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The Cannabinoid 1 Receptor and Progenitor Cells in the Adult Central Nervous System

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1. Introduction

The aims of this chapter are to: (1) examine the key developments leading up to the discovery the cannabinoid 1 receptor (CB1R) and (2) assess the potential therapeutic benefits of cannabinoid drugs with respect to neurogenesis in the adult brain and spinal cord. As one of the most abundant G-protein coupled receptors found in the central nervous system, localization of CB1R and its role in the mature and in the developing brain will be discussed. Pharmacological studies with cannabinergic drugs, and studies utilizing knock-out mice of various endocannabinoid system components will be reviewed in the context of adult brain neurogenesis. The apparent conflicting data reveal the complexity of endocannabinoid signaling in this process. Though many studies have focused on CB1R and neurogenesis in the brain, none have evaluated the potential ability for CB1R to modulate the fate, and specifically neuronal differentiation, of adult spinal cord progenitors. The implications for CB1R modulation of adult neurogenesis are pivotal for understanding the behavioral and cognitive effects of chronic marijuana use, but also for assessing the potential consequences of pharmacotherapeutics with CB1R agonists or antagonists

2. Discovery of an endogenous cannabinoid system

The history leading up to the discovery of the "endocannabinoid (eCB) system" is an interesting one, sprouting from a decades- long quest for the active constituents of the marijuana plant, *Cannabis sativa*. Though the cannabis plant has long been used for a variety of purposes dating back more than 4000 years (O'Shaughnessy 1842; Mechoulam and Hanus 2000), only recently was it found that delta 9- tetrahydrocannabinol (Δ^9-THC) was the ingredient responsible for the psychotropic effects associated and exploited with its use (Mechoulam and Gaoni 1965).

One of the original and most ancient uses of the *Cannabis sativa* plant was to induce a trance-like state, often an essential component to the elaborate religious rites in ancient cultures ranging from the Chinese, to the Ayurvedic Indians, to the Persians and Greeks (O'Shaughnessy 1842; Aldrich 2006). Herodotus referred to the use of the hemp plant by the

Scythians as incense in funeral rites, and also described the use of the hemp plant by the Phoenicians to make 'cordage' for building bridges (Herodotus 1824). The plant was extensively cultivated for its fiber which was used to make fabric for ship sails and clothing, but was also used for food, cooking oil, as a lubricant, and as an analgesic (Grinspoon 1993).

The earliest work to find the active ingredient began in the late 19th century after reports from Dr. O'Shaugnessy during his travels in India. In the true spirit of a responsible clinical researcher, before testing on humans, Dr. O'Shaugnessy described the use of hemp on various animals, a practice not standard for physicians at his time. Based on his findings, he believed that certain patients could benefit from the use of cannabis extracts (O'Shaughnessy 1842). His case studies described the use of the drug in humans for rheumatism, hydrophobia, cholera, tetanus, and infantile convulsions. He cautioned, however, of the "delirium occasioned by continued Hemp inebriation," which continues to be a great -but not insurmountable- obstacle for modern pharmacologists synthesizing drugs targeting the endocannabinoid system. He detailed the effects of cannabis preparations for a variety of ailments in a lecture given to the Medical College of Calcutta in 1839. Based on his work, a renewed interest in active cannabis extracts led to scientific inquiry in Europe and the United States, but an active component was not isolated mostly due to lack of effective techniques available at the time. It was not until 1965 that the major psychoactive constituent Δ^9-THC by Mechoulam's group (Mechoulam and Gaoni 1965). By the 1970s, many phytocannabinoids were characterized, and it was determined that they were lipid derivatives. Because of the lipophilic nature of these compounds, their mechanism of action was thought to be mediated by their ability to adhere to cellular membranes, much like the proposed mechanism of anesthetic action (Paton 1975). The isolation of Δ^9-THC was a key breakthrough in the discovery of an endogenous cannabinoid system because it allowed for the unexpected identification of a highly specific binding site in the body (Devane, Dysarz et al. 1988). This binding site was isolated and cloned in 1990, from both rat and human tissues (Matsuda, Lolait et al. 1990; Gerard, Mollereau et al. 1991) and was named the cannabinoid 1 receptor (CB1R).

Since it did not seem logical that the body would invest energy in the synthesis of receptors that specifically bind the constituents of this one plant, scientists began looking for compounds produced by the body that could also bind to CB1R. Binding studies with known neurotransmitters and hormones proved to be unfruitful, indicating that a unique ligand was utilizing this newly discovered CB1R. By using a highly specific probe for CB1R labeled with tritium (Devane, Breuer et al. 1992), competitive binding studies in pig brain fractions indicated the presence of endogenous compounds with cannabimimetic activity. Chromatography, nuclear magnetic resonance and mass spectrometry were used to identify arachidonoylethanolamide (Devane, Hanus et al. 1992). An amide group in this newly discovered compound and the historically acknowledged effect of cannabis use, led to the witty alternate name for the very first endocannabinoid 'anandamide', deriving from the Sanskrit word for 'bliss' (Devane, Hanus et al. 1992; Mechoulam 2000). Not only did anandamide work like Δ^9-THC in binding assays, it also mimicked its effects on motor functions, sedation and pain relief (Mechoulam 2000).

In 1993, shortly after the discovery of anandamide, another cannabinoid receptor was found and cloned from the periphery (rat spleen), and identified mostly on immune cells (Munro, Thomas et al. 1993). It was referred to as the CB2 receptor (CB2R). Two groups, made the separate discovery of another endocannabinoid, 2-arachidonoyl glycerol (2-AG), that was

capable of binding to both the original CB1R, and to this novel CB2R receptor [(Mechoulam, Ben-Shabat et al. 1995; Sugiura, Kondo et al. 1995) and see **Figure 1** for a timeline].

