Scuola Internazionale Superiore di Studi Avanzati - Trieste

Endocannabinoids and excitotoxicity: lessons from hypoglossal motoneurons

Thesis submitted for the degree of

'*Doctor Philosophiae*'

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Notes:

The work described in this thesis was carried out at the International School for Advanced Studies, Trieste, between August 2012 and April 2015. Data reported in the present thesis have been presented in a poster session during the 25th International Symposium on ALS/MND which took place in December 2014 in Brussels, Belgium. All data reported in this work arises from my own experiments and data analysis.

Contents

1. Abstract

Brainstem hypoglossal motoneurons (HMs) exclusively innervate tongue muscles and are severely damaged in the neurodegenerative disease called amyotrophic lateral sclerosis (ALS). One mechanism leading to such cell death is proposed to be glutamate-mediated excitotoxic stress. HMs are particularly vulnerable to excitotoxicity due to their expression of calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors and scarcity of intracellular Ca^{2+} binding proteins like parvalbumin and calbindin. Indeed, blocking glutamate uptake in medullary slices can lead to pathological bursting and motoneuron damage.

The endocannabinoid system is widely distributed in the brain and is believed to be an important regulator of synaptic transmission. Several studies reported neuroprotection mediated by the endocannabinoid system in such pathological insults like brain ischemia, traumatic brain injury or excitotoxicity. Moreover, in ALS animal models, up-regulation of the endocannabinoid system has been detected, suggesting it can play a role during disease development. Thus, detailed information on how the endocannabinoid system can affect cells during pathological insults like excitotoxicity is a valuable asset for future investigations of novel therapy approaches for ALS.

The objective of this work was to investigate the effect of modulation of the endocannabinoid system during excitotoxic stress in hypoglossal motoneurons *in vitro*. Thin medullary slices (for electrophysiology and viability assay) or whole brainstem isolates (for Western Blot) from postnatal Wistar rats were used. Each slice/brainstem containing hypoglossal nuclei was transferred to a recording/incubation chamber and superfused with oxygenated Krebs solution. Excitotoxic stress was evoked by application of DL-TBOA (DL-threo-β-benzyloxyaspartic acid, 50 μM), a potent and selective inhibitor of excitatory amino acid transporters, with consequent build-up of extracellular glutamate.

It was observed that modulation of endocannabinoid CB1 receptor (CB1R) function affected TBOA-evoked bursting, an event previously correlated with TBOA toxicity. Co-application of the endocannabinoid anandamide (AEA, 10 μM), a CB1R agonist, with TBOA resulted in lowered probability of the occurrence of pathological bursting, whereas co-application of the CB1R antagonist AM251 (10 μM) disrupted TBOA-induced bursts, leading to their “fragmentation”. Furthermore, AEA significantly decreased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) isolated by co-application of bicuculline and strychnine (10 μM and 0.4 μM, respectively) and caused occurrence of biphasic activity in spontaneous inhibitory postsynaptic

currents (sIPSCs; isolated by co-application of DNQX and APV at 10 μM and 50 μM, respectively) in some of the recorded cells. AM251 caused a decrease in the frequency of sIPSCs, but during application of bicuculline and strychnine it evoked activity which partly resembled bursting observed during TBOA application. Moreover, co-application of AEA with TBOA significantly decreased the number of damaged propidium iodide-positive cells with respect to counterstained Hoechst 33342-positive cells, which suggests a protective effect of this CB1R agonist against TBOA-induced toxicity. In addition, Western blot analysis showed a significant increase in CB1R protein levels after only 4 hours of TBOA incubation, indicating that the endocannabinoid system is activated during this excitotoxic insult. We suggest that a likely role of the endocannabinoid system in our brainstem preparation is to counteract the effects and consequences of elevated glutamate levels in the extracellular compartment.

2. List of abbreviations

Δ^9-THC - delta-9-tetrahydrocannabinol

$[Ca^{2+}]_i$ - intracellular calcium concentration

2-AG - 2-arachidonylglycerol

4-AP - 4-aminopyridine

ADP - afterdepolarization

AEA - anandamide

AHP - afterhyperpolarization

ALS - Amyotrophic Lateral Sclerosis

AM251 - N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide

AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

ASK1 - apoptosis signal-regulating kinase 1

ATF-3 - activating transcription factor 3

B - bicucullune

BAPTA-AM - 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis(acetoxymethyl ester)

BCA - bicinchoninic acid

cAMP - cyclic adenosine monophosphate

CB1/CB1R - cannabinoid receptor 1

CHI - closed head injury

CNS - central nervous system

COX-2 - cyclooxygenase 2

CPG - central pattern generator

D-APV - d-amino-phosphonovalerate

DAGL - diacylglycerol lipase

DL-TBOA - DL-threo-β-benzyloxyaspartate

DNQX - 6,7-dinitroquinoxaline-2,3-dione

EAAT2 - excitatory amino acid transporter 2

ECB - endocannabinoids

ER- endoplasmatic reticullum

FAAH - fatty acid amide hydrolase

fAHP - fast afterhyperpolarization

FALS – Familial Amyotrophic Lateral Sclerosis

FUS - fused in sarcoma

GABA - γ-Aminobutyric acid

GLT1- glutamate transporter 1

GluR2 - glutamate receptor 2 subunit

h - hour

HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HMs - hypoglossal motoneurons

I_A - A-type potassium current

I_{CaHVA} - high-threshold calcium current

I_{CaLVA} - low-threshold calcium current

I_{CaP} - persistant calcium current

I_D - D-type potassium current

I_{fast} - A-type, fast sodium current

I_h - hyperpolarization-activated, mixed-cation current

I_{In} - inward current

$I_{KCa(BK)}$ - calcium-activated potassium current with large conductance

$I_{K\ Ca(SK)}$ - calcium-activated potassium current with small conductance

I_{KDR} - delayed rectifier potassium current

I_{Kir} - inwardly rectifying potassium current

I_M - M-type potassium current

I_{Na} - fast sodium current

I_{NaP} - persistant potassium current

LOX - lipoxygenase

mAHP - afterhyperpolarization of medium duration

mGluR - metabotropic glutamate receptors

NMDA - N-methyl-D-aspartate

R_{In} - input resistance

ROS - reactive oxygen species

R_s – series resistance

S - strychnine

SDS-PAGE - sds polyacrylamide gel electrophoresis

sEPSC - spontaneous excitatory post-synaptic current

sIPSC - spontaneous inhibitory post-synaptic current

SOD1 –superoxide dismutase 1

sPSC - spontaneous post-synaptic current

TBST - Tris-buffered saline and Tween 20

TDP43 - transactive response DNA binding protein

TEA - tetraethylammonium

TTX - tetrodotoxin

UPR - the unfolded protein response

VAPB - (vesicle-associated membrane protein)-associated protein

VDAC - voltage-dependent anion channels

VDCC - voltage-dependent calcium channels

V_h - holding potential

V_m - membrane potential

WB - Western blot

3. Introduction

3.1 Amyotrophic lateral sclerosis

3.1.1. ALS: epidemiology

Jean-Martin Charcot was a French neurologist, whose accomplishments for modern neurology remain invaluable. One of his achievements was the first description of the disease which in 1874 was given its final name: Amyotrophic Lateral Sclerosis (ALS) (Kumar et al., 2011). The name 'ALS' refers to the atrophy of the denervated muscle fibers (Amyotrophic) and the hardening of the anterior and lateral corticospinal tracts (Lateral Sclerosis).

In the majority of cases, ALS is an adult onset disease, withamean age of symptoms starting between 55-65 years. Except for rare cases, the progression of the disease is rapid and leads to death due to respiratory failure within 2-3 (for bulbar onset) or 3-5 (for limb onset) years. It has rather uniform prevalence (around 5.2 per 100,000) and incidence (around 1.89 per 100,000/year) in Western countries (Wijesekera and Leigh, 2009).

3.1.2. Symptoms and diagnosis

Currently, the name ALS is used to describe a spectrum of neurodegenerative syndromes, which are characterised by progressive degeneration of motor neurons. The simplest classification divides ALS into spinal or bulbar onset. Most of the typical ALS patients (around 2/3) develop the spinal-onset form of the disease. The symptoms can start asymmetrically, but the other limbs will later develop weakness. Most patients also develop bulbar and eventually respiratory symptoms. In the case of bulbar onset ALS, patients initially present dysarthria (motor speech disorder), or rarely dysphagia for solid or liquids. At the same time of bulbar symptoms, limb symptoms can appear. The most common causes of death for ALS patients in both classifications are respiratory failure or other pulmonary complications (Wijesekera and Leigh, 2009).

Despite a large-scale search for specific markers, the diagnosis of ALS is still based on the presence of clinical features and the exclusion of other motor disorders. Most commonly, patients

are admitted to the doctor with weakness, twitching muscles (fasciculation), or hyper-reflexia of facial or limb muscles (Redler and Dokholyan, 2012). During years of studies on the ALS, El Escatorial research diagnostic criteria for ALS were developed by The World Federation of Neurology (WFN) Research Group on Motor Neuron Diseases. For the diagnosis of the disease, they require both upper and lower motor neuron degeneration, progressive spread of symptoms or signs within a region or to other regions, in the absence of evidence of other disease processes that might explain the symptoms. Moreover, they classify patients as 'possible', 'probable', and 'definite ALS (Redler and Dokholyan, 2012; Wijesekera and Leigh, 2009).

