10(1):57-66.*

Loebrich S, Bähring R, Katsuno T, Tsukita S, Kneussel M (2006) **Activated radixin is essential for GABAA receptor alpha5 subunit anchoring at the actin cytoskeleton** *EMBO J 25(5):987-99.*

Long P, Mercer A, Begum R, Stephens GJ, Sihra TS, Jovanovic JN (2009) **Nerve Terminal GABAA Receptors Activate Ca2+/Calmodulin-dependent Signaling to Inhibit Voltage-gated Ca2+ Influx and Glutamate Release** *J Biol Chem 284(13):8726-37.*

Lopez PH, Ahmad AS, Mehta NR, Toner M, Rowland EA, Zhang J, Doré S, Schnaar RL (2011) **Myelin-associated glycoprotein protects neurons from excitotoxicity** *J Neurochem 116(5):900-8.*

Loreti S, Ricordy R, De Stefano ME, Augusti-Tocco G, Tata AM (2007) **Acetylcholine inhibits cell cycle progression in rat Schwann cells by activation of the M2 receptor subtype** *Neuron Glia Biol 3(4):269-79.*

Loup F, Wieser HG, Yonekawa Y, Aguzzi A, Fritschy JM (2000**) Selective alterations in GABAA receptor subtypes in human temporal lobe epilepsy** *J Neurosci 20(14):5401-19.*

Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, Fritschy JM, Rülicke T, Bluethmann H, Möhler H, Rudolph U (2000) **Molecular and neuronal substrate for the selective attenuation of anxiety** *Science 290(5489):131-4.*

Lu JC, Hsiao YT, Chiang CW, Wang CT (2014) **GABAA receptor-mediated tonic depolarization in developing neural circuits** *Mol Neurobiol 49(2):702-23.*

Lundborg G (2000) **A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance** *J Hand Surg Am 25(3):391-414.*

Lupski JR, Garcia CA (2001) **The metabolic and molecular basis of inherited disease** (eds Scriver CR *et al.*) *McGraw-Hill, New York 5759-5788.*

Luyt K, Slade TP, Dorward JJ, Durant CF, Wu Y, Shigemoto R, Mundell SJ, Váradi A, Molnár E (2007) **Developing oligodendrocytes express functional GABA(B) receptors that stimulate cell proliferation and migration** *J Neurochem 100(3):822-40.*

Ma KT, Si JQ, Zhang ZQ, Zhao L, Fan P, Jin JL, Li XZ, Zhu L (2006) **Modulatory effect of CCK-8S on GABA-induced depolarization from rat dorsal root ganglion** *Brain Res 1121(1):66-75.*

Ma W, Saunders PA, Somogyi R, Poulter MO, Barker JL (1993) **Ontogeny of GABAA receptor subunit mRNAs in rat spinal cord and dorsal root ganglia** *J Comp Neurol 338(3):337-59.*

Ma X, Idle JR, Gonzalez FJ (2008) **The pregnane X receptor: from bench to bedside** *Expert Opin Drug Metab Toxicol 4(7):895-908.*

Macey DJ, Froestl W, Koob GF, Markou A (2001) **Both GABA(B) receptor agonist and antagonists decreased brain stimulation reward in the rat** *Neuropharmacology 40(5):676-85.*

Magnaghi V (2007) **GABA and neuroactive steroid interactions in glia: new roles for old players?** *Curr Neuropharmacol 5(1):47-64.*

Magnaghi V, Ballabio M, Camozzi F, Colleoni M, Consoli A, Gassmann M, Lauria G, Motta M, Procacci P, Trovato AE, Bettler B (2008) **Altered peripheral myelination in mice lacking GABAB receptors** *Mol Cell Neurosci 37(3):599-609.*

Magnaghi V, Ballabio M, Consoli A, Lambert JJ, Roglio I, Melcangi RC (2006) **GABA receptor-mediated effects in the peripheral nervous system: A cross-interaction with neuroactive steroids** *J Mol Neurosci 28(1):89-102.*

Magnaghi V, Ballabio M, Cavarretta IT, Froestl W, Lambert JJ, Zucchi I, Melcangi RC (2004) **GABAB receptors in Schwann cells influence proliferation and myelin protein expression** *Eur J Neurosci 19(10):2641-9.*

Magnaghi V, Cavarretta I, Galbiati M, Martini L, Melcangi RC (2001) **Neuroactive steroids and peripheral myelin proteins** *Brain Res Brain Res Rev 37(1-3):360-71.*

Magnaghi V, Cavarretta I, Zucchi I, Susani L, Rupprecht R, Hermann B, Martini L, Melcangi RC (1999) **Po gene expression is modulated by androgens in the sciatic nerve of adult male rats** *Brain Res Mol Brain Res 70(1):36-44.*

Magnaghi V, Parducz A, Frasca A, Ballabio M, Procacci P, Racagni G, Bonanno G, Fumagalli F (2010) **GABA synthesis in Schwann cells is induced by the neuroactive steroid allopregnanolone** *J Neurochem 112(4):980-90.*

Magnaghi V, Procacci P, Tata AM (2009) **Chapter 15: Novel pharmacological approaches to Schwann cells as neuroprotective agents for peripheral nerve regeneration** *Int Rev Neurobiol 87:295-315.*

Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) **Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety** *Nat Neurosci 8(6):797-804.*

Malan TP, Mata HP, Porreca F (2002) **Spinal GABA(A) and GABA(B) receptor pharmacology in a rat model of neuropathic pain** *Anesthesiology 96(5):1161-7.*

Mantuano E, Lam MS, Shibayama M, Campana WM, Gonias SL (2015) **The NMDA receptor functions independently and as an LRP1 co-receptor to promote Schwann cell survival and migration** *J Cell Sci 128(18):3478-88.*

Margeta-Mitrovic M, Mitrovic I, Riley RC, Jan LY, Basbaum AI (1999) **Immunohistochemical localization of GABA(B) receptors in the rat central nervous system** *J Comp Neurol 405(3):299-321.*

Marieb EN, Hoehn K (2007) **Human Anatomy & Physiology (7th ed.)** *Pearson, pp. 388–602.*

Marowsky A, Rudolph U, Fritschy JM, Arand M (2012) **Tonic inhibition in principal cells of the amygdala: a central role for α3 subunit-containing GABAA receptors** *J Neurosci 32(25):8611-9.*

Martini L, Magnaghi V, Melcangi RC (2003) **Actions of progesterone and its 5alpha-reduced metabolites on the major proteins of the myelin of the peripheral nervous system** *Steroids 68(10-13):825-9.*

Martini R, Mohajeri MH, Kasper S, Giese KP, Schachner M (1995) **Mice doubly deficient in the genes for P0 and myelin basic protein show that both proteins contribute to the formation of the major dense line in peripheral nerve myelin** *J Neurosci 15(6):4488-95.*