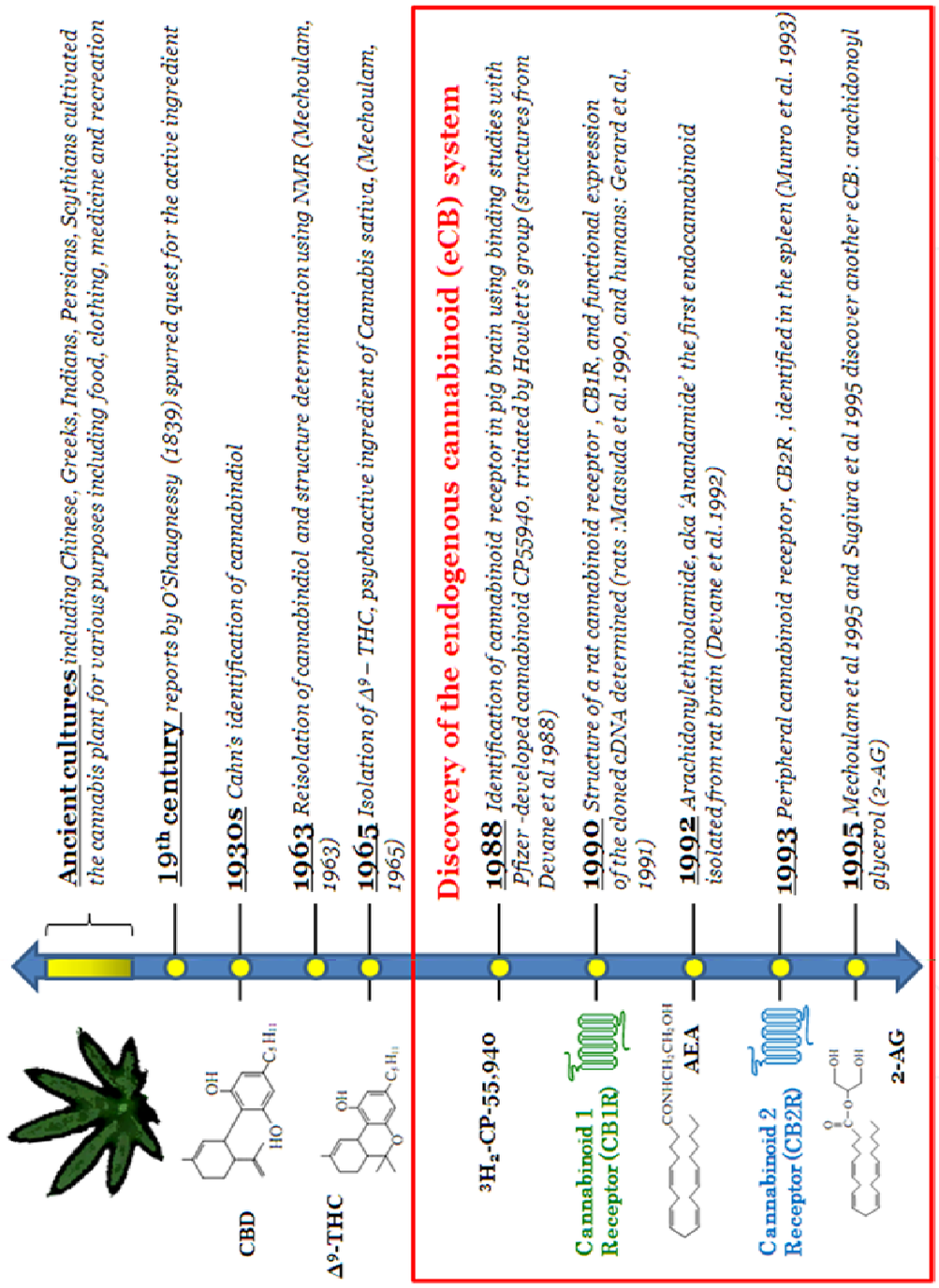

Fig. 1. **A Historical timeline of the eCB system.** Illustrated above are the major developments leading up to the discovery of an endogenous cannabinoid system, with the pivotal discoveries of the cannabinoid receptors, CB1R and CB2R, and the ligands AEA & 2-AG. Partly summarized from (Mechoulam and Hanus 2000). Receptors made with motifolio.com©.

By the end of the 20th century, the basic components of the endocannabinoid system- the receptors, endogenous ligands, and the enzymes responsible for their synthesis and degradation- were identified, paving the way for the groundbreaking discoveries that continually emerge, bringing forth the often surprising and unexpected ways in which this system works in the body.

3. CB1R localization in the central nervous system

With the discovery of the endocannabinoid system came the natural question as to what exactly these ligands and receptors are doing in the body. The location and density of CB1R could not only help explain some of the effects of cannabis use, but also has suggested the potential role of the endocannabinoids in learning, memory, motor function, emesis, reward behaviors and pain.

CB1R is a G-protein coupled receptor encoded by a single gene located on chromosome 4 in the mouse, 5 in the rat and 6 in humans**.** The mouse and rat display 95% nucleic acid homology, and 99.5% amino acid homology, while the mouse and human display 90% nucleic acid homology, and 97% amino acid homology (Onaivi, Leonard et al. 2002).

This receptor has been identified in both cortical and subcortical areas, the olfactory bulb, the retina, periaqueductal gray area, the cerebellum and the spinal cord (Mackie 2005). Original autoradiography studies revealed that the substantia nigra contains the highest density of CB1R in the central nervous system (CNS) (Herkenham, Lynn et al. 1991). The substantia nigra is a structure in the midbrain that plays an important role in movement, reward and addiction. CB1R is localized to the GABAergic (GABA = Gamma-aminobutyric acid) axons that project to the substantia nigra from the putamen. CB1R is also found in the caudate putamen, and on axons of medium spiny neurons projecting into the globus pallidus, on excitatory glutamatergic axons projecting from the sub-thalamic nucleus into the substantia nigra (Mailleux, Verslijpe et al. 1992; Sanudo-Pena, Tsou et al. 1997; Mackie 2005).

Much attention has been paid to the hippocampus and CB1R expression mainly because of the striking effects of marijuana on cognitive processes like memory. CB1R is widely distributed in the hippocampal structures. For example, high amounts of the receptor are found in the molecular and granule cell layer of the dentate gyrus (Mackie 2005), in the perisomatic region of CA1 indicative of expression that is post-synpaptic to basket cells, and may also be found on glutamatergic terminals of the perforant path (Kirby, Hampson et al. 1995). In the frontal cortex, double-immunocytochemical labeling experiments revealed GABAergic cholecystokinin (CCK) positive interneurons have somatic immunoreactivity for CB1R (Katona, Sperlagh et al. 1999; Tsou, Mackie et al. 1999). In terms of the laminar distribution within the neocortex high expression is found in layer II, upper III, layer IV and VI. Also it was found that the majority of cells in the neocortex which express the CB1R also express GAD65 (glutamic acid decarboxylase), the enzyme which converts L-glutamate to GABA, thus identifying inhibitory neurons in the CNS. In the cerebellum, there is a very high expression of CB1R in the molecular layer where the Purkinje neuron- parallel fiber synapse is found. Also, electrophysiological experiments infer that there are somatic CB1Rs on basket cells within the cerebellum. Therefore, there is strong evidence to suggest the presence of CB1R on GABAergic and glutamatergic neurons within the cerebellum (Mackie 2005).

Since it is believed that cannabis can be habit forming, evidence suggests that the brain area that processes addictive and reinforcing behaviors, the ventral tegmental area (VTA), contain GABAergic and glutamatergic terminals that express CB1R (Melis, Pistis et al. 2004). A potential, but yet unsubstantiated, role of CB1R in this area may be to facilitate other addictive behaviors such as alcoholism or illicit drug use (Mackie 2005). Cannabis and cannabinoid compounds have also been used as anti-emetics (Darmani 2001). Studies have illustrated that indeed the brain area responsible for emesis, the medullary nuclei of the brainstem (i.e. area postrema) contain high levels of the CB1R predominantly located on axon terminals. It is strongly believed that this anti-emesis may be attributed to CB1R activation in this area (Van Sickle, Oland et al. 2001; Van Sickle, Oland et al. 2003; Martin and Wiley 2004; Mackie 2005).