3.1.3. Causes of ALS

The disease is highly specific for motoneurons. In the majority of cases cognitive ability, sensation and autonomic nervous functions are preserved up to the very late stage. Nonetheless, 5-

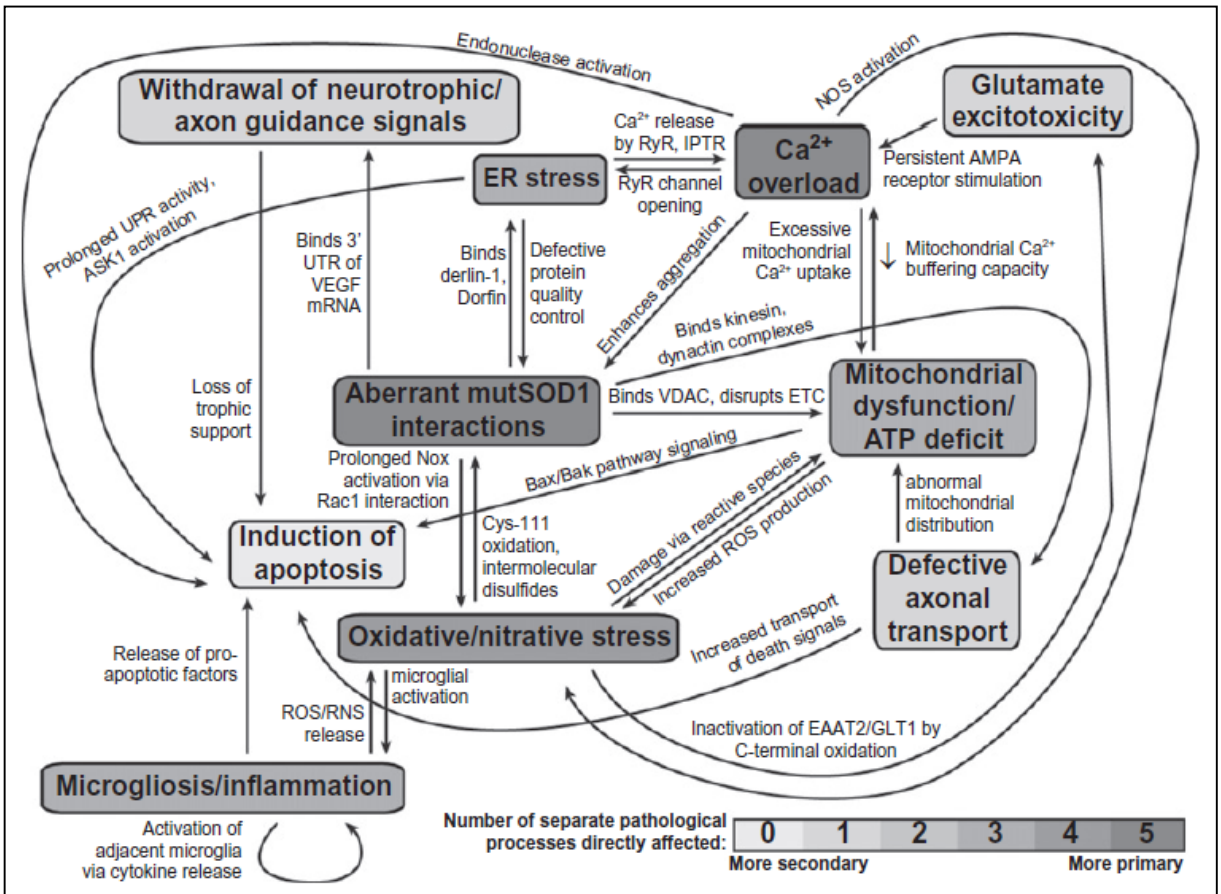

Fig 3.1 Diverse pathological processes in superoxide dismutase 1 (SOD1)-related familial ALS (FALS). FALS are highly interrelated and many step directly from SOD1 misfolding/aggregation and cytosolic calcium overload. Abbreviations: mutSOD1, mutant SOD1,; UTP, untranslated region; VDAC, voltage-dependent anion channel; ETC, electron transport chain; UPR, unfolded protein response; ROS/RNS, reactive oxygen species (adapted from Redler and Dokholyan, 2012).

10% of patients develop frontotemporal lobar dementia (FTLD) together with their ALS (Redler and Dokholyan, 2012).

ALS is a complex disease and its mechanisms are still elusive. Understanding this devastating condition is complicated by the fact that motoneuron death seems to result from a combination of numerous insults and not a single pathological event. During years of investigation numerous theories behind the development of ALS emerged, including oxidative insult, mitochondrial dysfunction, endoplasmic reticulum stress, protein aggregation, cytoskeletal dysfunction, environmental factors, neuromuscular junction alterations and excitotoxic stress. A lot of information was gathered thanks to the discovery of mutations causing ALS that led to the development of genetically-mutated rodents which gave significant input to the understanding of the pathogenic mechanisms involved in this disease (Fig 3.1).

3.1.3.1. Genetic causes

Genetic mutations are responsible for familial cases of ALS (FALS). The first gene associated with ALS was superoxide dismutase 1 (SOD1) responsible for around 20% of familial cases of the disease (Pasinelli and Brown, 2006). Currently there are over 30 genes reputed to be involved in the pathogenesis of ALS. In addition to SOD1, occurrence of ALS has been connected to TDP-43 (transactive response DNA binding protein), FUS, C9ORF72, alsin, VAPB, optineurin, alsin, ubiquitin, dynactin and many others. The vast diversity in function of those genes suggests that ALS can develop as the result of multiple cellular system disturbances (Wijesekera and Leigh, 2009).

3.1.3.2. Oxidative stress

Discovery of SOD1 involvement in ALS led to the theory of oxidative stress as the origin of motor neuron degeneration (Rosen et al., 1993). Studies have found wide-spread evidence for oxidative stress damage in both patients and animal models of the disease (Carrì et al., 2015; Carter et al., 2009; D'Amico et al., 2013; Liu et al., 1999; Poon et al., 2005; Tohgi et al., 1999). Excessive reactive oxygen species (ROS) can damage various cellular components including proteins, DNA, lipids, and cell membranes (Bogdanov et al., 2000; Girotti, 1998; Shaw et al., 1995). Oxidative stress isaserious threat to vulnerable neurons, regardless of whether it is a secondary consequence of

other pathological processes or a primary cause of the disease. Despite obvious oxidative insults related to ALS, trials with antioxidant therapies were not successful so far (Orrell et al., 2008).

3.1.3.3. Non-neuronal cell contribution

The discovery that astrocytes expressing mutated SOD1 protein are toxic to healthy neurons in co-culture has led to the theory of a glial-origin of ALS (Nagai et al., 2007). Activated microglia (which is one of the pathological features of ALS) can release a vast array of inflammatory and pro-apoptotic factors, which activate cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), which result in increased levels of prostaglandins and nitric oxide (Sargsyan et al., 2005). Moreover, programmed cell death signals such as Fas ligand (FasL) and tumor necrosis factor-alpha (TNF-α) are released and might contribute to motoneuron death in ALS (Raoul et al., 2002; Sargsyan et al., 2005).

3.1.3.4. Axonal structure and transport defects

Motoneurons have extremely long axons reaching up to 1m, with a very high energetic demand and established polarity (Shaw and Eggett, 2000). In such conditions, maintenance of axonal integrity is crucial to cell survival (Julien, 1997; Kawamura et al., 1981). Neurofilaments are fundamental structures of the neuronal cytoskeleton and their proper assembly is essential for the survival of large neurons like motoneurons. Accumulation of neurofilaments is one of the hallmarks in ALS pathology (Bruijn et al., 2004; Carpenter, 1968; Gurney et al., 1994; Hirano et al., 1984; Tu et al., 1997). Miss-assembly, mutations or deficient transport of individual subunits, hyper-phosphorylation causing detachment from motor complexes and aberrant self-association (Ackerley et al., 2003; Jung et al., 2005; Shea et al., 2004; Wagner et al., 2004) can all contribute to this process. Occurrence of such a neurofilament accumulation can further jeopardize axonal transport, leading to motoneuron death (Beaulieu et al., 1999; Collard et al., 1995; Côté et al., 1993; Lee et al., 1994; Millecamps et al., 2006; Yuan et al., 2003).

Neurofilaments are not the only ones affected by axonal transport malfunction. Mouse models of the disease showed alterations in both retrograde (mediated by dynein/dynactin motor protein complexes) and anterograde (mediated by kinesin motor protein complexes) transport (Perlson et al., 2009; Warita et al., 1999; Williamson and Cleveland, 1999) in the pre-symptomatic

stage of the disease. Retardation of mitochondrial and cytoskeletal component trafficking seems to be particularly vulnerable (De Vos et al., 2007; Magrané and Manfredi, 2009; Williamson and Cleveland, 1999). Distal accumulation of mitochondria, especially near the neuromuscular junction is essential to meet high energetic and calcium-buffering needs in this particular synaptic area (Rowland et al., 2000). Sporadic ALS patients show accumulation of that important organelle in proximal axons to support the role of axonal transport alterations in the pathological processes contributing to motoneuron death (Sasaki and Iwata, 1996).