Masaki T, Qu J, Cholewa-Waclaw J, Burr K, Raaum R, Rambukkana A (2013) **Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection** *Cell 152(1-2):51-67.*

Maurel P, Einheber S, Galinska J, Thaker P, Lam I, Rubin MB, Scherer SS, Murakami Y, Gutmann DH, Salzer JL (2007**) Nectin-like proteins mediate axon Schwann cell interactions along the internode and are essential for myelination** *J Cell Biol 178(5):861-74.*

Maurel P, Salzer JL (2000) **Axonal regulation of Schwann cell proliferation and survival and the initial events of myelination requires PI 3-kinase activity** *J Neurosci 20(12):4635-45.*

Mayhew TM, Sharma AK (1984) **Sampling schemes for estimating nerve fibre size. I. Methods for nerve trunks of mixed fascicularity** *J Anat 139 (Pt 1):45-58.*

McGuirk SM, Dolphin AC (1992) **G-protein mediation in nociceptive signal transduction: an investigation into the excitatory action of bradykinin in a subpopulation of cultured rat sensory neurons** *Neuroscience 49(1):117-28.*

McMahon S, Koltzenburg M (1990) **The changing role of primary afferent neurones in pain** *Pain 43(3):269-72.*

Meffre D, Delespierre B, Gouézou M, Leclerc P, Vinson GP, Schumacher M, Stein DG, Guennoun R (2005) **The membrane-associated progesterone-binding protein 25-Dx is expressed in brain regions involved in water homeostasis and is up-regulated after traumatic brain injury** *J Neurochem 93(5):1314-26.*

Meffre D, Labombarda F, Delespierre B, Chastre A, De Nicola AF, Stein DG, Schumacher M, Guennoun R (2013) **Distribution of membrane progesterone receptor alpha in the male mouse and rat brain and its regulation after traumatic brain injury** *Neuroscience 231:111-24.*

Mei L, Xiong WC (2008) **Neuregulin 1 in neural development, synaptic plasticity and schizophrenia** *Nat Rev Neurosci 9(6):437-52.*

Meier C, Parmantier E, Brennan A, Mirsky R, Jessen KR (1999) **Developing Schwann cells acquire the ability to survive without axons by establishing an autocrine circuit involving insulin-like growth factor, neurotrophin-3, and platelet-derived growth factor-BB** *J Neurosci 19(10):3847-59.*

Melcangi RC, Celotti F, Ballabio M, Poletti A, Martini L (1990) **Testosterone metabolism in peripheral nerves: presence of the 5 alpha-reductase-3 alpha-hydroxysteroid-dehydrogenase enzymatic system in the sciatic nerve of adult and aged rats** *J Steroid Biochem 35(1):145-8.*

Melcangi RC, Celotti F, Castano P, Martini L (1992) **Is the 5 alpha-reductase-3 alpha-hydroxysteroid dehydrogenase complex associated with the myelin in the peripheral nervous system of young and old male rats?** *Endocr Regul 26(3):119-25.*

Melcangi RC, Magnaghi V, Cavarretta I, Martini L, Piva F (1998) **Age-induced decrease of glycoprotein Po and myelin basic protein gene expression in the rat sciatic nerve. Repair by steroid derivatives** *Neuroscience 85(2):569-78.*

Melcangi RC, Magnaghi V, Cavarretta I, Zucchi I, Bovolin P, D'Urso D, Martini L (1999) **Progesterone derivatives are able to influence peripheral myelin protein 22 and P0 gene expression: possible mechanisms of action** *J Neurosci Res 56(4):349-57.*

Melcangi RC, Magnaghi V, Galbiati M, Martini L (2001) **Formation and effects of neuroactive steroids in the central and peripheral nervous system** *Int Rev Neurobiol 46:145-76.*

Melcangi RC, Magnaghi V, Martini L (2000) **Aging in peripheral nerves: regulation of myelin protein genes by steroid hormones** *Prog Neurobiol 60(3):291-308.*

Melcangi RC, Riva MA, Fumagalli F, Magnaghi V, Racagni G, Martini L (1996) **Effect of progesterone, testosterone and their 5 alpha-reduced metabolites on GFAP gene expression in type 1 astrocytes** *Brain Res 711(1-2):10-5.*

Mellon SH, Gong W, Schonemann MD (2008) **Endogenous and synthetic neurosteroids in treatment of Niemann-Pick Type C disease** *Brain Res Rev 57(2):410-20.*

Mellon SH, Griffin LD, Compagnone NA (2001) **Biosynthesis and action of neurosteroids** *Brain Res Brain Res Rev 37(1-3):3-12.*

Mellor H, Parker PJ (1998) **The extended protein kinase C superfamily** *Biochem J 332 (Pt 2):281-92.*

Merskey H (1994) **Logic, truth and language in concepts of pain** *Qual Life Res 3 Suppl 1:S69-76.*

Meyer C, Schmid R, Scriba PC, Wehling M (1996) **Purification and partial sequencing of high-affinity progesterone-binding site(s) from porcine liver membranes**

Meyer RA, Ringkamp M, Campbell JN, Raja SN (2008*)* **Peripheral mechanisms of cutaneous nociception** *In Wall and Melzack's Textbook of Pain, S.B. McMahon and M. Koltzenburg, eds. (Philadelphia: Elsevier), pp. 3–34.*

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA (2004) **Axonal neuregulin-1 regulates myelin sheath thickness** *Science 304(5671):700-3.*

Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE (1999) **Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice** *Proc Natl Acad Sci USA 96(22):12905-10.*

Millan MJ (1999) **The induction of pain: an integrative review** *Prog Neurobiol 57(1):1-164.*

Millesi H (2007) **Bridging defects: autologous nerve grafts** *Acta Neurochir Suppl 100:37-8.*

Mochly-Rosen D, Basbaum AI, Koshland DE Jr (1987) **Distinct cellular and regional localization of immunoreactive protein kinase C in rat brain** *Proc Natl Acad Sci USA 84(13):4660-4.*

Mogha A, Benesh AE, Patra C, Engel FB, Schöneberg T, Liebscher I, Monk KR (2013) **Gpr126 functions in Schwann cells to control differentiation and myelination via G-protein activation** *J Neurosci 33(46):17976-85.*

Monje PV, Soto J, Bacallao K, Wood PM (2010) **Schwann cell dedifferentiation is independent of mitogenic signaling and uncoupled to proliferation: role of cAMP and JNK in the maintenance of the differentiated state** *J Biol Chem 285(40):31024-36.*

Monk KR, Feltri ML, Taveggia C (2015) **New insights on Schwann cell development** *Glia 63(8):1376-93.*

Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, Moens CB, Talbot WS (2009) **A G protein-coupled receptor is essential for Schwann cells to initiate myelination** *Science 325(5946):1402-5.*

Monnet FP, Maurice T (2006) **The sigma1 protein as a target for the non-genomic effects of neuro(active)steroids: molecular, physiological, and behavioral aspects** *J Pharmacol Sci 100(2):93-118.*

Monuki ES, Weinmaster G, Kuhn R, Lemke G (1989) **SCIP: a glial POU domain gene regulated by cyclic AMP** *Neuron 3(6):783-93.*

Morenilla-Palao C, Planells-Cases R, García-Sanz N, Ferrer-Montiel A (2004) **Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity** *J Biol Chem 279(24):25665-72.*

Morris ME, Di Costanzo GA, Fox S, Werman R (1983) **Depolarizing action of GABA (gamma-aminobutyric acid) on myelinated fibers of peripheral nerves** *Brain Res 278(1-2):117-26.*

Mortensen M, Ebert B, Wafford K, Smart TG (2010) **Distinct activities of GABA agonists at synaptic- and extrasynaptic-type GABAA receptors** *J Physiol 588(Pt 8):1251-68.*

Mtchedlishvili Z, Bertram EH, Kapur J (2001) **Diminished allopregnanolone enhancement of GABA(A) receptor currents in a rat model of chronic temporal lobe epilepsy** *J Physiol 537(Pt 2):453-65.*

Nagai T, Delay RJ, Welton J, Roper SD (1998) **Uptake and release of neurotransmitter candidates, [3H]serotonin, [3H]glutamate, and [3H]gamma-aminobutyric acid, in taste buds of the mudpuppy, Necturus maculosus** *J Comp Neurol 392(2):199-208.*

Narita M, Ohsawa M, Mizoguchi H, Kamei J, Tseng LF (1997) **Pretreatment with protein kinase C activator phorbol 12,13-dibutyrate attenuates the antinociception induced by mu- but not epsilon-opioid receptor agonist in the mouse** *Neuroscience 76(1):291-8.*

Newbern J, Birchmeier C (2010) **Nrg1/ErbB signaling networks in Schwann cell development and myelination** *Semin Cell Dev Biol 21(9):922-8.*

Newbern JM, Li X, Shoemaker SE, Zhou J, Zhong J, Wu Y, Bonder D, Hollenback S, Coppola G, Geschwind DH, Landreth GE, Snider WD (2011) **Specific functions for ERK/MAPK signaling during PNS development** *Neuron 69(1):91-105.*

Nguyen L, Malgrange B, Breuskin I, Bettendorff L, Moonen G, Belachew S, Rigo JM (2003) **Autocrine/paracrine activation of the GABA(A) receptor inhibits the proliferation of neurogenic polysialylated neural cell adhesion molecule-positive (PSA-NCAM+) precursor cells from postnatal striatum** *J Neurosci 23(8):3278-94.*

Nguyen T, Mehta NR, Conant K, Kim KJ, Jones M, Calabresi PA, Melli G, Hoke A, Schnaar RL, Ming GL, Song H, Keswani SC, Griffin JW (2009) **Axonal protective effects of the myelin-associated glycoprotein** *J Neurosci 29(3):630-7.*

Nishizuka Y (1995) **Protein kinase C and lipid signaling for sustained cellular responses** *FASEB J 9(7):484-96.*

Notter T, Panzanelli P, Pfister S, Mircsof D, Fritschy JM (2014) **A protocol for concurrent high-quality immunohistochemical and biochemical analyses in adult mouse central nervous system** *Eur J Neurosci 39(2):165-75.*

Nusser Z, Ahmad Z, Tretter V, Fuchs K, Wisden W, Sieghart W, Somogyi P (1999) **Alterations in the expression of GABAA receptor subunits in cerebellar granule cells after the disruption of the alpha6 subunit gene** *Eur J Neurosci 11(5):1685-97.*

Obata K, Yamanaka H, Fukuoka T, Yi D, Tokunaga A, Hashimoto N, Yoshikawa H, Noguchi K (2003) **Contribution of injured and uninjured dorsal root ganglion neurons to pain behavior and the changes in gene expression following chronic constriction injury of the sciatic nerve in rats** *Pain 101(1-2):65-77.*

Oberlander JG, Porter DM, Onakomaiya MM, Penatti CA, Vithlani M, Moss SJ, Clark AS, Henderson LP (2012) **Estrous cycle variations in GABA(A) receptor phosphorylation enable rapid modulation by anabolic androgenic steroids in the medial preoptic area** *Neuroscience 226:397-410.*

Obradovic AL, Scarpa J, Osuru HP, Weaver JL, Park JY, Pathirathna S, Peterkin A, Lim Y, Jagodic MM, Todorovic SM, Jevtovic-Todorovic V (2015) **Silencing the α2 subunit of γ-aminobutyric acid type A receptors in rat dorsal root ganglia reveals its major role in antinociception posttraumatic nerve injury** *Anesthesiology 123(3):654-67.*

Ogata T, Yamamoto S, Nakamura K, Tanaka S (2006) **Signaling axis in schwann cell proliferation and differentiation** *Mol Neurobiol 33(1):51-62.*

Olah Z, Karai L, Iadarola MJ (2002) **Protein kinase C(alpha) is required for vanilloid receptor 1 activation. Evidence for multiple signaling pathways** *J Biol Chem 277(38):35752-9.*

Olsen RW, Sieghart W (2008) **International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update** *Pharmacol Rev 60(3):243-60.*

Olsen RW, Snowhill EW, Wamsley JK (1984) **Autoradiographic localization of low affinity GABA receptors with [3H]bicuculline methochloride** *Eur J Pharmacol 99(2-3):247-8.*

Pan B, Fromholt SE, Hess EJ, Crawford TO, Griffin JW, Sheikh KA, Schnaar RL (2005) **Myelin-associated glycoprotein and complementary axonal ligands, gangliosides, mediate axon stability in the CNS and PNS: neuropathology and behavioral deficits in single- and double-null mice** *Exp Neurol 195(1):208-17.*

Pang Y, Dong J, Thomas P (2008) **Estrogen signaling characteristics of Atlantic croaker G protein-coupled receptor 30 (GPR30) and evidence it is involved in maintenance of oocyte meiotic arrest** *Endocrinology 149(7):3410-26.*

Pang Y, Dong J, Thomas P (2013) **Characterization, neurosteroid binding and brain distribution of human membrane progesterone receptors δ and {epsilon} (mPRδ and mPR{epsilon}) and mPRδ involvement in neurosteroid inhibition of apoptosis** *Endocrinology 154(1):283-95.*