In the spinal cord, several studies have been published demonstrating that CB1R is found throughout the gray matter, but at higher densities in the dorsal areas relative to the ventral areas (Herkenham, Lynn et al. 1991; Tsou, Brown et al. 1998; Ong and Mackie 1999; Farquhar-Smith, Egertova et al. 2000; Mackie 2005; Hegyi, Kis et al. 2009). Many of our essential functions depend on an intact and healthy spinal cord, such as sensation (modulated primarily by the dorsal spinal cord) and locomotion (modulated primarily by the ventral spinal cord). This is evident particularly is diseases of the spinal cord or after traumatic injury, in which the most severe cases render the individual incapable of feeling or moving, or even death. At the spinal cord level, endocannabinoid tone and receptor expression appear to play a role in modulating movement (El Manira, Kyriakatos et al. 2008; El Manira and Kyriakatos 2010), but also nociception (Pernia-Andrade, Kato et al. 2009). Therefore, understanding the role of the eCB system in the adult spinal cord is clinically relevant, and deserves as much attention as other areas of the CNS.

Though strong evidence exists for neuronal CB1R expression, evidence also exists for its expression on astrocytes in the rat striatum (Rodriguez, Mackie et al. 2001), hippocampus (Navarrete and Araque 2008), and spinal cord (Salio, Doly et al. 2002). In addition, microglia derived from neonatal rat brains, were also found to be immunoreactive for CB1R (Waksman, Olson et al. 1999). RIP-positive or APC-positive oligodendrocytes in healthy adult rat brains and spinal cords, respectively, constitutively express CB1R (Molina-Holgado, Vela et al. 2002).

4. Role of CB1R activation in adult neurons

CB1R is included among the most abundant receptors in the brain, with picomolar ranges per milligram of tissue (as determined from rat brain (Herkenham, Lynn et al. 1991; Pazos, Nunez et al. 2005). Interestingly, compared to the abundance of CB1R, under physiological conditions, the amounts of eCBs (AEA and 2-AG) reach only into the low femtomolar range (Bisogno, Berrendero et al. 1999; Pazos, Nunez et al. 2005). This discrepancy - higher amounts of receptor and lower amounts of endogenous ligands- can be reconciled by understanding the function of the endocannabinoid system as an elegant and efficient negative feedback mechanism to control the levels of neurotransmitters released into the synaptic cleft.

Neurotransmitters are synthesized in the pre-synaptic neuron, and stored in vesicles ready to be released into the synaptic cleft after depolarization leads to an influx of calcium

through voltage-dependent calcium channels. In contrast, eCBs are synthesized on demand in the post-synaptic neuron using lipid precursors from cell membranes (Di Marzo, Bifulco et al. 2004) - **Figure 2**.

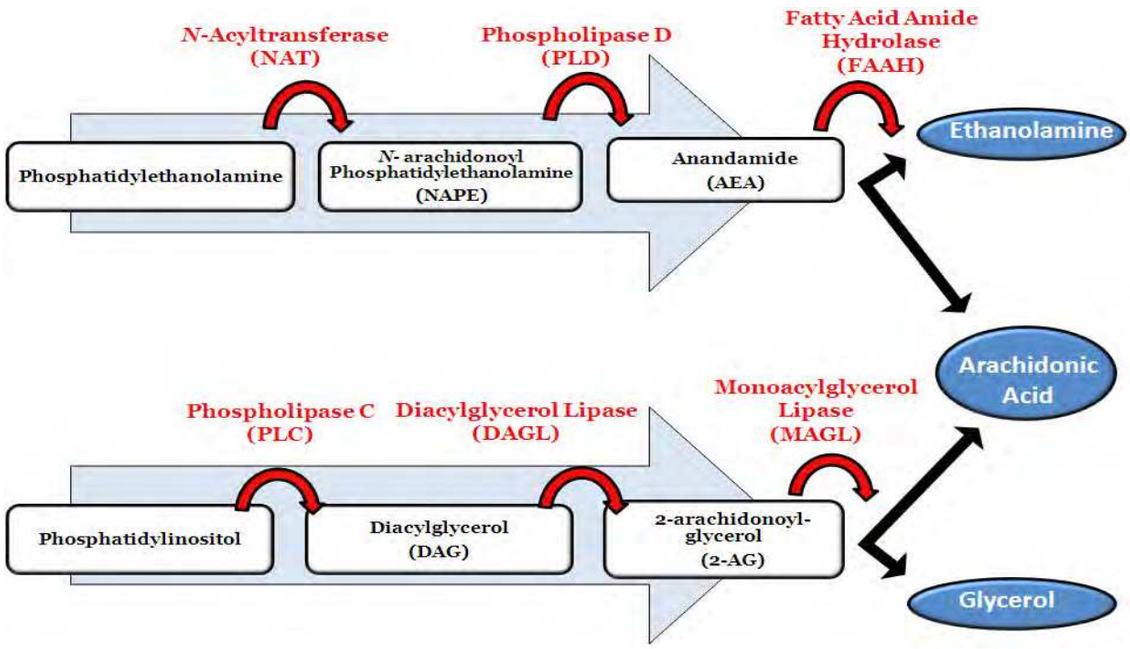

Fig. 2. The enzymes responsible for the synthesis and degradation of the two major eCBs, AEA and 2-AG. They are made on-demand from membrane lipid precursors in the post-synaptic neuron. The endocannabinoid membrane transporter (EMT) facilitates their re-uptake into either the post-synaptic (2-AG) or pre-synaptic (AEA) neuron for degradation by MAGL, or FAAH, respectively (Di Marzo, Bifulco et al. 2004; El Manira and Kyriakatos 2010)

Endocannabinoids readily pass through the post-synaptic membrane, travel retrogradely into the synaptic cleft, and bind to pre-synaptically located CB1Rs (Wilson and Nicoll 2002). As a G-protein coupled receptor, activation of CB1R by the endocannabinoids results in various cellular consequences, two of which are the ability to inhibit voltage-dependent calcium channels, or activate inwardly rectifying potassium channels. These processes affect the pre-synaptic neuron by ultimately decreasing the probability of neurotransmitter release (**Figure 3**).

The magnitude and duration of CB1R activation affects the machinery responsible for the release of several neurotransmitters such as glutamate, GABA, glycine, acetylcholine, noradrenaline and serotonin (Szabo and Schlicker 2005). Therefore, within a neuronal circuit, cells are able to regulate the strength of their synaptic inputs by on-demand release of eCBs which can then bind to CB1R (Freund, Katona et al. 2003). The high abundance of CB1Rs coupled with the relatively low-levels of detectable eCBs can be attributed to the fact that released ligand does not accumulate, but rather acts rapidly and transiently to mediate synaptic plasticity (Pazos, Nunez et al. 2005). In order to achieve such a highly efficient modulation of activity without accumulation of ligand, there must be a high density of receptors. This is precisely the state of the endocannabinoid system under physiological conditions.