3.1.3.5. Mitochondrial dysfunction

Mitochondria are key organelles in motoneurons. Not only do they supply energy to maintain all physiological processes in the cells, but they also play a crucial role in calcium homeostasis. Abnormalities such as vacuolization and swelling are well documented not only in ALS patients, but also in numerous rodent models of the disease (Bendotti et al., 2001; Jaarsma et al., 2000; Martin, 2007; Martin et al., 2007; Wong et al., 1995). Misfolded SOD1 proteins are known to accumulate at the cytoplasmic site of the outer mitochondrial membrane, and directly interact with voltage-dependent anion channels (VDAC), thus depolarizing the membrane and perturbing the electron transport chain (ETC) (Jung et al., 2002; Liu et al., 2004; Mattiazzi et al., 2002; Vande Velde et al., 2008). Moreover, the mutant G93A of SOD1 has the ability to bind cytosolic malate dehydrogenase, an event which disrupts the malate-aspartate shuttle (Mali and Zisapels, 2008).

Additionally, mitochondria are key players in intracellular calcium homeostasis. Experiments on animal models of ALS showed a CNS-specific decrease in mitochondrial calcium loading capacity (Damiano et al., 2006). Mitochondrial damage is detected concomitantly with increased intracellular calcium (Damiano et al., 2006; Siklós et al., 1996), although it is not clear which one of them occurs first.

Disturbed mitochondrial function can release cytochrome c (Kirkinezos et al., 2005) which can trigger apoptosis. Mitochondrial pathologies are observed in sporadic and familial cases of ALS (Borthwick et al., 1999; Swerdlow et al., 1998; Vielhaber et al., 2000). Moreover, the mitochondrial genome instability in the ageing process accords with the late disease onset in the majority of sporadic cases of ALS (de Grey, 2004; Khrapko and Vijg, 2007; Kujoth et al., 2005; Trifunovic et al., 2004; Vermulst et al., 2007).

3.1.3.6. Deficient protein quality control and protein aggregation

The ubiquitin-proteasome system (UPS) is the intracellular machinery that degrades miss-folded and old proteins. Malfunction or overloading of this system has been implicated in the development of ALS. Protein aggregates have been found in familial and sporadic cases, implying that disturbance in this protein clearance pathway is a common feature of neurodegeneration (Bendotti et al., 2004; Bruijn et al., 1997; Leigh et al., 1991; Mendonça et al., 2006; Sasaki, 2010; Watanabe et al., 2001). Nevertheless, studies on SOD1 mutant mice have revealed that proteosomal impairment occurs after the onset of the symptoms, making it unlikely to be the initiator of the pathology in familial SOD1-related cases of ALS (Cheroni et al., 2009; Sau et al., 2007). As the disease progresses another proteasome system is activated, namely the immunoproteasome that consists of inflammatory cytokine-responsive subunits and it replaces the active proteasomal components. Furthermore, this complex has another role, *i.e.* to prepare fragments of the degraded protein to the class 1 major histocompatibility complex (Cheroni et al., 2009; Kabashi et al., 2008; Puttaparthi and Elliott, 2005). This immunoproteasome may be seen as a response to glia-mediated inflammation in the ALS-affected CNS (Papadimitriou et al., 2010).

The ubiquitin-proteasome system is not the only protein control system which is affected by ALS. Earlier studies show that mutated SOD1 interferes directly with ER-associated degradation (Kikuchi et al., 2006; Nishitoh et al., 2008). This system plays a critical part in protein quality control. Its dysfunction or overloading can result in triggering the unfolded protein response (UPR) which can lead to apoptosis via ASK1 activation (Kozutsumi et al., 1988; Schröder and Kaufman, 2005). The activated UPR system has been detected even before symptom onset in mouse models (Saxena et al., 2009), and mutations in its component VAPB have been linked to ALS (Nishimura et al., 2004). Moreover, sporadic ALS patients have up-regulated UPR components (Atkin et al., 2008; Ilieva et al., 2007; Sasaki, 2010), thus hinting that ER stress may be a primary contributor to the pathogenesis of this disease.

3.1.3.7. Neuromuscular junction

Denervation of some neuromuscular synapses (especially fast-fatigable synapses) is a very early event in the mutated SOD1 gene model of ALS with onset prior to the loss of the motoneuron cell body (Fischer et al., 2004). Moreover, indication of dying-back mechanisms of the disease was found in one ALS patient, who died unexpectedly. His motoneurons were not expressing

pathological changes, but there were changes in the innervation of the neuromuscular junction (Fischer et al., 2004). Other studies showed that changes in electrophysiological parameters of the neuron-muscle synapses predicted the onset and survival time of the rodent model of ALS (Casas et al., 2013; Mancuso et al., 2014, 2011). These data are intriguing because they raise the possibility of the disease starting not at the level of the motoneuron soma, but more distally at the level of the peripheral synapse.

3.1.3.8. Environmental and life-style risk factors

The sporadic occurrence of ALS suggested the theory that environmental exposure or life-style factors might influence the development of this disease. To date, there have been several associations between external factors and ALS. For instance, chemical exposure to pesticides (McGuire et al., 1997; Park et al., 2005) or heavy metals (Callaghan et al., 2011; Kamel et al., 2008; Mitsumoto et al., 1988; Vinceti et al., 2000), military service (Coffman et al., 2005; Horner et al., 2003), sport trauma and intensive exercise (Abel, 2007; Armon and Nelson, 2012; Belli and Vanacore, 2005; Chiò et al., 2005; Mattsson et al., 2012), diet (Morozova et al., 2008; Okamoto et al., 2007b) and smoking (Schmidt et al., 2010; Sutedja et al., 2007; Wang et al., 2011) have been proposed as causative factors. Although significant correlations between those factors and the increases in ALS occurrence were reported, their statistical impact was not very strong, thus indicating that coexistence of several factors must occur for the ALS to develop.

3.1.3.9. Excitotoxicity

Excitotoxicity has been shown to play an important role in many neuronal disorders including neurodegenerative diseases like ALS, stroke, neurotrauma, and epilepsy (Coyle and Puttfarcken, 1993; Doble, 1999; Lipton and Rosenberg, 1994). It is propagated mainly via calcium-permeable membrane channels. Excessive intracellular calcium induces many deleterious effects on cells as it activates catabolic enzymes like proteases, phospholipases, and endonucleases. Moreover, disruption of the mitochondrial calcium-buffering system can lead to production of ROS, electron chain dysfunction and eventually apoptosis (Clapham, 2007; Emerit et al., 2004).

Initially, N-methyl-D-aspartate (NMDA) was thought to play a major role in glutamate mediated excitotoxicity, because of the calcium permeability of the channel associated to the

NMDA receptor (Choi, 1988). More recently, certain α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA) receptors were found to be also permeable to calcium. This AMPA receptor lacks the GluR2 subunit, which is responsible for low permeability to calcium (Burnashev et al., 1992a, 1992b; Hollmann et al., 1991). Some neurons, like hypoglossal motoneurons, which do not express this subunit, are particularly vulnerable to excitotoxicity mediated by glutamate (Essin et al., 2002; Laslo et al., 2001). Nonetheless, complete lack of GluR2 expression is not sufficient to cause motor neuron disease (Jia et al., 1996), but it does accelerate motoneuron damage in SOD1 mutated animals (Van Damme et al., 2005). Moreover, intraperitoneal injections of AMPA antagonists prolong survival of the ALS animal model (Van Damme et al., 2003).

Due to their calcium permeability, NMDA receptors are contributors to excitotoxicity (Hardingham and Bading, 2003). For the activation of these receptors not only glutamate is needed, but also a co-agonist like D-serine or glycine (Mothet et al., 2000; Panatier et al., 2006), with the former having 3 times lower affinity for NMDA binding (Furukawa and Gouaux, 2003; Matsui et al., 1995; Mothet et al., 2000; Panatier et al., 2006). Binding of D-serine contributes to NMDA excitotoxicity, while glycine does not (Shleper et al., 2005). In normal conditions, the brainstem and spinal cord have relatively low levels of D-serine (Schell et al., 1995). During excitotoxicity, activation of AMPA receptors increases the activity of serine racemase (SRR), an enzyme which produces D-serine (Kim et al., 2005). Activation of microglia and inflammatory stimuli can up regulate the expression of SRR in microglia, which further increases the release of D-serine (Sasabe et al., 2007; Wu and Barger, 2004). Indeed, in the spinal cord of ALS patients and SOD1 mouse models, raised levels of D-serine have been detected (Sasabe et al., 2007). Removal of this compound from spinal cord cultures of the SOD1 mouse ALS model attenuates NMDA-mediated death of motoneurons, supporting the view that D-serine can amplify excitotoxicity mediated by NMDA receptors (Sasabe et al., 2007). Further confirmation of involvement of those receptors in ALS is indicated by the fact that a NMDA blocker prolongs the life of SOD1 animals (Joo et al., 2007; Wang and Zhang, 2005).

When considering excitotoxic stress in ALS, one cannot forget the influence of inhibitory neurotransmission since, in ALS patients, abnormal levels of glycine and GABA have been found in the serum (Niebroj-Dobosz et al., 1999), and a decreased level of glycine in the spinal cord of autopsied ALS patients (Malessa et al., 1991). GABA-A receptor mRNA is decreased in spinal cords and motor cortex of ALS patients, together with low binding of GABA (Lloyd et al., 2000; Petri et al., 2006, 2003; Whitehouse et al., 1983). Also decreased levels of glycine receptors in lower motoneurons of ALS patients have been detected (Hayashi et al., 1981; Whitehouse et al.,

1983). One can surmise that impaired inhibitory circuits can trigger overstimulation of the nervous system and excitotoxicity.