Pang Y, Dong J, Thomas P (2015) **Progesterone increases nitric oxide synthesis in human vascular endothelial cells through activation of membrane progesterone receptor-α** *Am J Physiol Endocrinol Metab 308(10):E899-911.*

Panzanelli P, Gunn BG, Schlatter MC, Benke D, Tyagarajan SK, Scheiffele P, Belelli D, Lambert JJ, Rudolph U, Fritschy JM (2011) **Distinct mechanisms regulate GABAA receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells** *J Physiol 589(Pt 20):4959-80.*

Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, Gavish M (2006) **Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function** *Trends Pharmacol Sci 27(8):402-9.*

Parada CA, Yeh JJ, Joseph EK, Levine JD (2003) **Tumor necrosis factor receptor type-1 in sensory neurons contributes to induction of chronic enhancement of inflammatory hyperalgesia in rat** *Eur J Neurosci 17(9):1847-52.*

Pareyson D, Marchesi C, Salsano E (2013) **Dominant Charcot-Marie-Tooth syndrome and cognate disorders** *Handb Clin Neurol 115:817-45.*

Park-Chung M, Malayev A, Purdy RH, Gibbs TT, Farb DH (1999) **Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites** *Brain Res 830(1):72-87.*

Parkinson DB, Bhaskaran A, Arthur-Farraj P, Noon LA, Woodhoo A, Lloyd AC, Feltri ML, Wrabetz L, Behrens A, Mirsky R, Jessen KR (2008) **c-Jun is a negative regulator of myelination** *J Cell Biol 181(4):625-37.*

Patel B, Mortensen M, Smart TG (2014) **Stoichiometry of δ subunit containing GABA(A) receptors** *Br J Pharmacol 171(4):985-94.*

Patel S, Naeem S, Kesingland A, Froestl W, Capogna M, Urban L, Fox A (2001) **The effects of GABA(B) agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat** *Pain 90(3):217-26.*

Patte-Mensah C, Meyer L, Taleb O, Mensah-Nyagan AG (2014) **Potential role of allopregnanolone for a safe and effective therapy of neuropathic pain** *Prog Neurobiol 113:70-8.*

Peden DR, Petitjean CM, Herd MB, Durakoglugil MS, Rosahl TW, Wafford K, Homanics GE, Belelli D, Fritschy JM, Lambert JJ (2008) **Developmental maturation of synaptic and extrasynaptic GABAA receptors in mouse thalamic ventrobasal neurons** *J Physiol 586(4):965-87.*

Peluso JJ, Liu X, Gawkowska A, Johnston-MacAnanny E (2009) **Progesterone activates a progesterone receptor membrane component 1-dependent mechanism that promotes human granulosa/luteal cell survival but not progesterone secretion** *J Clin Endocrinol Metab 94(7):2644-9.*

Peluso JJ, Pappalardo A, Losel R, Wehling M (2006) **Progesterone membrane receptor component 1 expression in the immature rat ovary and its role in mediating progesterone's antiapoptotic action** *Endocrinology 147(6):3133-40.*

Peluso JJ, Romak J, Liu X (2008) **Progesterone receptor membrane component-1 (PGRMC1) is the mediator of progesterone's antiapoptotic action in spontaneously immortalized granulosa cells as revealed by PGRMC1 small interfering ribonucleic acid treatment and functional analysis of PGRMC1 mutations** *Endocrinology 149(2):534-43.*

Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR (2002) **GABA(A) receptor changes in delta subunit-deficient mice: altered expression of alpha4 and gamma2 subunits in the forebrain** *J Comp Neurol 446(2):179-97.*

Peng Z, Huang CS, Stell BM, Mody I, Houser CR (2004) **Altered expression of the delta subunit of the GABAA receptor in a mouse model of temporal lobe epilepsy** *J Neurosci 24(39):8629-39.*

Perego C, Di Cairano ES, Ballabio M, Magnaghi V (2012) **Neurosteroid allopregnanolone regulates EAAC1-mediated glutamate uptake and triggers actin changes in Schwann cells** *J Cell Physiol 227(4):1740-51.*

Pereira JA, Lebrun-Julien F, Suter U (2012) **Molecular mechanisms regulating myelination in the peripheral nervous system** *Trends Neurosci 35(2):123-34.*

Pérez-Garci E, Gassmann M, Bettler B, Larkum ME (2006) **The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca2+ spikes in layer 5 somatosensory pyramidal neurons** *Neuron 50(4):603-16.*

Perl ER (2007) **Ideas about pain, a historical view** *Nat Rev Neurosci 8(1):71-80.*

Perroy J, Adam L, Qanbar R, Chénier S, Bouvier M (2003) **Phosphorylation-independent desensitization of GABA(B) receptor by GRK4** *EMBO J 22(15):3816-24.*

Petersen SL, Intlekofer KA, Moura-Conlon PJ, Brewer DN, Del Pino Sans J, Lopez JA (2013) **Novel progesterone receptors: neural localization and possible functions** *Front Neurosci 7:164.*

Petroff OA (2002) **GABA and glutamate in the human brain** *Neuroscientist 8(6):562-73.*

Pfaff T, Malitschek B, Kaupmann K, Prézeau L, Pin JP, Bettler B, Karschin A (1999) **Alternative splicing generates a novel isoform of the rat metabotropic GABA(B)R1 receptor** *Eur J Neurosci 11(8):2874-82.*

Pfaffl MW (2001) **A new mathematical model for relative quantification in real-time RT-PCR** *Nucleic Acids Res 29(9):e45.*

Pinal CS, Tobin AJ (1998) **Uniqueness and redundancy in GABA production** *Perspect Dev Neurobiol 5(2-3):109-18*

Poisbeau P, Patte-Mensah C, Keller AF, Barrot M, Breton JD, Luis-Delgado OE, Freund-Mercier MJ, Mensah-Nyagan AG, Schlichter R (2005) **Inflammatory pain upregulates spinal inhibition via endogenous neurosteroid production** *J Neurosci 25(50):11768-76.*

Pontier SM, Lahaie N, Ginham R, St-Gelais F, Bonin H, Bell DJ, Flynn H, Trudeau LE, McIlhinney J, White JH, Bouvier M (2006) **Coordinated action of NSF and PKC regulates GABAB receptor signaling efficacy** *EMBO J 25(12):2698-709.*

Price T (2013) **Progesterone receptor membrane component 1: is metabolism integral to its function and what other steroids are involved?** *Menopause 20(5):486-7.*

Procacci P, Ballabio M, Castelnovo LF, Mantovani C, Magnaghi V (2012) **GABA-B receptors in the PNS have a role in Schwann cells differentiation?** *Front Cell Neurosci 6:68.*