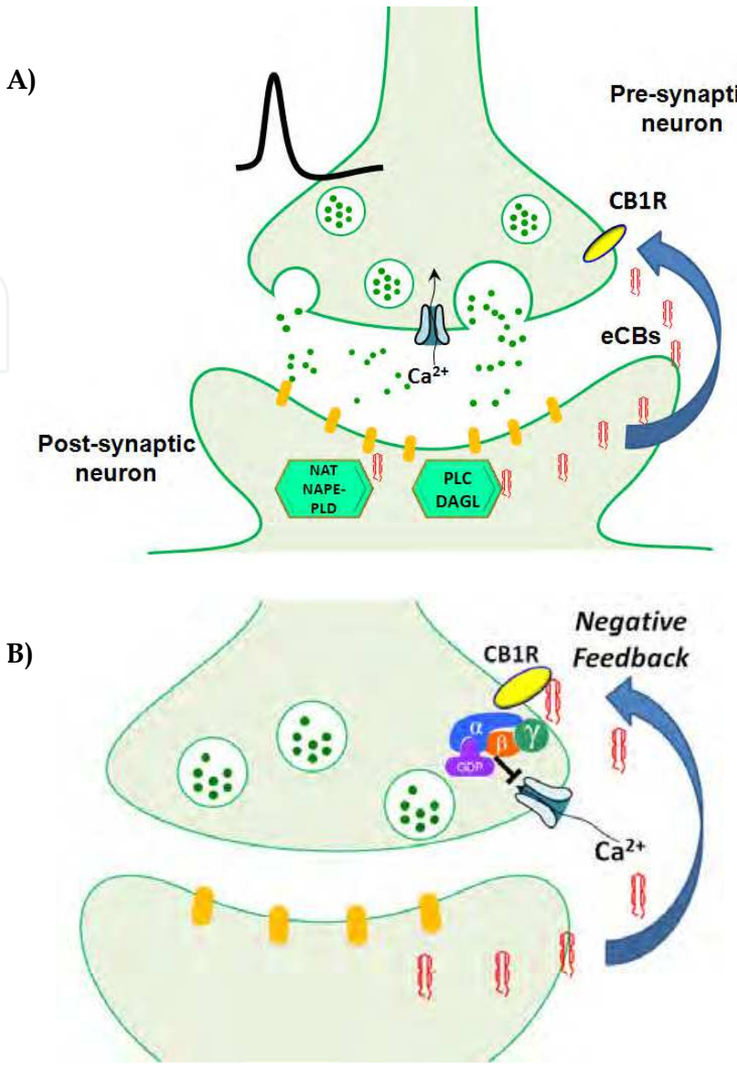

Fig. 3. **Endocannabinoids act as retrograde messengers in the CNS. (A)** Neurotransmitters bind to their postsynaptic receptors causing the synthesis of AEA or 2-AG via their synthetic enzymes, NAT/NAPE-PLD and PLC DAGL, respectively, before traveling retrogradely to bind to CB1Rs. **(B)** Inhibition of voltage-dependent calcium channels is one way by which neurotransmitter release probability is decreased. Binding of ligand to CB1R can result in the inactivation of N and P/Q- type, but not L-type calcium (Ca^{2+}) channels (Caulfield and Brown 1992; Mackie and Hille 1992; Mackie, Devane et al. 1993; Pertwee 1997). The particular channel involved is related to the brain region: in rat striatum, CB1Rs modulate N-type Ca^{2+} channels (Huang, Lo et al. 2001; Schlicker and Kathmann 2001) and not L, P or Q-type Ca^{2+} channels. In cultured rat hippocampal neurons, the CB1R modulates N- and Q-, but not P-type calcium channels (Sullivan 1999; Schlicker and Kathmann 2001). However, CB1R does not modulate any of the voltage- dependent Ca^{2+} channels found in the nucleus accumbens (Robbe, Alonso et al. 2001; Schlicker and Kathmann 2001). In contrast, newer evidence suggests that CB1R activation modulates all of the voltage-dependent Ca^{2+} channels found at the granule cell-Purkinje cell synapse of the cerebellum: the N-, P/Q- and R-type Ca^{2+} channels (Brown, Safo et al. 2004).

Furthermore, CB1R activation causes cAMP levels to drop because CB1R is negatively coupled to adenylate cyclase (AC) through heterotrimeric $G_{i/o}$ proteins, (Matsuda, Lolait et al. 1990; Munro, Thomas et al. 1993; Guzman, Sanchez et al. 2002). CB1R activation is also associated with activation of extracellular signal-related kinase (ERK) (Bouaboula, Poinot-Chazel et al. 1995; Wartmann, Campbell et al. 1995) c-Jun N-terminal kinase (Jnk) p38 mitogen activated-protein kinase (p38) (Rueda, Navarro et al. 2002), protein kinase B (Gomez del Pulgar, Velasco et al. 2000), and increased levels of the second messenger ceramide (Sanchez, Galve-Roperh et al. 1998; Guzman, Sanchez et al. 2002) (**Figure 4**). These pathways have been shown to modulate various cellular functions including cell fate, apoptosis and survival in different cell types (Guzman, Sanchez et al. 2001).

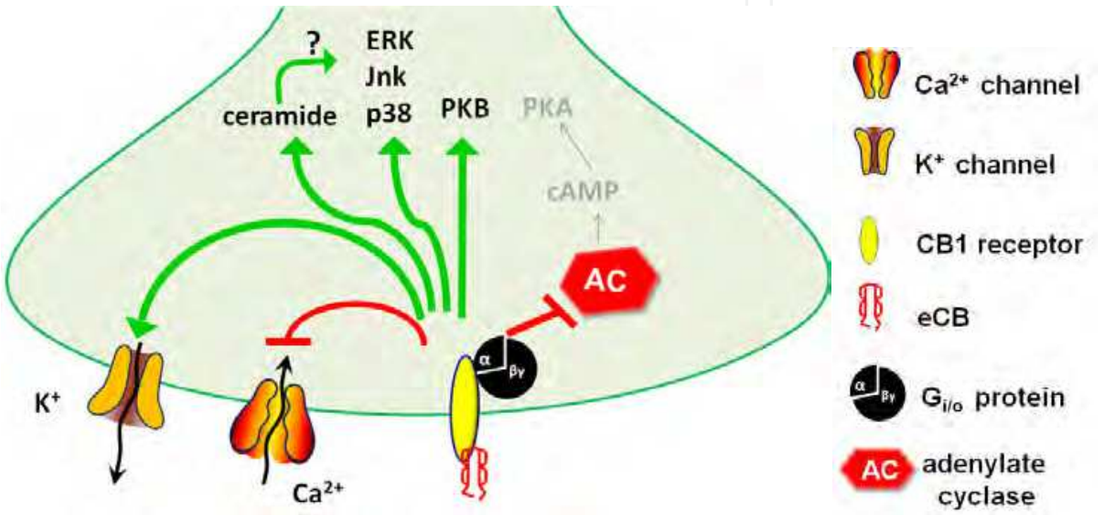

Fig. 4. **The effects of pre-synaptic CB1 receptor activation**. CB1R activation on pre-synaptic neurons inhibits voltage dependent calcium channels, and adenylate cyclase, but can also activate inwardly rectifying potassium channels, and the MAPK pathway. (Image adapted from DiMarzo et al. 2004, and Guzman et al. 2002. and created with motifolio.com©)

5. Role of cannabinoid receptors during pathological states

Because of the ubiquitous expression of the receptor throughout the CNS, several pre-clinical and clinical studies have addressed the potential therapeutic value in modulating the endocannabinoid system for analgesia, weight loss, appetite stimulation, neuroprotection after ischemic injuries, and for anti-emetic, anti-epileptic and anti-spasmodic purposes (Nogueiras, Diaz-Arteaga et al. 2009; Bisogno and Di Marzo 2010; Karst, Wippermann et al. 2010; Scotter, Abood et al. 2010). The premise of many of these therapeutic approaches lies in the neuromodulatory function of CB1R, or in the anti-inflammatory effects on CB2R activation.