The main excitatory input to motoneurons is mediated by glutamate. Clinical data have shown elevated glutamate levels in the cerebrospinal fluid of ALS patients (Perry et al., 1990; Spreux-Varoquaux et al., 2002). This effect was attributed to decreased levels of the excitatory amino acid transporter 2 (EAAT2) in patients and mutant SOD1 mice (Fray et al., 1998; Howland et al., 2002; Rothstein et al., 1995; Sasaki et al., 2000). Further studies confirmed that deletion of the gene encoding this protein was sufficient to induce progressive neurodegeneration (Rothstein et al., 1996). Analysis of glial excitatory amino acid transporter promoter activity and transcript quality in SOD1 mutant mice revealed a substantial decrease which overlapped with the onset of the disease (Yang et al., 2009). This transporter protein is susceptible to oxidative stress with alterations in the C-terminus region impairing glutamate uptake (Trotti et al., 1998, 1997; Volterra et al., 1994). A truncated version of the protein, which is inactive, can also be due to caspase-3 activation, which takes place during the disease development (Boston-Howes et al., 2006). Moreover, neuronal signalling significantly affects EAAT2 expression in the astrocytes, and synaptic dysfunction can result in diminished expression of this protein (Yang et al., 2009).

PET (positron emission tomography) scans of ALS patients show abnormally widespread activation, while transcranial magnetic stimulation of the motor cortex shows hyperexcitability (Bae et al., 2013).

Currently there are several therapies under investigation for ALS. Nonetheless, the first agent marketed and still widely used is riluzole, which stimulates glutamate uptake (Lacomblez et al., 1996; Meininger et al., 2000). It is noteworthy that this drug extends the lifespan for patients for a few months only (Van Den Bosch et al., 2006).

3.2 Hypoglossal motoneurons

3.2.1 Anatomy and presynaptic inputs

The hypoglossal nucleus is located near the medullary midline bilaterally. Its caudal end is oriented ventro-laterally with the rostral end localized ventrally to the IVth ventricle. Axons of

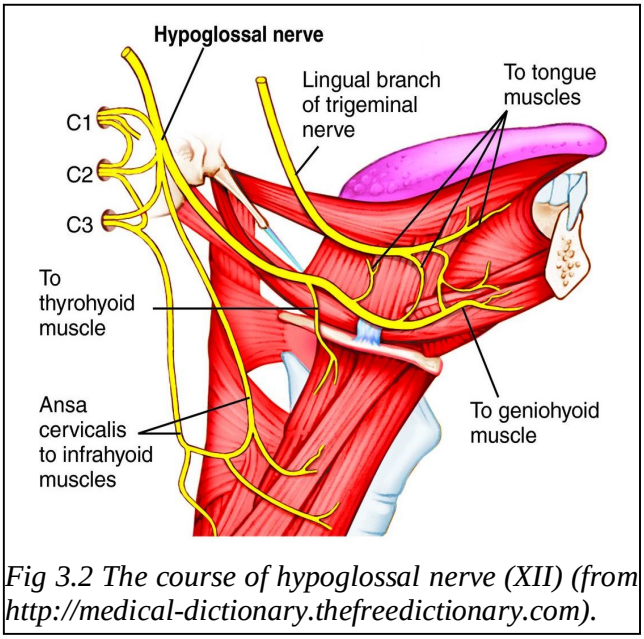

Fig 3.2 The course of hypoglossal nerve (XII) (from http://medical-dictionary.thefreedictionary.com).

hypoglossal motoneurons exit the medulla via the XIIth cranial nerve (hypoglossal nerve) and innervate intrinsic and extrinsic muscles of the tongue (Berger et al., 1996). Intrinsic muscles include: longitudinal, transverse, vertical muscles. They are located in the body of the tongue and possess no bone attachments. They alter the shape of the tongue. Extrinsic muscles consist of genioglossus (tongue protruder), hyoglossus (tongue retractor and depressing tongue), and styloglossus (tongue retractor and elevating tongue): they all have bony attachment (Berger et al., 1996; Lowe, 1980; Sokoloff and Deacon, 1992).

The organization of the hypoglossal nucleus is somatotopical, *i.e.* different subregions of the nucleus are responsible for innervating distinct tongue muscles (Altschuler et al., 1994; Berger et al., 1996; Krammer et al., 1979; Sokoloff and Deacon, 1992). The adult-like somatotopic organization of this brainstem region is achieved very early in the postnatal period within the first few days (Sokoloff, 1993). The vast majority of neurons in the hypoglossal nucleus (over 90%) are motoneurons. They are large, multipolar cells with a soma of around 25-50 μm, whose dendrites spread extensively within the hypoglossal nucleus and into the reticular formation (Boone and Aldes, 1984; Withington-Wray et al., 1988). During the first two postnatal weeks, motoneurons undergo morphological changes as the dendritic tree of cells innervating genioglossal muscles is pruned. The remaining dendrites become more elongated, so there is no net change in total dendritic surface. Later, dendritic branches and diameters increase so that the dendritic surface area doubles (Mazza et al., 1992; Núñez-Abades et al., 1994; Núñez-Abades and Cameron, 1995). Surprisingly the soma of these neurons remains unchanged (Núñez-Abades and Cameron, 1995). Among motoneurons, there are intermingled small interneurons, which represent around 5% of neuronal population of this nucleus, and are mainly restricted to its dorsolateral, lateral and ventral margins (Popratiloff et al., 2001; Sawczuk and Mosier, 2001; Takasu and Hashimoto, 1988).

In the initial postnatal days, motoneurons are electrically and dye coupled as detected with Lucifer yellow or neurobiotin. This connectivity is later lost and is thought to allow their firing synchronization (Mazza et al., 1992).

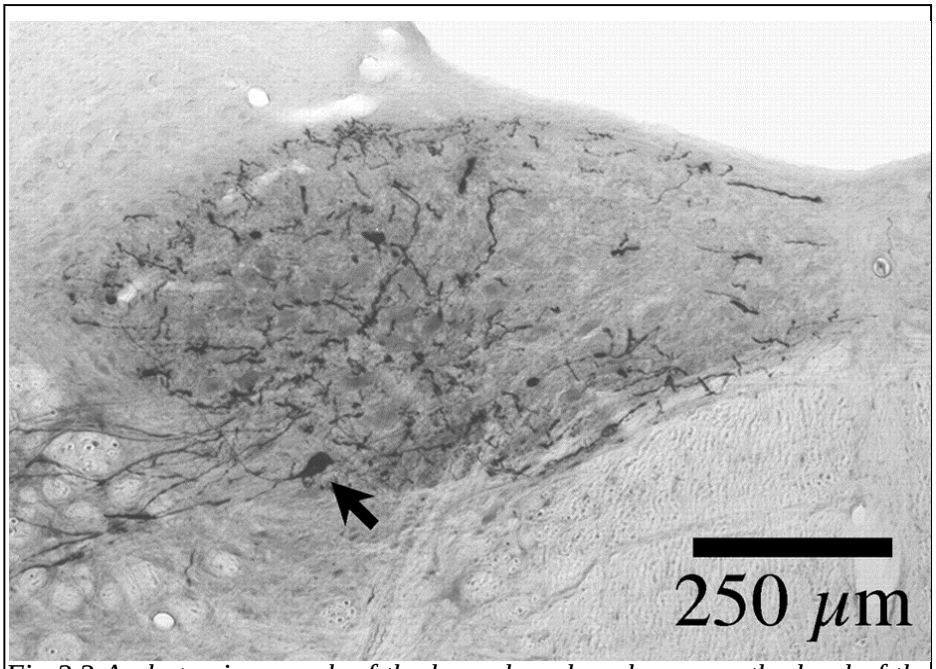

Fig 3.3 A photomicrograph of the hypoglossal nucleus near the level of the obex, illustrating motoneuron cell bodies and processes infected by injection of pseudorabies virus (PRV)-152 into the genioglossal muscle. Neurons were labelled by using immunoperoxidase procedures. An arrow indicates a motoneuron cell body that could be readily distinguished (modified from Shintani et al., 2003).

The greatest distribution of hypoglossal premotor neurons is located in the medullary and pontine reticular formation (Chamberlin et al., 2007; Dobbins and Feldman, 1995; Peever et al., 2002; Rekling et al., 2000), whose projections are largely bilateral (Cunningham and Sawchenko, 2000). Other sources of input are the spinal trigeminal nucleus (Borke et al., 1983), the nucleus of the solitary tract (Borke et al., 1983), the pre-Bötzinger complex (Smith et al., 2007), interneurons within the hypoglossal nucleus (Peever et al., 2002) and the nucleus of Roller which is a source of inhibitory interneurons (both glycinergic and GABAergic) and lies just ventrolateral to XII nucleus (Aldes et al., 1988; Umemiya and Berger, 1995; van Brederode et al., 2011).

It has been shown that electrical stimulation of some components of trigeminal nerve (alveolar and masseter nerve) results in complex post-synaptic potentials in HMs, both inhibitory and excitatory (Sumino and Nakamura, 1974). Stimulating the lingual nerve (part of the trigeminal nerve) also evokes depolarizing and hyperpolarizing synaptic potentials in HMs (Porter, 1967). Thus, at least some premotoneurons process sensory information from the periphery and then project it to HMs. Medullary interneurons, which are excited by sensory afferent fibers, generate monosynaptic EPSCs (glutamatergic) and IPSCs (glycinergic and GABAergic) on HMs.