Puia G, Ravazzini F, Castelnovo LF, Magnaghi V (2015) **PKCε and allopregnanolone: functional cross-talk at the GABAA receptor level** *Front Cell Neurosci 9:83.*

Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E (1990) **Neurosteroids act on recombinant human GABAA receptors** *Neuron 4(5):759-65.*

Qi ZH, Song M, Wallace MJ, Wang D, Newton PM, McMahon T, Chou WH, Zhang C, Shokat KM, Messing RO (2007) **Protein kinase C epsilon regulates gamma-aminobutyrate type A receptor**

sensitivity to ethanol and benzodiazepines through phosphorylation of gamma2 subunits *J Biol Chem 282(45):33052-63.*

Quarles RH (2002) **Myelin sheaths: glycoproteins involved in their formation, maintenance and degeneration** *Cell Mol Life Sci 59(11):1851-71.*

Quarles RH (2007) **Myelin-associated glycoprotein (MAG): past, present and beyond** *J Neurochem 100(6):1431-48.*

Rang HP, Ritchie JM (1988) **Depolarization of nonmyelinated fibers of the rat vagus nerve produced by activation of protein kinase C** *J Neurosci 8(7):2606-17.*

Raphael AR, Lyons DA, Talbot WS (2011) **ErbB signaling has a role in radial sorting independent of Schwann cell number** *Glia 59(7):1047-55.*

Reddy DS (2004) **Anticonvulsant activity of the testosterone-derived neurosteroid 3alpha-androstanediol** *Neuroreport 15(3):515-8.*

Reddy DS (2008) **Mass spectrometric assay and physiological-pharmacological activity of androgenic neurosteroids** *Neurochem Int 52(4-5):541-53.*

Reddy DS (2009) **The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy** *Epilepsy Res 85(1):1-30.*

Reddy DS (2011) **Role of anticonvulsant and antiepileptogenic neurosteroids in the pathophysiology and treatment of epilepsy** *Front Endocrinol (Lausanne) 2:38.*

Reddy DS, Kulkarni SK (2000) **Development of neurosteroid-based novel psychotropic drugs** *Prog Med Chem 37:135-75.*

Ren X, Mody I (2003) **Gamma-hydroxybutyrate reduces mitogen-activated protein kinase phosphorylation via GABA B receptor activation in mouse frontal cortex and hippocampus** *J Biol Chem 278(43):42006-11.*

Roberts E, Frankel S (1950) **gamma-Aminobutyric acid in brain: its formation from glutamic acid** *J Biol Chem 187(1):55-63.*

Roglio I, Bianchi R, Gotti S, Scurati S, Giatti S, Pesaresi M, Caruso D, Panzica GC, Melcangi RC (2008**) Neuroprotective effects of dihydroprogesterone and progesterone in an experimental model of nerve crush injury** *Neuroscience 155(3):673-85.*

Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Möhler H (1999) **Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes** *Nature 401(6755):796-800.*

Rudolph U, Möhler H (2004) **Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics** *Annu Rev Pharmacol Toxicol 44:475-98.*

Ruiz A, Campanac E, Scott RS, Rusakov DA, Kullmann DM (2010**) Presynaptic GABAA receptors enhance transmission and LTP induction at hippocampal mossy fiber synapses** *Nat Neurosci 13(4):431-8.*

Rupprecht R, di Michele F, Hermann B, Ströhle A, Lancel M, Romeo E, Holsboer F (2001) **Neuroactive steroids: molecular mechanisms of action and implications for neuropsychopharmacology** *Brain Res Brain Res Rev 37(1-3):59-67.*

Rupprecht R, Reul JM, Trapp T, van Steensel B, Wetzel C, Damm K, Zieglgänsberger W, Holsboer F (1993**) Progesterone receptor-mediated effects of neuroactive steroids** *Neuron 11(3):523-30.*

Saher G, Simons M (2010) **Cholesterol and myelin biogenesis** *Subcell Biochem 51:489-508.*

Saito N, Itouji A, Totani Y, Osawa I, Koide H, Fujisawa N, Ogita K, Tanaka C (1993) **Cellular and intracellular localization of epsilon-subspecies of protein kinase C in the rat brain; presynaptic localization of the epsilon-subspecies** *Brain Res 607(1-2):241-8.*

Saito Y, Teshima R, Takagi K, Ikebuchi H, Yamazaki T, Sawada J (1998) **Activation of protein kinase C alpha enhances human growth hormone-binding protein release** *Mol Cell Endocrinol 146(1-2):197-205.*

Sakaba T, Neher E (2003) **Direct modulation of synaptic vesicle priming by GABA(B) receptor activation at a glutamatergic synapse** *Nature 424(6950):775-8.*

Salzer JL (2015) **Schwann cell myelination** *Cold Spring Harb Perspect Biol 7(8):a020529.*

Saporta MA, Shy ME (2013) **Inherited peripheral neuropathies** *Neurol Clin 31(2):597-619*

Sarkar J, Wakefield S, MacKenzie G, Moss SJ, Maguire J (2011) **Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive GABAA receptors** *J Neurosci 31(50):18198-210.*

Schepelmann K, Messlinger K, Schmidt RF (1993) **The effects of phorbol ester on slowly conducting afferents of the cat's knee joint** *Exp Brain Res 92(3):391-8.*

Schneider Gasser EM, Duveau V, Prenosil GA, Fritschy JM (2007) **Reorganization of GABAergic circuits maintains GABAA receptor-mediated transmission onto CA1 interneurons in alpha1-subunit-null mice** *Eur J Neurosci 25(11):3287-304.*

Schofield CM, Kleiman-Weiner M, Rudolph U, Huguenard JR (2009) **A gain in GABAA receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures** *Proc Natl Acad Sci USA 106(18):7630-5.*

Scholz J, Woolf CJ (2007) **The neuropathic pain triad: neurons, immune cells and glia** *Nat Neurosci 10(11):1361-8.*

Schuler V, Lüscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, Fox A, Spooren W, Jaton AL, Vigouret J, Pozza M, Kelly PH, Mosbacher J, Froestl W, Käslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H, Bettler B (2001) **Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1))** *Neuron 31(1):47-58.*

Schumacher M, Guennoun R, Robert F, Carelli C, Gago N, Ghoumari A, Gonzalez Deniselle MC, Gonzalez SL, Ibanez C, Labombarda F, Coirini H, Baulieu EE, De Nicola AF (2004) **Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination** *Growth Horm IGF Res 14 Suppl A:S18-33.*

Schumacher M, Guennoun R, Stein DG, De Nicola AF (2007) **Progesterone: therapeutic opportunities for neuroprotection and myelin repair** *Pharmacol Ther 116(1):77-106.*