During disease or following injury, cannabinoid receptor expression and levels of eCBs are altered. For example, after rat spinal cord injury, cannabinoid receptor expression is altered at the spinal level, but also in brain areas: in the spinal cord, CB1R becomes expressed in reactive astrocytes, and CB2R becomes strongly upregulated in microglia, astrocytes and macrophages. In the brain, CB1R is upregulated in thalamic and hippocampal areas, while

downregulated in the amygdala and Periaqueductal Gray Area (Garcia-Ovejero, Arevalo-Martin et al. 2009; Knerlich-Lukoschus, Noack et al. 2011). In healthy spinal cords, several studies indicate that there are very low levels of CB2R, but peripheral nerve injury, for example, leads to significant upregulation of this receptor, corresponding to significant microglial activation in the spinal cord (Zhang, Hoffert et al. 2003; Romero-Sandoval, Nutile-McMenemy et al. 2008). Microglial cells contribute to the inflammatory response by producing and secreting the pro-inflammatory cytokines that contribute to excitotoxic damage in the CNS, but also to the differentiation of pathogenic lymphocytes entering the CNS (Arevalo-Martin, Garcia-Ovejero et al. 2008). Activation of CB2R in cultured microglial cells inhibits these inflammatory cytokines, making CB2R activation an anti-inflammatory target. However, the potential role of CB2R in microglial cells following injury is not clear. Cultured rat microglial cells can produce the eCBs 2-AG and AEA, which in turn auto-stimulate their CB2Rs to induce proliferation (Carrier, Kearn et al. 2004).

Whether these changes reflect an adaptive defense mechanism or contribute to pathology is still a matter of debate. These studies implicate CB1R and CB2R as double edged swords for CNS insult, and whether their activation promotes protection or contributes to damage likely depends on the etiology and progression of the disease or injury, but also in the localization of each receptor on specific cell types.

6. Adult CNS progenitor cells and CB1R

Progenitor cells in the adult CNS are promising targets as endogenous repair mechanisms following insult, and their proliferation and differentiation may provide an avenue to do so. The functional significance of constitutive or pathologically-induced neurogenesis in the adult brain has been associated with wide ranging processes such as memory formation and consolidation, depression, anxiety, and seizure- like activity (Ming and Song 2011). Endocannabinoid system elements have recently been discovered in adult brain progenitor cells (Aguado, Monory et al. 2005; Aguado, Palazuelos et al. 2006; Palazuelos, Aguado et al. 2006). There is an emerging and critical role for the eCB system and specifically, CB1R in adult brain progenitor cells, revealing a novel strategy to help the brain repair itself (Galve-Roperh, Aguado et al. 2007).

In the adult brain, the subgranular zone (SGZ) of the hippocampus, and the subventricular zone (SVZ) contain two different populations of progenitor cells. The first population is referred to as the type 1 or type B cells (SGZ and SVZ, respectively). These cells resemble their developmental counterparts; the radial glia. They are characterized by their slow proliferation kinetics, their morphological hallmarks (tiny processes extending from their somata in the SVZ), and these cells express both Nestin and Glial Fibrillary Acidic Protein (GFAP). The type 2 or C cells (SGZ and SVZ, respectively) are actively dividing, non-radial cells that maintain their Nestin expression, but do not express GFAP. They are occasionally positive for the immature neuronal marker Doublecortin (DCX). Ablation studies indicate that these two different populations are distinct in their characteristics, but they are developmentally connected to one another. The type 1, B cells give rise to the type 2, C cells, and if the latter are destroyed, they can eventually be replenished by the former (Suh, Deng et al. 2009). These progenitor cells give birth to new neurons continually throughout adulthood, in a process known as adult neurogenesis.

It is imperative to distinguish these progenitor populations when assessing the role of the various eCB components in the neurogenic process. This distinction is rarely made in the literature, and yet it is very plausible that the various eCB system components affect these progenitor populations differently. The results from the following studies indicate that the distinct processes involved in adult brain neurogenesis cannot be grouped together with regards to endocannabinoid modulation.

The role of the eCB system, and in particular CB1R, on adult brain neurogenesis is not clear, partly because the separation between effects on progenitor proliferation and neuronal differentiation have not always been made. A study published in 2004 concluded that that there is defective neurogenesis in the CB1R knockout (KO) mouse (Jin, Xie et al. 2004). A major limitation to this study is that the authors equated changes in BrdU (thymidine analog) incorporation with changes in neurogenesis. Their data strongly support the view that CB1R is critical in progenitor proliferation in the hippocampus, but nothing more can be deduced with regards to which progenitor population is affected, nor about neuronal differentiation and maturation of the remaining progenitor cells.

Pharmacological studies in wild-type mice support the conclusions from CB1R KO mice. Treatment with CB1R agonists (either with the endocannabinoid anandamide, the synthetic agonists WIN 55, 212-2 or HU-210) increased the number of BrdU positive(+)/NeuN negative(-) hippocampal cells, but decreased the number of co-labeled, newly generated BrdU(+)/NeuN(+) neurons *in vivo* (Rueda, Navarro et al. 2002; Aguado, Palazuelos et al. 2006; Galve-Roperh, Aguado et al. 2006). Furthermore, these studies showed that a CB1R antagonist, SR141716, reversed the agonist actions- the number of co-labeled cells increased, while BrdU(+)/NeuN(-) cells decreased. Similarly, in a study by Jiang et al, 2005, CB1R activation resulted in increased BrdU(+) cells, which was interpreted as enhanced neurogenesis by cannabinoids; however, the authors themselves never show increases in co-labeled cells, and also point out that relative to no treatment, CB1R agonists do not change the percentage of cells expressing immature neuronal markers (Jiang, Zhang et al. 2005).

Adult hippocampal progenitor cells from mouse brains express CB1R *in vitro* and *in vivo* (Aguado, Monory et al. 2005; Aguado, Palazuelos et al. 2006). CB1R activation induced proliferation of these progenitors assessed by quantifying the amount of cells expressing Nestin and incorporating the thymidine analog BrdU. Interestingly, these studies showed CB1R and FAAH are selectively enriched in type 1 (Nestin(+)/GFAP(+)) progenitors *in vivo* compared to type 2 (Nestin(+)/ GFAP(-)) (Aguado, Palazuelos et al. 2006). Utilizing various markers for immature neurons and glia, CB1R activation appears to promote astroglial differentiation, while inhibition of the receptor appears to promote neuronal differentiation (Aguado, Palazuelos et al. 2006). In contrast, a recent study indicated that CB1R was preferentially expressed on type 2b/3 cells that are also expressing DCX, suggesting that CB1Rs have a role in later stages of neuronal differentiation, and migration of the nascent neuron (Wolf, Bick-Sander et al. 2010). This study examined the levels of DCX expressing cells in the hippocampi of CB1R KO mice, and also in Nestin-GFP reporter mice treated with the CB1R antagonist AM251. According to the authors, genetic deletion of CB1R resulted in increased proliferation but decreased net neurogenesis relative to wild-type mice. But, administration of the CB1R specific

antagonist AM251 to wild-type mice promoted proliferation of type 2b/3 DCX(+) cells 7 days after BrdU administration (**Figure 5**).