GABAergic neurons within the XII motor nucleus are few (van Brederode et al., 2011). This observation is compatible with the data indicating that HMs do not possess axon collaterals, implying lack of local feedback inhibition (Mosfeldt Laursen and Rekling, 1989; Withington-Wray et al., 1988).

3.2.2 Excitatory synaptic transmission in hypoglossal motoneurons

The primary excitatory drive to hypoglossal motoneurons is mediated by glutamate neurotransmission (Berger, 2000). Ionotrophic glutamate receptors, which mediate fast excitatory synaptic transmission, are heterotetramers and are divided into three classes: AMPA receptors, kainate receptors (often referred collectively as non-NMDA receptors) and NMDA receptors (Traynelis et al., 2010).

Non-NMDA receptors have fast activation and deactivation rates, followed by strong and rapid desensitization. On the contrary, NMDA receptors have slower activation and deactivation rates followed by slow and modest desensitization (Traynelis et al., 2010). At the glutamatergic synapses in HMs, all three types of glutamate receptors are present, as demonstrated electrophysiologically (Berger et al., 1998; Funk et al., 1993; Greer et al., 1991; O'Brien et al., 1997; Rekling, 1992) and immunocytochemically (García Del Caño et al., 1999; Williams et al., 1996). Synaptic transmission in the neonatal rat is mainly mediated by non-NMDA receptors (Funk et al., 1993; Greer et al., 1991). This condition changes in adulthood, when all three classes of receptors are involved in the respiratory drive to HMs (Chitravanshi and Sapru, 1996; Steenland et al., 2008, 2006). During the postnatal period, NMDA receptors have another role because their block inhibits motoneuron dendritic branching and somatic growth (Kalb, 1994). Activation of NMDA receptors during this postnatal period can lead to bursting behaviour in the majority of hypoglossal motoneurons, which resembles the pattern involved in suckling (Sharifullina et al., 2008). While NMDA receptors need glycine for their activation, the glycine binding site is not saturated during the first two postnatal weeks (Lim et al., 2004).

Another receptor group activated by glutamate comprises metabotropic glutamate receptors (mGluRs). It is well known that in forebrain networks, mGluR activation can lead to oscillatory activity (Beierlein et al., 2000; Cobb et al., 2000; Hughes et al., 2002; Whittington et al., 1995), including network synchronization of inhibitory transmission (Beierlein et al., 2000; Whittington et al., 1995). In hypoglossal motoneurons, mGluRs are known to modulate excitability of those cells

and facilitate glycinergic inhibitory neurotransmission (Donato et al., 2003; Donato and Nistri, 2000). In addition, it has been shown that stimulation of group I metabotropic glutamate receptors with their selective agonist (dihydroxyphenylglycine – DHPG) generates persistent, regular oscillations, with large outward slow currents alternated with fast, repeated inward currents. These events require intact transmission via AMPA receptors and electrical coupling via gap junctions of neighbouring neurons (Sharifullina et al., 2005). It is likely that the role of mGluR Iis to facilitate rhythmic firing, for example, during suckling behaviour (Berger et al., 1996; Sharifullina et al., 2005).

3.2.3 Inhibitory synaptic transmission in hypoglossal motoneurons

The main inhibitory transmission on hypoglossal motoneurons is mediated by glycinergic and GABAergic synapses originating from reticular formation neurons (Li et al., 1997), respiratory centers like the pre Bötzinger complex (Paton and Richter, 1995) and hypoglossal interneurons (Peever et al., 2002). Both neurotransmitters might be co-released from a low number of fibers (O'Brien and Berger, 1999) and are transported by the same vesicular transporter (Berger, 2000). Although receptors for both transmitters are found on HMs (Muller et al., 2004), these receptors have different kinetics, and generate responses of distinct frequency and amplitude. Glycinergic events have higher amplitude and frequency in comparison to GABAergic ones (Donato and Nistri, 2000; O'Brien and Berger, 1999). Since glycinergic transmission is very fast, co-transmission mediated by GABA can enable those inhibitory signals to occur for much longer duration. The GABA receptor, which belongs mainly to the $GABA_A$ class, is blocked reversibly by bicuculline whereas glycine receptors are blocked by strychnine (Barnard et al., 1993; Donato and Nistri, 2000; Kuhse et al., 1995). GABA and glycine receptors are both Cl^- channels and, in the neonatal animal, events elicited by such receptors are always depolarizing, and later become hyperpolarizing due to maturation of the chloride ion transport system (Singer and Berger, 2000). Nonetheless, their action is always inhibitory due to increased membrane conductance, thus making cells less responsive to excitatory signals, an effect which is defined as shunting inhibition (Marchetti et al., 2002). Shunting conductances can have profound effects not only on the excitability of neurons, but also on neuronal gain control (Bonin et al., 2007; Bright et al., 2007; Stell et al., 2003) and network excitability (Semyanov et al., 2003).

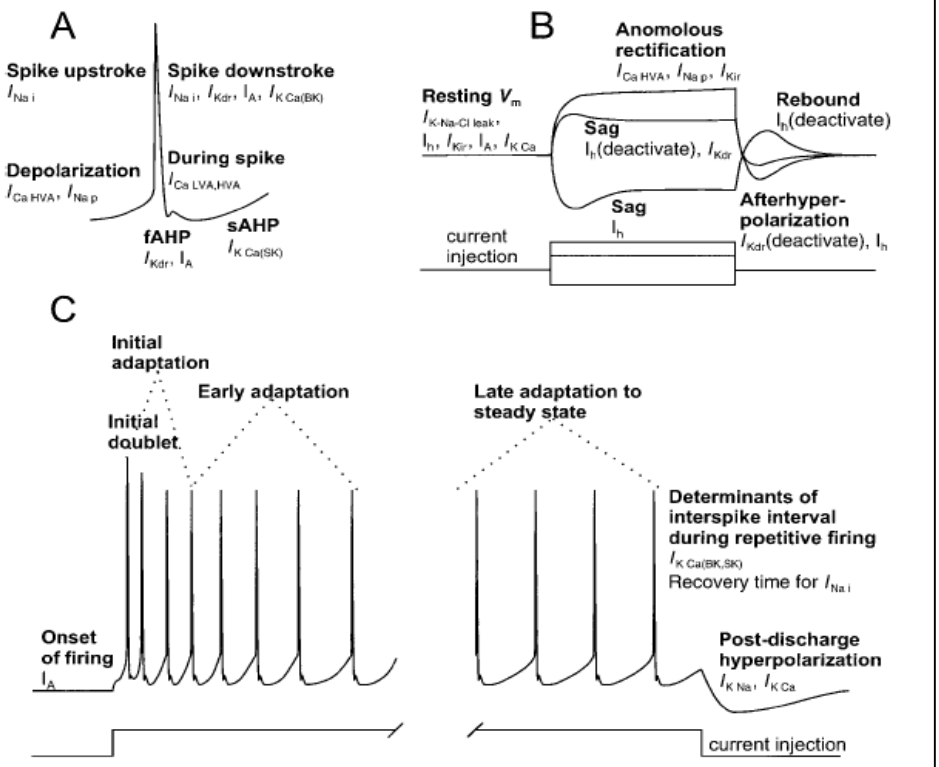

Fig 3.4 Supra- and subthreshold membrane behaviour of motoneurons

Ionic currents underlying the action potential waveform (A).Tonic currents underlying subthreshold membrane behaviour, in this case, elicited by a short-lasting depolarizing/hyperpolarizing square current pulse (B). Different phases of adaptation during repetitive firing and postdischarge hyperpolarization after a long-lasting current pulse (C) (adapted from Rekling et al., 2000).

3.2.4 Intrinsic properties of hypoglossal motoneurons

Hypoglossal motoneurons display a wide range of currents which contribute to the intrinsic properties of these cells (see Table 3.1 and Fig 3.4). Their role is to integrate the synaptic inputs and generate firing behaviour. Beside voltage-activated fast sodium and potassium ion flow, responsible for generation of action potentials, other conductances include calcium conductances elicited by both low-threshold activated (LVA; T-type) and high-threshold activated (HVA; N-, P/Q- and L-types) (Rekling et al., 2000; Umemiya and Berger, 1994) calcium channels, K^+ conductances responsible for afterhyperpolarization (AHP) which follow the action potential, hyperpolarization-activated current comprising mixed cationic current of Na^+ and K^+ (I_h) (Bayliss et al., 1994), and persistent inward conductances mediated by Na^+ and /or Ca^{2+} channels (I_{NaP} and I_{CaP}) (Del Negro et

al., 2005; Lamanauskas and Nistri, 2008; Powers and Binder, 2003).

Neonatal and mature hypoglossal motoneurons have different firing patterns elicited by membrane depolarization. In neonatal animals, after stimulation of motoneurons by a supra-threshold current, most cells steadily fire a set of discharges with a linear-current frequency relation, with an initial brief period of frequency adaptation (Haddad et al., 1990; Mosfeldt Laursen and Rekling, 1989; Núñez-Abades and Cameron, 1995; Viana et al., 1995, 1993a, 1993b). Only a smaller group shows progressive acceleration of firing to a steady state level (Viana et al., 1995). In the case of adult motoneurons, these fire at a higher rate with three different phases of adaptation (Sawczuk et al., 1995; Viana et al., 1995). Neonatal HM adaptation is controlled by Ca^{2+}-dependent afterhyperpolarization of medium duration (mAHP) (Lape and Nistri, 2000; Viana et al., 1995).