Schwarzer C, Tsunashima K, Wanzenböck C, Fuchs K, Sieghart W, Sperk G (1997) **GABA(A) receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced temporal lobe epilepsy** *Neuroscience 80(4):1001-17.*

Sedlácek M, Korínek M, Petrovic M, Cais O, Adamusová E, Chodounská H, Vyklický L Jr (2008) **Neurosteroid modulation of ionotropic glutamate receptors and excitatory synaptic transmission** *Physiol Res 57 Suppl 3:S49-57.*

Seltzer Z, Dubner R, Shir Y (1990) **A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury** *Pain 43(2):205-18.*

Sereda MW, Meyer zu Hörste G, Suter U, Uzma N, Nave KA (2003) **Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A)** *Nat Med 9(12):1533-7.*

Serrano A, Haddjeri N, Lacaille JC, Robitaille R (2006) **GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression** *J Neurosci 26(20):5370-82.*

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) **Synaptic and nonsynaptic localization of GABAA receptors containing the alpha5 subunit in the rat brain** *J Comp Neurol 499(3):458-70.*

Shaban H, Humeau Y, Herry C, Cassasus G, Shigemoto R, Ciocchi S, Barbieri S, van der Putten H, Kaupmann K, Bettler B, Lüthi A (2006) **Generalization of amygdala LTP and conditioned fear in the absence of presynaptic inhibition** *Nat Neurosci 9(8):1028-35.*

Shah NM, Marchionni MA, Isaacs I, Stroobant P, Anderson DJ (1994) **Glial growth factor restricts mammalian neural crest stem cells to a glial fate** *Cell 77(3):349-60.*

Shapiro L, Doyle JP, Hensley P, Colman DR, Hendrickson WA (1996) **Crystal structure of the extracellular domain from P0, the major structural protein of peripheral nerve myelin** *Neuron 17(3):435-49.*

Sheean ME, McShane E, Cheret C, Walcher J, Müller T, Wulf-Goldenberg A, Hoelper S, Garratt AN, Krüger M, Rajewsky K, Meijer D, Birchmeier W, Lewin GR, Selbach M, Birchmeier C (2014) **Activation of MAPK overrides the termination of myelin growth and replaces Nrg1/ErbB3 signals during Schwann cell development and myelination** *Genes Dev 28(3):290-303.*

Shu HJ, Eisenman LN, Jinadasa D, Covey DF, Zorumski CF, Mennerick S (2004) **Slow actions of neuroactive steroids at GABAA receptors** *J Neurosci 24(30):6667-75.*

Shy ME (2006) **Peripheral neuropathies caused by mutations in the myelin protein zero** *J Neurol Sci 242(1-2):55-66.*

Si JQ, Zhang ZQ, Li CX, Wang LF, Yang YL, Li ZW (2004) **Modulatory effect of substance P on GABA-activated currents from rat dorsal root ganglion** *Acta Pharmacol Sin 25(5):623-9.*

Simons M, Trajkovic K (2006) **Neuron-glia communication in the control of oligodendrocyte function and myelin biogenesis** *J Cell Sci 119(Pt 21):4381-9.*

Sindrup SH, Jensen TS (2002) **Pharmacotherapy of trigeminal neuralgia** *Clin J Pain 18(1):22-7.*

Singh R, Kishore L, Kaur N (2014**) Diabetic peripheral neuropathy: current perspective and future directions** *Pharmacol Res 80:21-35.*

Sleiter N, Pang Y, Park C, Horton TH, Dong J, Thomas P, Levine JE (2009) **Progesterone receptor**

A (PRA) and PRB-independent effects of progesterone on gonadotropin-releasing hormone release *Endocrinology 150:3833–3844.*

Smith JL, Kupchak BR, Garitaonandia I, Hoang LK, Maina AS, Regalla LM, Lyons TJ (2008) **Heterologous expression of human mPRα, mPRβ and mPRγ in yeast confirms their ability to function as membrane progesterone receptors** *Steroids 73:1160–1173.*

Spiegel I, Adamsky K, Eshed Y, Milo R, Sabanay H, Sarig-Nadir O, Horresh I, Scherer SS, Rasband MN, Peles E (2007) **A central role for Necl4 (SynCAM4) in Schwann cell-axon interaction and myelination** *Nat Neurosci 10(7):861-9.*

Sposato LA, Fustinoni O (2014) **Iatrogenic neurology** *Handb Clin Neurol 121:1635-71.*

Stassart RM, Fledrich R, Velanac V, Brinkmann BG, Schwab MH, Meijer D, Sereda MW, Nave KA (2013) **A role for Schwann cell-derived neuregulin-1 in remyelination** *Nat Neurosci 16(1):48-54.*

Steckelbroeck S, Watzka M, Reichelt R, Hans VH, Stoffel-Wagner B, Heidrich DD, Schramm J, Bidlingmaier F, Klingmüller D (2001) **Characterization of the 5alpha-reductase-3alpha-hydroxysteroid dehydrogenase complex in the human brain** *J Clin Endocrinol Metab 86(3):1324-31.*

Steiger JL, Bandyopadhyay S, Farb DH, Russek SJ (2004) **cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal GABABR1a and GABABR1b subunit gene expression through alternative promoters** *J Neurosci 24(27):6115-26.*

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) **Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors** *Proc Natl Acad Sci USA 100(24):14439-44.*

Stevens B, Ishibashi T, Chen JF, Fields RD (2004) **Adenosine: an activity-dependent axonal signal regulating MAP kinase and proliferation in developing Schwann cells** *Neuron Glia Biol 1(1):23-34.*

Stewart HJ, Eccleston PA, Jessen KR, Mirsky R (1991) **Interaction between cAMP elevation, identified growth factors, and serum components in regulating Schwann cell growth** *J Neurosci Res 30(2):346-52.*

Stoffel-Wagner B, Watzka M, Steckelbroeck S, Ludwig M, Clusmann H, Bidlingmaier F, Casarosa E, Luisi S, Elger CE, Beyenburg S (2003) **Allopregnanolone serum levels and expression of 5**

alpha-reductase and 3 alpha-hydroxysteroid dehydrogenase isoforms in hippocampal and temporal cortex of patients with epilepsy *Epilepsy Res 54(1):11-9.*

Stone DJ, Rozovsky I, Morgan TE, Anderson CP, Hajian H, Finch CE (1997) **Astrocytes and microglia respond to estrogen with increased apoE mRNA in vivo and in vitro** *Exp Neurol 143(2):313-8.*

Studer R, von Boehmer L, Haenggi T, Schweizer C, Benke D, Rudolph U, Fritschy JM (2006) **Alteration of GABAergic synapses and gephyrin clusters in the thalamic reticular nucleus of GABAA receptor alpha3 subunit-null mice** *Eur J Neurosci 24(5):1307-15.*