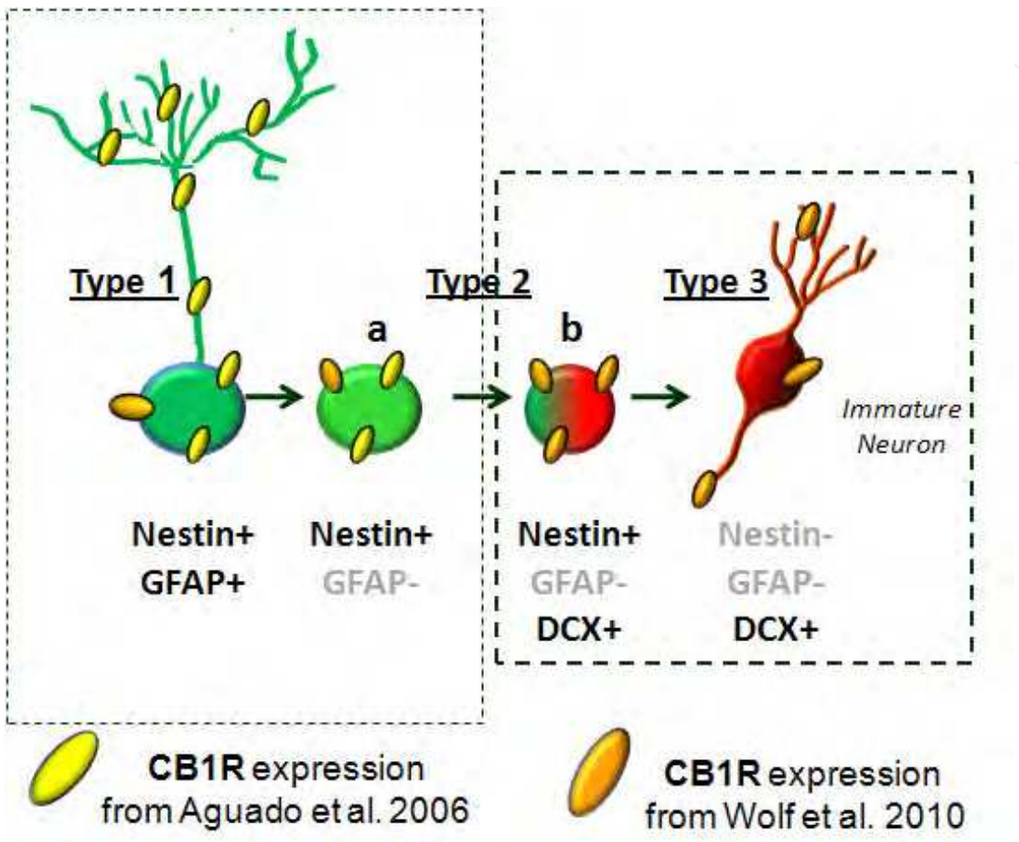

Fig. 5. CB1R is expressed throughout neuronal development (Harkany, Guzman et al. 2007; Harkany, Keimpema et al. 2008), and also at all stages of adult hippocampal neurogenesis. It is not clear whether CB1R is enriched in certain progenitor populations, and if so, how endogenous cannabinoids differentially affect these populations. Equally compelling is how exogenous CB1R agonists or antagonists may affect these different populations, and what the functional outcomes of such interventions may be. Image created with motifolio.com©.

Clarification through additional studies must be made to reconcile these seemingly disparate results. Species, sex and strain of the animals used, chronic versus acute treatment with cannabinergic drugs, specificity, dose/concentration of cannabinergic drugs, BrdU injection protocol and immunohistochemical markers must all be considered when

interpreting the many studies published on CB1R's role on adult hippocampal neurogenesis. Table 1 summarizes several knock-out mice that have been developed that target endocannabinoid system components, and the consequences on progenitor proliferation, neuronal differentiation and glial differentiation.

✓ = successful induction ✗ = impaired induction nc = no change N.D. = Not determined		CB1 -/- (Cannabinoid 1 Receptor)	CB2-/- (Cannabinoid 2 Receptor)	FAAH -/- (Fatty Acid Amide Hydrolase)	DAGLαβ -/- [d] (Diacyl-glycerol Lipase)	
					in vivo, SVZ	*in vivo*, Hippocampus
Neural Progenitor Proliferation	Embryonic/ Postnatal	✗ *in vivo* P2 hippocampus[a]	✗ *in vitro*,[c]	N.D.	N.D.	
	Adult	✗ *in vivo* 3 months old hippocampus[a]	✗ *in vitro* & *in vivo*[c]	✓ *in vivo*, 3 months old hippocampus[a]	α-/- ✗	✗
					β -/- nc	✗
Glial differentiation or Gliogenesis	Embryonic/ Postnatal	✗ *in vivo* P2 hippocampus[a]	N.D.	N.D.	N.D.	
	Adult	✗ *in vivo*, 3 months old hippocampus[a]	N.D.	✓ *in vivo*, 3months old hippocampus[a]	N.D.	
Neuronal Differentiaiton or Neurogenesis	Embryonic/ Postnatal	✓ *in vivo*, P2 hippocampus[a]	N.D	N.D.	N.D.	
	Adult	✓ *in vivo* 3 months old hippocampus[a]	N.D	✗ *in vivo*, 3 months old hippocampus[a]	α-/- ✗	✗
		✗ *in vivo* hippocampus[b]			β -/- N.D.	N.D.

Table 1. **eCB Knock-out mice and adult CNS progenitor cells.** [a] (Aguado, Palazuelos et al. 2006); [b](Jin, Xie et al. 2004); [c](Palazuelos, Aguado et al. 2006); [d](Gao, Vasilyev et al. 2010); KO= Knockout; SVZ = subventricular zone. The apparent conflicting results in the adult CB1 -/- brains may be attributed to the interpretation of 'neurogenesis'(see Section 6).

7. Neurogenesis in the adult spinal cord

Compared to the brain, even though progenitor cells also exist in the adult spinal cord, the spinal cord environment does not seem to support robust constitutive neurogenesis, nor does it seem to support neurogenesis following region specific injury or disease. Though injury results in different functional consequences for the brain and spinal cord, it is not clear why one region of the CNS is capable of generating new neurons, while another area is

not. There are several clinical examples where new neuron formation in the adult spinal cord could potentially ameliorate disease symptoms or progression, or replace damaged neurons following trauma. Replacement of dead or damaged neurons in the compromised spinal cord may be able to promote functional motor recovery, but also reduce pain (Hofstetter, Holmstrom et al. 2005; Scholz, Broom et al. 2005; Ohori, Yamamoto et al. 2006; Meisner, Marsh et al. 2010). Manipulating the spinal cord environment to coerce neurogenesis from endogenous progenitors is a promising therapeutic intervention, which may bypass the many obstacles inherent to transplantation of exogenous stem/progenitor cells (Obermair, Schroter et al. 2008).