Table 3.1 Main membrane currents in hypoglossal motoneurons and their proposed function (from Cifra, 2011)

Current	Abbreviation	Description and function	References
Fast Na^+ current	I_{Na}	TTX-sensitive, fast-activating and inactivating, responsible for the depolarizing phase of AP	Mosfeldt et al., 1989; Haddad et al., 1990; Lape and Nistri, 2001
Delayed rectifier K^+ current	I_{KDR}, I_{slow}	TEA-sensitive, AP repolarization, fAHP	Mosfeldt et al., 1989; Haddad et al., 1990; Viana et at. 1993a; Lape and Nistri, 1999
Fast K^+ current	A-type, I_{fast}	4-AP-sensitive, initial spike frequency adaptation, fAHP	Mosfeldt et al., 1989; Viana et at. 1993a; Lape and Nistri, 1999
Ca^{2+}- activated K^+ current	$I_{K\ Ca(BK)}$ $I_{K\ Ca(SK)}$	mAHP ($I_{K\ Ca(BK)}$), repetitive firing properties,	Mosfeldt et al., 1989; Viana et at. 1993a;

(two types of channels: BK= large conductance; SK= small conductance		AP repolarization ($I_{K\ Ca(SK)}$)	Umemiya and Berger, 1994; Lape and Nistri, 2000; Rekling et al., 2000
Low- threshold Ca^{2+} current	I_{CaLVA}	Rebound depolarization bursting behaviour, AP repolarization (via activation of $I_{K,\ Ca(BK)}$), ADP	Viana et al., 1993b, Umemiya and Berger, 1994
High- threshold Ca^{2+} current	I_{CaHVA}	ADP, mAHP (via activation of $I_{K\ Ca(SK)}$)	Viana et al., 1993b; Umemiya and Berger, 1994
Na^+ / K^+ hyperpolarization-activated current	I_h	Rebound potentials, stabilize V_m around rest, reduces mAHPs as well as response for inhibitory synaptic inputs	Bayliss et al., 1994; Viana et al., 1994
Persistent inward currents (Na^+ and Ca^{2+})	I_{NaP} I_{CaP}	Integration of synaptic inputs, rhythmic discharges	Powers and Binder, 2003; Zeng et al., 2005; Moritz et al., 2007

TTX: tetrodotoxin; AP: action potential; TEA: tetraethylammonium; fAHP: fast afterhyperpolarization; 4-AP: 4-aminopyridine; mAHP: medium AHP; ADP: afterdepolarization: V_M: membrane potential

3.2.5 Function of hypoglossal motoneurons

Hypoglossal motoneurons are involved in several activities like breathing, mastication, swallowing, vomiting, licking, vocalization and coughing (Gestreau et al., 2005). These activities involve tongue rhythmic contractions and proper coordination, and are crucial for survival shortly after birth (Berger, 2000; Feldman and Del Negro, 2006; Horner, 2008).

There are numerous studies investigating the discharge patterns of hypoglossal motoneurons during breathing (Hwang et al., 1983; Peever et al., 2002; Withington-Wray et al., 1988). During inspiration, genioglossus muscle activity is enhanced in order to increase airway patency, the retrusor muscles help stiffening of the pharyngeal wall, and the activity of the intrinsic tongue muscles contributes to lingual stiffness (Bailey and Fregosi, 2004). During sleep, especially the rapid-eye-movement phase, the activity of respiratory muscles changes. The airway narrows and the resistance to breathing increases (Horner, 2008). This change can lead to pathological conditions like obstructive sleep apnea in individuals with constitutively narrow upper airways (Remmers et al., 1978). Experiments on naturally sleeping animals demonstrated that some tonic excitatory drive to HMs is withdrawn during sleep and the additional dominance of inhibitory GABAergic inputs results in suppression of respiratory muscle activity (Horner, 2009).

During swallowing of food or fluids, inspiration is inhibited in order to prevent aspiration of food and the tongue retrusor muscles propel food towards the pharyngeal cavity. Additionally, the geniohyoid and thyrohyoid muscles close the laryngeal vestibule and elevate the entire larynx facilitating the upper esophageal sphincter opening (Gestreau et al., 2005). During those activities, hypoglossal motoneurons discharge complex patterns of firing in order to coordinate the tongue muscles (Amri et al., 1991; Tomomune and Takata, 1988). Involvement of hypoglossal motoneurons in other activities, including coughing, have been also investigated, but to a lesser extent (Dick et al., 1993; Dinardo and Travers, 1994; Hayashi and McCrimmon, 1996; Ono et al., 1998; Roda et al., 2002; Satoh et al., 1998; Umezaki et al., 1998).

Recording from hypoglossal motoneurons shows that 20% of HMs change their membrane potentials only during one behaviour (swallowing), 30% receive synaptic drive during both breathing and swallowing, 35% exhibit membrane potential changes in relation to the three tested behaviours (coughing, breathing and swallowing), and 15% show no change in their membrane potential (Gestreau et al., 2005; Roda et al., 2002). All motoneurons active during coughing display respiratory-related activity, with similar frequencies and amplitudes of synaptic potentials indicating that they formed a common subset with equal synaptic drives (Roda et al., 2002). Other investigations revealed a distinct subset of HMs recruited only during swallowing (Gestreau et al., 2000). The results from those studies indicate that hypoglossal motoneurons represent distinct functional pools activated during different behaviours (Baekey et al., 2001; Gestreau et al., 2000; Ono et al., 1998; Roda et al., 2002; Tomomune and Takata, 1988; Umezaki et al., 1998). Hypoglossal motoneurons do not possess intrinsic rhythmic activity as they discharge only when driven by central pattern generators (CPGs) like those involved in breathing (Rekling and Feldman,

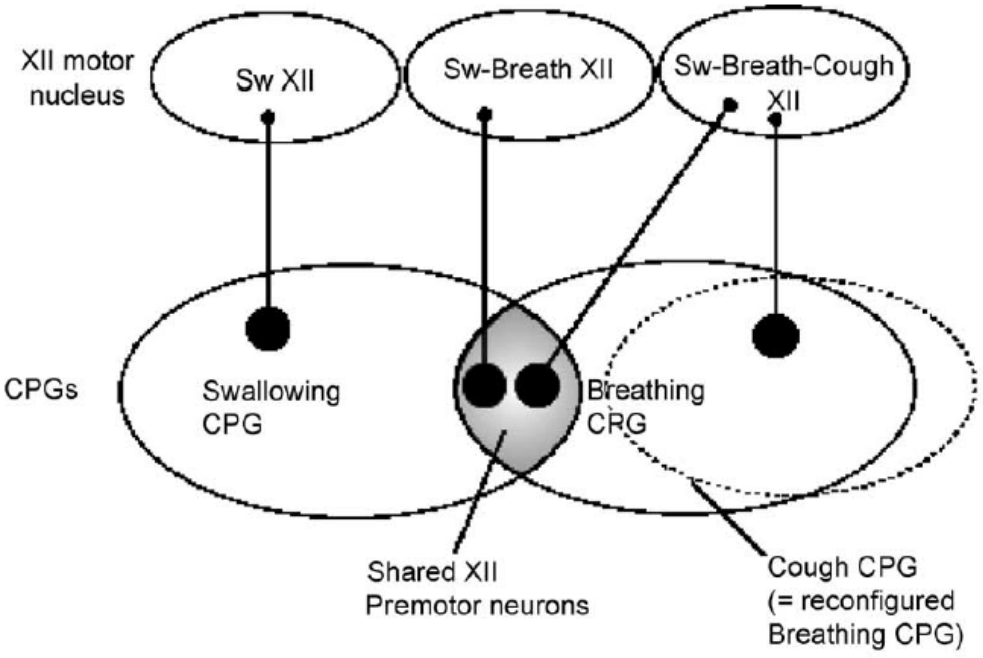

Fig. 3.5 Output patterns of lingual muscles are achieved by activation of various subsets of XII motoneurons.

Brainstem neural networks (or central pattern generators, CPGs) producing breathing, swallowing and coughing motor activities form neural ensembles including XII premotor neurons (black circles). The latter send convergent and divergent projections onto subsets of motoneurons. Several results suggest the existence of multifunctional (or shared) premotor neurons (see overlapping CPGs). The cough motor pattern is likely generated by reconfiguration of the breathing CPG (adapted from Gestreau et al., 2005).

1998; Smith et al., 1991; Suzue, 1984), swallowing (Jean, 2001) and mastication (Nakamura and Katakura, 1995). A single CPG can have divergent inputs on distinct pools of HMs and a subset of HMs can receive convergent excitatory and inhibitory synaptic drives arising from divergent CPGs (see Fig 3.5) (Gestreau et al., 2005; Roda et al., 2002). Moreover, several results suggest the existence of multifunctional XII premotoneurons, which share several CPGs inputs (Ono et al., 1998; Roda et al., 2002). A very useful feature of the medullary slice is the preservation of CPGs. Many studies used such a preparation and recorded from the XII nerve in order to study outputs of the respiratory centers and functional changes in respiratory rhythm generation and modulation (Funk et al., 1993; Ramirez et al., 1997; Shao and Feldman, 2005; Smith et al., 1991; Telgkamp and Ramirez, 1999).