Su C, Cunningham RL, Rybalchenko N, Singh M (2012**) Progesterone increases the release of brain-derived neurotrophic factor from glia via progesterone receptor membrane component 1 (Pgrmc1)-dependent ERK5 signaling** *Endocrinology 153(9):4389-400.*

Suter U, Scherer SS (2003) **Disease mechanisms in inherited neuropathies** *Nat Rev Neurosci 4(9):714-26.*

Svaren J, Meijer D (2008) **The molecular machinery of myelin gene transcription in Schwann cells** *Glia 56(14):1541-51.*

Tamura S, Watanabe M, Kanbara K, Yanagawa T, Watanabe K, Otsuki Y, Kinoshita M (2009) **Expression and distribution of GABAergic system in rat knee joint synovial membrane** *Histol Histopatho 24(8):1009-19.*

Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC, Funk WD (2005) **PAQR proteins: novel membrane receptor family defined by an ancient 7-transmembrane pass motif** *J Mol Evol 61:372–380.*

Tao W, Higgs MH, Spain WJ, Ransom CB (2013) **Postsynaptic GABAB receptors enhance extrasynaptic GABAA receptor function in dentate gyrus granule cells** *J Neurosci 33(9):3738-43.*

Tapinos N, Rambukkana A (2005) **Insights into regulation of human Schwann cell proliferation by Erk1/2 via a MEK-independent and p56Lck-dependent pathway from leprosy bacilli** *Proc Natl Acad Sci USA 102(26):9188-93.*

Taskén K, Aandahl EM (2004) **Localized effects of cAMP mediated by distinct routes of protein kinase A** *Physiol Rev 84(1):137-67.*

Taveggia C (2016) **Schwann cells-axon interaction in myelination** *Curr Opin Neurobiol 39:24-9.*

Taveggia C, Feltri ML, Wrabetz L (2010) **Signals to promote myelin formation and repair** *Nat Rev Neurol 6(5):276-87.*

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P, Chao MV, Falls DL, Role L, Salzer JL (2005) **Neuregulin-1 type III determines the ensheathment fate of axons** *Neuron 47(5):681-94.*

Terada N, Saitoh Y, Ohno N, Komada M, Yamauchi J, Ohno S (2013) **Involvement of Src in the membrane skeletal complex, MPP6-4.1G, in Schmidt-Lanterman incisures of mouse myelinated nerve fibers in PNS** *Histochem Cell Biol 140(2):213-22.*

Thi AD, Jung-Testas I, Baulieu EE (1998**) Neuronal signals are required for estrogen-mediated induction of progesterone receptor in cultured rat Schwann cells** *J Steroid Biochem Mol Biol 67(3):201-11.*

Thomas P, Pang Y (2012) **Membrane progesterone receptors: evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells** *Neuroendocrinology 96(2):162-71.*

Thomas P, Pang Y, Dong J (2014) **Enhancement of cell surface expression and receptor functions of membrane progestin receptor α (mPRα) by progesterone receptor membrane component 1 (PGRMC1): evidence for a role of PGRMC1 as an adaptor protein for steroid receptors** *Endocrinology 155(3):1107-19.*

Thomas P, Pang Y, Dong J, Groenen P, Kelder J, de Vlieg J, Zhu Y, Tubbs C (2007) **Steroid and G protein binding characteristics of the seatrout and human progestin membrane receptor alpha subtypes and their evolutionary origins** *Endocrinology 148(2):705-18.*

Thomas P, Tubbs C, Detweiler C, Das S, Ford L, Breckenridge-Miller D (2005) **Binding characteristics, hormonal regulation and identity of the sperm membrane progestin receptor in Atlantic croaker** *Steroids 70(5-7):427-33.*

Toda K, Small JA, Goda S, Quarles RH (1994) **Biochemical and cellular properties of three immortalized Schwann cell lines expressing different levels of the myelin-associated glycoprotein** *J Neurochem 63(5):1646-57.*

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) **The cloned capsaicin receptor integrates multiple pain-producing stimuli** *Neuron 21(3):531-43.*

Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P (1994) **Krox-20 controls myelination in the peripheral nervous system** *Nature 371(6500):796-9.*

Towers S, Princivalle A, Billinton A, Edmunds M, Bettler B, Urban L, Castro-Lopes J, Bowery NG (2000) **GABAB receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia** *Eur J Neurosci 12(9):3201-10.*

Trigo FF, Marty A, Stell BM (2008) **Axonal GABAA receptors** *Eur J Neurosci 28(5):841-8.*

Twyman RE, Macdonald RL (1992) **Neurosteroid regulation of GABAA receptor single-channel kinetic properties of mouse spinal cord neurons in culture** *J Physiol 456:215-45.*

Ukena K, Usui M, Kohchi C, Tsutsui K (1998) **Cytochrome P450 side-chain cleavage enzyme in the cerebellar Purkinje neuron and its neonatal change in rats** *Endocrinology 139(1):137-47.*

Van Kolen K, Pullan S, Neefs JM, Dautzenberg FM (2008) **Nociceptive and behavioural sensitisation by protein kinase Cepsilon signalling in the CNS** *J Neurochem 104(1):1-13.*

Vegeto E, Bonincontro C, Pollio G, Sala A, Viappiani S, Nardi F, Brusadelli A, Viviani B, Ciana P, Maggi A (2001) **Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia** *J Neurosci 21(6):1809-18.*

Vegeto E, Pollio G, Pellicciari C, Maggi A (1999) **Estrogen and progesterone induction of survival of monoblastoid cells undergoing TNF-alpha-induced apoptosis** *FASEB J 13(8):793-803.*

Verdi JM, Campagnoni AT (1990) **Translational regulation by steroids. Identification of a steroid modulatory element in the 5'-untranslated region of the myelin basic protein messenger RNA** *J Biol Chem 265(33):20314-20.*

Vergnano AM, Schlichter R, Poisbeau P (2007) **PKC activation sets an upper limit to the functional plasticity of GABAergic transmission induced by endogenous neurosteroids** *Eur J Neurosci 26(5):1173-82.*

Vigot R, Barbieri S, Bräuner-Osborne H, Turecek R, Shigemoto R, Zhang YP, Luján R, Jacobson LH, Biermann B, Fritschy JM, Vacher CM, Müller M, Sansig G, Guetg N, Cryan JF, Kaupmann K, Gassmann M, Oertner TG, Bettler B (2006) **Differential compartmentalization and distinct functions of GABAB receptor variants** *Neuron 50(4):589-601.*

Von Blankenfeld G, Trotter J, Kettenmann H (1991) **Expression and Developmental Regulation of a GABAA Receptor in Cultured Murine Cells of the Oligodendrocyte Lineage** *Eur J Neurosci 3(4):310-316.*