Several models propose distinct locations for the endogenous spinal cord progenitors, and how they respond to physiological and pathological stimuli (Namiki and Tator 1999; Horner, Power et al. 2000; Horky, Galimi et al. 2006; Meletis, Barnabe-Heider et al. 2008; Hamilton, Truong et al. 2009; Barnabe-Heider, Goritz et al. 2010; Hugnot and Franzen 2011). The overwhelming majority of progenitor cells do not differentiate into neurons *in vivo*. Nevertheless, these progenitors have neurogenic potential revealed from *in vitro* studies, but also from *in vivo* transplantation studies. Progenitors isolated from all levels and areas of the adult spinal cord can give rise to neurons in culture (Weiss, Dunne et al. 1996; Yamamoto, Yamamoto et al. 2001). When spinal cord progenitors were transplanted into the hippocampus- a pro-neurogenic environment, they readily formed neurons (Shihabuddin, Horner et al. 2000). These studies imply that the spinal cord environment is restricting the neurogenic potential of the endogenous progenitors, and astrocytes may be one of the culprits (Song, Stevens et al. 2002).

New evidence is emerging to challenge the idea that new neurons cannot be generated in the adult spinal cord. Direct injury to the spinal cord results in massive progenitor proliferation leading to astrocyte differentiation, and a massive inflammatory response which contributes to glial scar formation (Barnabe-Heider, Goritz et al. 2010; Wang, Cheng et al. 2011). This injured environment has been demonstrated as non-neurogenic (Yamamoto, Nagao et al. 2001; Hannila, Siddiq et al. 2007); however, there are instances in which an environment filled with inflammatory cytokines can still elicit neurogenesis in the adult spinal cord. For example, in an experimental rat model of multiple sclerosis (experimental autoimmune encephalomyelitis), newly generated neurons migrated towards the neuroinflammatory lesion (Danilov, Covacu et al. 2006). Also there are instances of indirect injury to the adult spinal cord, such as dorsal rhizotomy (cutting of the dorsal root at the cervical spinal level) in which neurogenesis is observed in the dorsal horn at the corresponding spinal level (Vessal, Aycock et al. 2007). Recent papers showed that in non-injured, intact adult spinal cords, immature neurons can be found in the area surrounding the central canal (Shechter, Ziv et al. 2007; Marichal, Garcia et al. 2009), but also throughout the spinal cord, with a preferential dorsal gray matter localization and exclusive GABAergic phenotype (Shechter, Ziv et al. 2007; Shechter, Baruch et al. 2011). The exact roles of these immature neurons in the healthy spinal cord have not been determined, but may indicate physiological roles for new GABAergic neurons in nociception (Shechter, Baruch et al. 2011), and also for movement. The existence of these cells is exciting, as it sets the tone for more intensive studies to characterize their function and promote their differentiation.

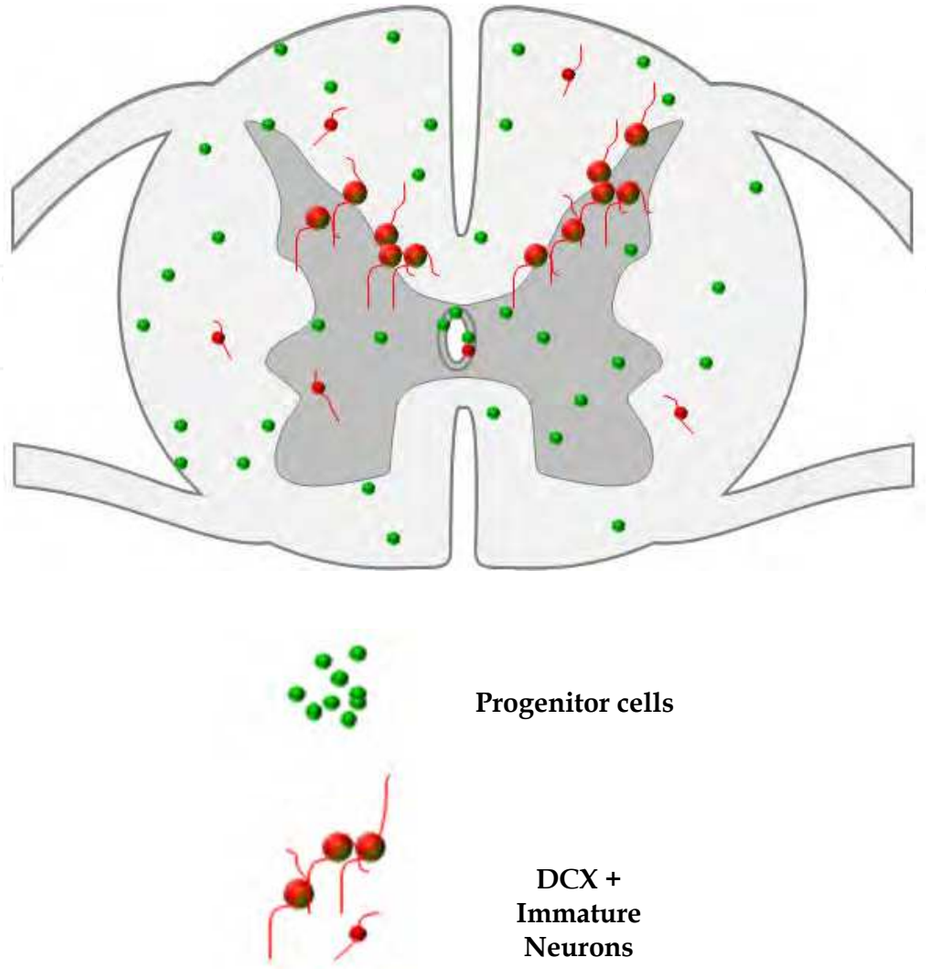

Fig. 6. Transverse section of an adult mouse spinal cord, depicting a model for progenitor cell and immature neuron location. Based on the work by Shechter et al, 2007, 2011, the majority of the GABAergic, BrdU(+)/ DCX(+) immature neurons reside in the gray matter of the dorsal horn. Under physiological conditions, the levels of these cells depend on the type of and exposure to sensory environmental enrichment. Image generated with motifolio.com©.

8. CB1R and adult spinal cord neurogenesis

Taking the adult brain as an example of endocannabinoid system involvement in progenitor cell proliferation and differentiation, there is a possibility that the spinal cord progenitors may also be modulated by this system. There is an overwhelming lack of published studies addressing the presence and roles of the endocannabinoid system in adult spinal cord progenitor cells. Of particular importance is that CB1R is widely distributed on cells throughout the spinal cord, but also in lamina X, which includes the putative progenitor cell niche. We have identified CB1R on adult spinal cord-derived Nestin(+) progenitor cells in primary cultures (**Figure 7**).

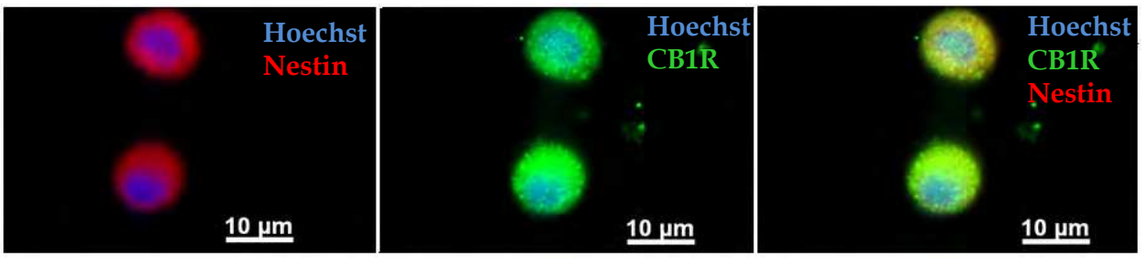

Fig. 7. Primary adult spinal cord cultures from rats contain Nestin(+) progenitor cells (red), which also express CB1R (green). The role of CB1R on these progenitors has not been examined, and further studies are needed to determine how the receptor is involved in progenitor cell quiescence, proliferation or differentiation. Image obtained after 6 days *in vitro* with 63X objective.