3.2.6 Hypoglossal motoneurons in pathological conditions

As mentioned above, hypoglossal motoneurons mediate several functions important for

survival. Thus, dysfunction of these cells contributes to certain pathological conditions and ALS is certainly one of them. Little is known about the early pathogenesis of HMs during ALS onset, although they are among the most strongly affected neurons, especially in bulbar form of the disease (Krieger et al., 1994; Laslo et al., 2001; Lips and Keller, 1998). Even in the case of limb-onset ALS, most patients eventually develop bulbar symptoms (Haverkamp et al., 1995; Yamauchi et al., 2014). Respiratory failure is the most common cause of death in ALS and deterioration of hypoglossal motoneurons creates life-threatening events like choking more probable (Haverkamp et al., 1995; Yamauchi et al., 2014). Tongue muscle motoneurons play a major role in sleep apnea, a breathing disorder associated among others with sleepiness, stroke, cardiovascular disorders, depression, attention deficit disorder, and learning impairments (Saboisky et al., 2009). Under normal conditions in the awake state, the respiratory output is controlled, among others, by hypoglossal motoneurons, whose activity prevents airway collapse. During sleep, the central respiratory drive to the muscles weakens and may result in occlusion, especially in the individuals with narrowed airways (Ramirez et al., 2013).

3.2.7 Hypoglossal motoneurons and excitotoxicity studies

Our laboratory has previously reported a simple *in vitro* model of excitotoxicity (Sharifullina and Nistri, 2006). By applying a glutamate uptake inhibitor DL-threo-β-benzyloxyaspartate (DL-TBOA) via the perfusion system to the brainstem medullary slice of the neonatal rat, a gradual rise in extracellular glutamate concentration is elicited. DL-TBOA is suitable for this kind of experimental investigation, because it does not affect glutamate receptors and is not transported by glutamate carriers (Anderson et al., 2001). Hence, following TBOA application, about half of the HM population develops strong bursting. These events are synchronous due to electrical coupling among neighbouring motoneurons, and are accompanied by strong enhancement in excitatory synaptic transmission (Cifra et al., 2011a, 2011b; Sharifullina and Nistri, 2006). Bursts have a network origin, since tetrodotoxin (TTX) application or the cell-permeable Ca^{2+} chelator (BAPTA-AM) suppress them. Development of bursts requires interplay of several glutamate receptors including AMPA, NMDA and metabotropic glutamate receptor 1 (mGluR1), while the role of GABA and glycine is to inhibit bursting occurrence. Previous data have indicated that NMDA and mGluR are not accessible for glutamate during normal, fast synaptic transmission, but they can be activated by ambient glutamate (Brasnjo and Otis, 2001; Campbell and Hablitz, 2004; Huang and Bordey, 2004; Huang et al., 2004). NMDA most likely supports bursting by spreading membrane

depolarization, while the mGluR activation increases membrane resistance, thus making neurons electrotonically more compact and sensitive to excitatory inputs (Sharifullina and Nistri, 2006). In addition, gap junctions are also important for bursting occurrence, most likely by recruiting cells and synchronizing their discharges (Sharifullina and Nistri, 2006). Co-activation of all three types of glutamate receptor combined with electrical coupling triggers bursting. Riluzole, a drug which is used for ALS therapy, suppresses bursting evoked by TBOA (Cifra et al., 2011a, 2009). Processes which most likely contributed to termination of the TBOA-evoked bursts are the cyclic operation of the Na^+-K^+ pump and synaptic fatigue, a mechanism which may control bursting in the rat spinal motoneurons (Rozzo et al., 2002).

In almost half of the motoneuron population, TBOA induces an irreversible rise in calcium levels, apparently leading to motoneuron death (Sharifullina et al., 2005). After application of TBOA, Ca^{2+} waves are observed in HMs, possibly because of a persistent activation of mGluRs which trigger calcium release from intracellular stores (Schoepp et al., 1999), depolarization-dependent Ca^{2+} influx, and opening of Ca^{2+}-permeable AMPA and NMDA receptors (Sharifullina et al., 2005). With ongoing TBOA application, Ca^{2+} imaging reveals a rise in $[Ca^{2+}]_i$ baseline in some of the cells indicating patchy excitotoxic effects (Sharifullina et al., 2005). Motoneurons with large bursts and Ca^{2+} deregulation are probably more likely to be affected and prone to die during the insult (Sharifullina and Nistri, 2006). TBOA also significantly increases the number of propidium iodide (a dye which penetrates cells via disrupted cell membranes to bind to DNA) positive cells in medullary slices, an effect blocked by drugs which suppressed bursting (carbenoxolone - a gap junction blocker, glutamate receptor antagonists, or riluzole) (Cifra et al., 2009; Sharifullina and Nistri, 2006). Also immunohistochemical studies with ATF-3 (a well-known marker for motoneuron distress), or analysis of occurrence of pyknotic nuclei (condensation of chromatin associated with cell death) confirm that riluzole diminishes the pathological action of TBOA (Cifra et al., 2011a).

The TBOA model offers a number of advantages for the investigation of pathological mechanisms correlated with excitotoxicity and presymptomatic stage of ALS. First of all, pharmacological inhibition of glutamate uptake resembles the gradual build-up of the glutamate levels observed in the cerebrospinal fluid of ALS patients, a phenomenon which is most likely due to down-regulation of glutamate uptake in ALS (Rothstein et al., 1995, 1992). Moreover, the patchy pathology of hypoglossal motoneurons is analogous to the human pathology (Swash and Ingram, 1988). Furthermore, medullary slices allow for tight control over experimental conditions and fast changes in parameters such as ion composition or drug application. Thus, despite the large

simplification of neuronal networks in our preparation, it represents a useful tool for studying pathological processes and potential new approaches to treat ALS.

3.3. Endocannabinoids in central nervous system

3.3.1. Endocannabinoids

Cannabis sativa is a plant known and used for thousands of years for medical and mood-altering purposes. Its psychoactive component is delta-9-tetrahydrocannabinol or Δ^9-THC which, by binding to cannabinoid type 1 receptors (CB1Rs), is responsible for its psychoactive actions (Gaoni and Mechoulam, 1964). The first endogenous compound which activated cannabinoid receptors (endocannabinoid) was anandamide (AEA) or *N*-arachidonylethanolamide (Devane et al., 1992). Later, a second endocannabinoid, namely 2-arachidonylglycerol (2-AG) was identified (Mechoulam et al., 1995; Sugiura et al., 1995). Endocannabinoids induce a variety of actions on the nervous system, such as reduced motor activities, immobility, hypothermia, analgesia, impairment of memory, stimulation of appetite, inhibition of adenylyl cyclase, inhibition of voltage-gated Ca^{2+} channels, activation of an inwardly rectifying K^+ current, reduction of gap junction permeability, inhibition of neurotransmitter release, and the inhibition of long-term potentiation (LTP) in hippocampal slices *in vitro* (Di Marzo, 1998; Felder and Glass, 1998; Hillard, 2000; Hillard and Campbell, 1997; Mechoulam et al., 1998; Pertwee, 1997; Piomelli et al., 1998; Schmid et al., 2002). AEA behaves as a partial agonist for both CB1 and CB2 receptors (Sugiura et al., 2002) and is also an endogenous ligand for transient receptor potential vanilloid receptor 1 (TPRV1) receptors although with significantly lower affinity (Di Marzo et al., 2001; Ross, 2003). 2-AG is much more abundant in the brain than AEA and reaches nM concentrations per gram of tissue (Sugiura et al., 2006). Currently there are also other compounds considered to be endocannabinoids. These include: dihomo-linolenoyl ethanolamide (Hanus et al., 1993), docosatetraenoyl ethanolamide (Hanus et al., 1993), 2-arachidonyl glycerol ether (noladin ether) (Hanus et al., 2001), *O*-arachidonoylethanolamine (virodhamine) (Porter et al., 2002), and *N*-arachidonoyldopamine (Huang et al., 2002).

3.3.2. Synthesis of endocannabinoids

Both AEA and 2-AG are synthesised on demand in an activity-dependent manner and are released into the extracellular space (Di Marzo et al., 1994; Piomelli, 2003).

During studies on the synthesis of AEA it was discovered that a rise in intracellular calcium is necessary for its production, so it can be induced by the Ca^{2+} ionophore ionomycin, depolarization with high potassium solution, electrical stimulation and/or carbachol (muscarinic receptor agonist) or glutamate application (Di Marzo et al., 1994; Di et al., 2005; Stella and Piomelli, 2001). Currently it is known that synthesis of endocannabinoids from postsynaptic neurons can be triggered by strong depolarization with associated elevation in intracellular Ca^{2+} concentration (Ca^{2+}-driven endocannabinoid release) (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001), strong activation of postsynaptic Gq/11 protein-coupled receptors at basal Ca^{2+} level (basal receptor-driven endocannabinoid release) (Maejima et al., 2001; Varma et al., 2001), or simultaneous Ca^{2+} elevation and Gq/11 protein-coupled receptor activation (Ca^{2+}-assisted receptor-driven endocannabinoid release) (Kim et al., 2002; Ohno-Shosaku et al., 2002; Varma et al., 2001).

Synthesis of AEA is composed of two reactions. Increase in intracellular calcium activates *N*-acyltransferase (NAT), which transfers an arachidonate group from phospholipids like *N*-arachidonoyl-phosphatidylethanolamine (NAPE) to the primary amino group of phosphatidylethanolamine (PE), creating *N*-arachidonoyl PE. During the second step, the enzyme *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) hydrolyses *N*-arachidonoyl PE to anandamide and phosphatidic acid (Cadas et al., 1997; Fonseca et al., 2013). NAT activation is thought to be rate-limiting for AEA production. NAPE-PLD-knockout mice (Leung et al., 2006) do not show any obvious behavioural abnormalities, suggesting that there are other NAPE-PLD-independent pathways for AEA synthesis (Okamoto et al., 2007a).