Wakita M, Kotani N, Kogure K, Akaike N (2014) **Inhibition of excitatory synaptic transmission in hippocampal neurons by levetiracetam involves Zn^{2+}-dependent GABA type A receptor-mediated presynaptic modulation** *J Pharmacol Exp Ther 348(2):246-59.*

Wallquist W, Plantman S, Thams S, Thyboll J, Kortesmaa J, Länngren J, Domogatskaya A, Ogren SO, Risling M, Hammarberg H, Tryggvason K, Cullheim S (2005) **Impeded interaction between Schwann cells and axons in the absence of laminin alpha4** *J Neurosci 25(14):3692-700.*

Walker MC, Ruiz A, Kullmann DM (2002) **Do mossy fibers release GABA?** *Epilepsia 43 Suppl 5:196-202.*

Watson JC, Dyck PJ (2015) **Peripheral Neuropathy: A Practical Approach to Diagnosis and Symptom Management** *Mayo Clin Proc 90(7):940-51.*

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) **The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon** *J Neurosci 12(3):1040-62.*

White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH (1998) **Heterodimerization is required for the formation of a functional GABA(B) receptor** *Nature 396(6712):679-82.*

Witschi R, Punnakkal P, Paul J, Walczak JS, Cervero F, Fritschy JM, Kuner R, Keist R, Rudolph U, Zeilhofer HU (2011) **Presynaptic alpha2-GABAA receptors in primary afferent depolarization and spinal pain control** *J Neurosci 31(22):8134-42.*

Witzel C, Rohde C, Brushart TM (2005) **Pathway sampling by regenerating peripheral axons** *J Comp Neurol 485(3):183-90.*

Wlodarczyk AI, Sylantyev S, Herd MB, Kersanté F, Lambert JJ, Rusakov DA, Linthorst AC, Semyanov A, Belelli D, Pavlov I, Walker MC (2013) **GABA-independent GABAA receptor openings maintain tonic currents** *J Neurosci 33(9):3905-14*

Wohlfarth KM, Bianchi MT, Macdonald RL (2002) **Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit** *J Neurosci 22(5):1541-9.*

Woodhoo A, Alonso MB, Droggiti A, Turmaine M, D'Antonio M, Parkinson DB, Wilton DK, Al-Shawi R, Simons P, Shen J, Guillemot F, Radtke F, Meijer D, Feltri ML, Wrabetz L, Mirsky R,

Jessen KR (2009) **Notch controls embryonic Schwann cell differentiation, postnatal myelination and adult plasticity** *Nat Neurosci 12(7):839-47.*

Woodhoo A, Sommer L (2008) **Development of the Schwann cell lineage: from the neural crest to the myelinated nerve** *Glia 56(14):1481-90.*

Woolf CJ, Mannion RJ (1999) **Neuropathic pain: aetiology, symptoms, mechanisms, and management** *Lancet 353(9168):1959-64.*

Wu G, Ringkamp M, Murinson BB, Pogatzki EM, Hartke TV, Weerahandi HM, Campbell JN, Griffin JW, Meyer RA (2002) **Degeneration of myelinated efferent fibers induces spontaneous activity in uninjured C-fiber afferents** *J Neurosci 22(17):7746-53.*

Xu J, Zeng C, Chu W, Pan F, Rothfuss JM, Zhang F, Tu Z, Zhou D, Zeng D, Vangveravong S, Johnston F, Spitzer D, Chang KC, Hotchkiss RS, Hawkins WG, Wheeler KT, Mach RH (2011) **Identification of the PGRMC1 protein complex as the putative sigma-2 receptor binding site** *Nat Commun 2:380.*

Yamada K, Akasu T (1996) **Substance P suppresses GABAA receptor function via protein kinase C in primary sensory neurones of bullfrogs** *J Physiol 496 (Pt 2):439-49.*

Yang D, Bierman J, Tarumi YS, Zhong YP, Rangwala R, Proctor TM, Miyagoe-Suzuki Y, Takeda S, Miner JH, Sherman LS, Gold BG, Patton BL (2005) **Coordinate control of axon defasciculation and myelination by laminin-2 and -8** *J Cell Biol 168(4):655-66.*

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL (2012) **Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction** *BMC Bioinformatics 13:134.*

Yee BK, Keist R, von Boehmer L, Studer R, Benke D, Hagenbuch N, Dong Y, Malenka RC, Fritschy JM, Bluethmann H, Feldon J, Möhler H, Rudolph U (2005) **A schizophrenia-related sensorimotor deficit links alpha 3-containing GABAA receptors to a dopamine hyperfunction** *Proc Natl Acad Sci USA 102(47):17154-9.*

Zeller A, Arras M, Jurd R, Rudolph U (2007) **Mapping the contribution of beta3-containing GABAA receptors to volatile and intravenous general anesthetic actions** *BMC Pharmacol 7:2.*

Zeller A, Crestani F, Camenisch I, Iwasato T, Itohara S, Fritschy JM, Rudolph U (2008) **Cortical glutamatergic neurons mediate the motor sedative action of diazepam** *Mol Pharmacol 73(2):282-91.*

Zhang LJ, Wang XJ, Han JS (1990) **Phorbol ester suppression of opioid analgesia in rats** *Life Sci 47(19):1775-82.*

Zhou Y, Zhou ZS, Zhao ZQ (2001) **PKC regulates capsaicin-induced currents of dorsal root ganglion neurons in rats** *Neuropharmacology 41(5):601-8.*

Zhu WJ, Vicini S (1997) **Neurosteroid prolongs GABAA channel deactivation by altering kinetics of desensitized states** *J Neurosci 17(11):4022-31.*

Zhu Y, Bond J, Thomas P (2003a) **Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor** *Proc Natl Acad Sci USA 100(5):2237-42.*

Zhu Y, Rice CD, Pang Y, Pace M, Thomas P (2003b) **Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes** *Proc Natl Acad Sci USA 100(5):2231-6.*

Zigmond RE (2012) **Cytokines that promote nerve regeneration** *Exp Neurol 238(2):101-6.*

Zilberter Y, Kaiser KM, Sakmann B (1999) **Dendritic GABA release depresses excitatory transmission between layer 2/3 pyramidal and bitufted neurons in rat neocortex** *Neuron 24(4), 979-88.*

Zuloaga DG, Yahn SL, Pang Y, Quihuis AM, Oyola MG, Reyna A, Thomas P, Handa RJ, Mani SK (2012) **Distribution and estrogen regulation of membrane progesterone receptor-β in the female rat brain** *Endocrinology 153(9):4432-43.*

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sørgård M, Di Marzo V, Julius D, Högestätt ED (1999) **Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide** *Nature 400(6743):452-7.*