In response to injury, not only do progenitor cells proliferate in the spinal cord (Frisen, Johansson et al. 1995; Johansson, Momma et al. 1999; Namiki and Tator 1999; Shibuya, Miyamoto et al. 2002), but levels of endocannabinoids, receptors and enzymes are also altered as described earlier (in Section 4). Rigorous studies are needed to address if and how adult spinal cord progenitor cells respond to endogenous cannabinoid tone or to exogenously administered cannabinoids. Does endocannabinoid tone contribute to the non-neurogenic spinal cord environment? Are endo/exo-cannabinoids capable of promoting spinal cord neurogenesis or gliogenesis? These are just a few critical and novel avenues for potentially promoting neurogenesis in the adult spinal cord.

9. The effect of chronic cannabinergic drug use on the CNS- implications for the treatment of chronic pain

Cannabis is used both acutely and chronically for recreational or medicinal purposes. There is controversy regarding medical marijuana because of the documented cognitive side effects of chronic recreational use (Jager and Ramsey 2008; Hester, Nestor et al. 2009; Battisti, Roodenrys et al. 2010). However, all drugs come with a risk-benefit consideration, and a plethora of historical and emerging evidence indicates that the medicinal value of cannabis cannot be ignored. Many studies have demonstrated that endocannabinoids and application of exogenous cannabinoids (usually mixed CB1R/CB2R agonists) reduce pain sensation (Guindon and Hohmann 2009). Presently, such an approach is becoming more clinically accepted for treating chronic pain states (Aggarwal, Carter et al. 2009; Karst, Wippermann et al. 2010; Lynch and Campbell 2011). While CB2R activation attenuates nociception mostly by modulating the inflammatory response (Guindon and Hohmann 2008), the role of CB1R is more complex because its location on various cells along the pain pathways appears to contribute differently to nociception. Moreover, many cannabinergic drugs are not only mixed agonists, but may bind non-specifically to other receptors, including TRP-channels (Patwardhan, Jeske et al. 2006; Patil, Patwardhan et al. 2011).

The use of several CB1R knock-outs (global and conditional) has helped to clarify the role of these receptors in nociception. Recent worked demonstrated that cannabinoids mediate analgesia by activating CB1Rs located on peripheral nociceptors (dorsal root ganglia sensory neurons) (Agarwal, Pacher et al. 2007). Interestingly, by using *in vitro* spinal cord slices and *in vivo* recordings of dorsal horn neurons, activation of CB1Rs on spinal cord dorsal horn

neurons actually enhances (not reduces) nociceptive responses (Pernia-Andrade, Kato et al. 2009; Zhang, Chen et al. 2010). Stimulation of spinal cord CB1Rs inhibits the release of GABA, glycine (Pernia-Andrade 2009), and opioids, while enhancing the release of substance P (Zhang, Chen et al. 2010). Therefore, CB1R activation may contribute to nociception by increasing excitability at the spinal cord level. Consequently, CB1R antagonists have shown anti-nociceptive efficacy in several experimental pain models (Costa, Trovato et al. 2005; Croci and Zarini 2007; Pernia-Andrade, Kato et al. 2009). On the contrary, another recent study also using *in vivo* recordings demonstrated that blocking spinal CB1Rs enhanced the evoked response of the spinal cord dorsal horn neurons in neuropathic rats, indicative of a pro-nociceptive role of the receptor (Sagar, Jhaveri et al. 2010). One explanation for these different results could be attributed to the anesthetic used. Pernia-Andrade et al 2009 used a mixture of pentobarbital with pancuronium (a muscle relaxant), while Sagar et al.'s study only used isoflurane. The use of a muscle relaxant would allow the use of lower levels of the anesthetic to achieve immobility (required for the *in vivo* recordings). It is possible that the level of anesthesia used in Sagar et al's 2010 recordings may have depressed the neuronal activity relevant to pain sensation. Consistent with this possibility is that there was no difference in the firing rate of dorsal horn neurons in anesthetized neuropathic and sham operated animals at various levels of stimulation.

The chronic use of mixed cannabinoid drugs should be further investigated in light of the fact that the majority readily cross the blood-brain barrier. These compounds may be capable of providing pain relief, but they may also be affecting other important cellular functions, such as neurogenesis in the brain and spinal cord. Neurogenesis from endogenous progenitor cells is associated with a wide range of functions, and perturbations of this process are correlated with disease symptoms. Interference of physiological neurogenesis may be a highly undesirable side-effect of chronic endocannabinoid system manipulation by the use of CB1R/CB2R agonists or antagonists. For example, following peripheral nerve injury or direct spinal cord injury, a specific loss of inhibitory GABAergic interneurons in the spinal cord dorsal horn is postulated to be a major contributor to chronic pain (Moore, Kohno et al. 2002; Scholz, Broom et al. 2005; Meisner, Marsh et al. 2010). Replacement of these neurons through neurogenesis is an attractive therapeutic strategy because it attempts to go beyond the management of symptoms; it targets an underlying biological phenomenon of neuronal death following injury. Given the controversy regarding how cannabinoids modulate neurogenesis, it is possible that while treatment with mixed cannabinoids can ameliorate pain, long term usage may prevent the replacement of damaged inhibitory neurons by blocking neurogenesis, and thus contribute to an underlying etiology of chronic pain. Hence understading the role of the individual CBRs in adult neurogenesis, but also during pain states, could help discern how to more succesfully use these agents clinically.

10. Conclusions

CB1R expression on adult CNS-derived progenitor cells is not only indicative of endogenous cannabinoid modulation, but also points to potential consequences of cannabinoid pharmacotherapy on progenitor proliferation and differentiation- whether beneficial or deleterious. The complex results published about adult brain progenitors and the lack of data on adult spinal cord progenitors demonstrate that extensive basic research is

still needed to understand how the endocannabinoid system affects these cells normally and in response to injury and disease.

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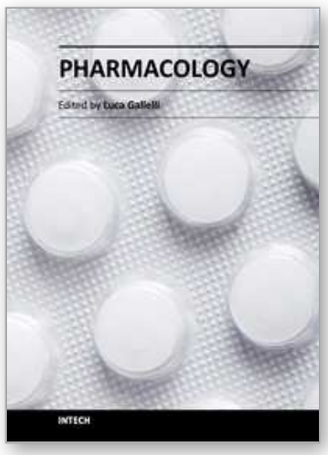

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The history of pharmacology travels together to history of scientific method and the latest frontiers of pharmacology open a new world in the search of drugs. New technologies and continuing progress in the field of pharmacology has also changed radically the way of designing a new drug. In fact, modern drug discovery is based on deep knowledge of the disease and of both cellular and molecular mechanisms involved in its development. The purpose of this book was to give a new idea from the beginning of the pharmacology, starting from pharmacodynamic and reaching the new field of pharmacogenetic and ethnopharmacology.

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