Currently there are several pathways for 2-AG synthesis known. The main one consists of initial hydrolysis of the arachidonic acid-containing membrane phospholipid (such as phosphatidylinositol) by phospholipase C (PLC) enzyme and creation of the arachidonic acid-containing diacylglycerol. Further on, diacylglycerol lipase (DAGL) hydrolyses this compound to 2-AG (Bisogno et al., 2003; Jung et al., 2007, 2005b; Kondo et al., 1998; N. Stella et al., 1997). Other pathways for 2-AG synthesis involve sequential reactions by the phospholipase A1 (PLA1) and the lysoPI-specific PLC (Sugiura et al., 1995; Tsutsumi et al., 1994; Ueda et al., 1993), conversion from 2-arachidonoyl lysophosphatidic acid by phosphatase (Nakane et al., 2002), and

formation of 2-AG from 2-arachidonoyl phosphatidic acid through 1-acyl-2-arachidonoylglycerol (Bisogno et al., 1999; Carrier et al., 2004).

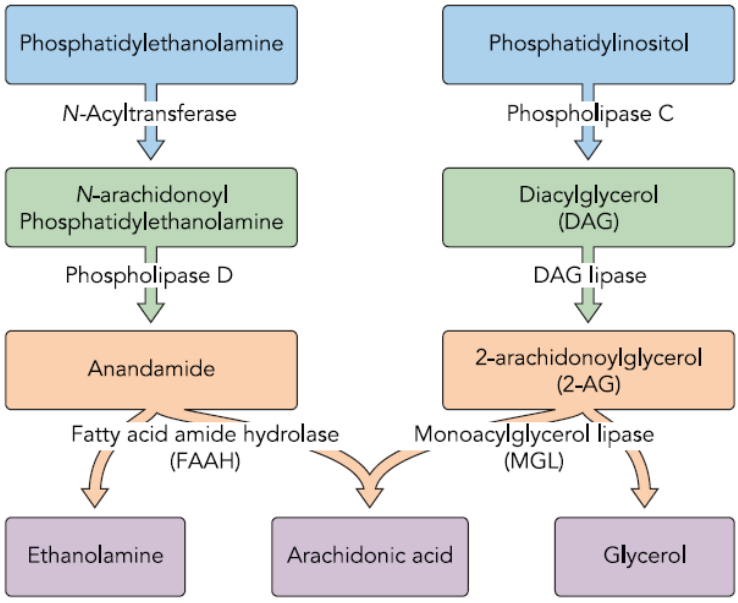

Fig 3.6 ECB synthesis and degradation

Main pathways of synthesis and degradation of the endocannabinoids AEA and 2-AG (adapted from El Manira and Kyriakatos, 2010).

3.3.3. Transport and degradation of endocannabinoids

Endocannabinoid removal from the extracellular space is thought to be a two step process, which consists of the transport into the cells followed by the enzymatic degradation (McFarland and Barker, 2004; Hillard and Jarrahian, 2000; Fowler and Jacobsson, 2002). Uptake of AEA has been observed in many preparations, including neuronal primary cultures, and described as saturable and temperature dependent (Beltramo et al., 1997; Di Marzo et al., 1994; Di Marzo and Matias, 2005). Currently there are several models proposed for AEA uptake. The first one implies the existence of a carrier protein which transports AEA (Fegley et al., 2004; Ligresti et al., 2004). The second model neglects the existence of the carrier and considers that AEA passes through the membrane by simple diffusion, followed by intracellular degradation (Glaser et al., 2003). The third model implies that AEA undergoes endocytosis through a caveolae-related uptake process (McFarland et al., 2004). There is not much information concerning any 2-AG uptake mechanism, although

several studies suggest that it can be transported by the system involved in the AEA uptake (Beltramo and Piomelli, 2000; Bisogno et al., 2001; Piomelli et al., 1999).

Two pathways for endocannabinoid degradation exist, namely hydrolysis and oxidation (Vandevoorde and Lambert, 2007). The AEA degradation enzyme was cloned from rat liver and named fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996). FAAH primary substrate is AEA, but it can recognize a variety of other fatty acid amides. The main enzyme involved in the hydrolysis of 2-AG is termed monoacylglycerol lipase (MGL) (Dinh et al., 2004, 2002; Vandevoorde and Lambert, 2007). In the oxidative degradation of endocannabinoids two enzymes are involved: cyclooxygenase 2 (COX-2) and lipoxygenase (LOX). For the COX-2 enzyme the preferred substrate is arachidonic acid, nonetheless it can act on both 2-AG and AEA, although with low affinity for the former one (Vandevoorde and Lambert, 2007). There are known several kinds of LOX enzymes, which can use both 2-AG and AEA as a substrate (Vandevoorde and Lambert, 2007).

3.3.4. Endocannabinoid receptors

There are two major cannabinoid receptors (CBs): CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993). Both are seven trans-membrane domain receptors coupled to G proteins; while CB2 is distributed primarily within the immune system, CB1 is abundant in the central nervous system (CNS) (Glass et al., 1997; Kano, 2014; Herkenham, 1990; Pertwee, 1997). Studies with CB1R-knock-out mice and CB1R-specific antagonists indicate that this receptor is mainly responsible for the psychoactive responses to exogenous cannabinoids and physiological actions of endocannabinoids (Elphick and Egertová, 2001; Ledent et al., 1999). The CB1R of the rat is composed of 473 amino acids and its human and mouse analogues are sharing 97-99% sequence identity (Chakrabarti et al., 1995; Gérard et al., 1990; Matsuda et al., 1990). *In vivo* CB1Rs exist as homodimers (Mackie, 2005; Wager-Miller et al., 2002) or in form of heterodimers, for example with D2 (dopamine 2) receptors (Kearn et al., 2005).

Anther receptor which can be activated by AEA is the transient receptor potential vanillinoid receptor 1 (TRPV1R) (Starowicz et al., 2007). It is a Ca^{2+} permeable, non-selective cation channel (Caterina et al., 1997). It is best known for its role in the sensory nociceptive neurons of the peripheral nervous system, although it is accepted that it has a broad distribution in the central nervous system, and is involved in several functions like modulation of the neuronal and glial

activities (Martins et al., 2014).

Other cannabinoid receptors are also likely to exist, but their role and characterization is still under investigation (Hájos et al., 2001).

The first insights into the distribution of CB1Rs in the brain came from studies using the synthetic radio-labelled cannabinoid ligand [^{3}H]CP55,940 (Herkenham et al., 1991, 1990; Mailleux and Vanderhaeghen, 1992). Those data have shown that CB1Rs are widely distributed across the brain areas. Moderate levels of labelling were detected in the brainstem and spinal cord areas. CB1Rs can be very often found in the terminals and pre-terminal axons as indicated by studies in the hippocampus dentate gyrus, pyramidal neurons and cerebellum (Egertová et al., 2003; Eggan and Lewis, 2007; Harkany et al., 2005; Katona et al., 2000, 1999; Mailleux et al., 1992; Mailleux and Vanderhaeghen, 1992; Marsicano and Lutz, 1999; Pettit et al., 1998; Tsou et al., 1999, 1998).

3.3.5. Intracellular signalling elicited by endocannabinoids with focus on CB1 receptors

One of the best known effects elicited by CB1Rs is inhibition of neurotransmitter release. Many studies associate CB1R activation with suppression of the release of glutamate (Lévénés et al., 1998), GABA (Szabo et al., 1998), glycine (Jennings et al., 2001), acetylcholine (Gifford and Ashby, 1996), norepinephrine (Ishac et al., 1996), dopamine (Cadogan et al., 1997), serotonin (Nakazi et al., 2000), and cholecystokinin (Beinfeld and Connolly, 2001). Research on neurons and transfected cells also link activation of the CB1Rs with activation of A-type (Hampson et al., 1995) and inwardly rectifying K^+ channels (Mackie et al., 1995), inhibition of N- and P/Q-type Ca^{2+} channels (Twitchell et al., 1997) and D- and M-type K^+ channels (Mu et al., 1999; Schweitzer, 2000), modulation of activity of focal adhesion kinase (Derkinderen et al., 1996), mitogen-activated protein kinase (Sánchez et al., 1998), phosphatidylinositol 3-kinase (Bouaboula et al., 1995), and some enzymes involved in energy metabolism (Guzmán and Sánchez, 1999). At biochemical level, the CB1R signalling pathway significantly inhibits cAMP synthesis, thus being considered the principal effector of CB1R activation.

3.3.5.1. Regulation of intracellular cAMP

The first pathway connected to the cannabinoid receptor system was the one mediated by $G_{i/o}$protein (negatively coupled to adenylyl cyclase) (Howlett et al., 1986; Howlett and Fleming, 1984). Both exo- and endocannabinoids inhibit adenylyl cyclase activity in cells in culture and in brain homogenates. In 1988, Howlett and his colleagues presented data on the potency of various cannabinoids to inhibit cAMP formation and correlated it with antinociceptive effects of those

drugs in vivo (Howlett et al., 1988) indicating that this pathway is responsible for the analgesic effects of these compounds. Both 2-AG and AEA induce CB1-mediated inhibition of adenylyl cyclase (Felder et al., 1993; Mechoulam et al., 1995; Stella et al., 1997; Vogel et al., 1993).

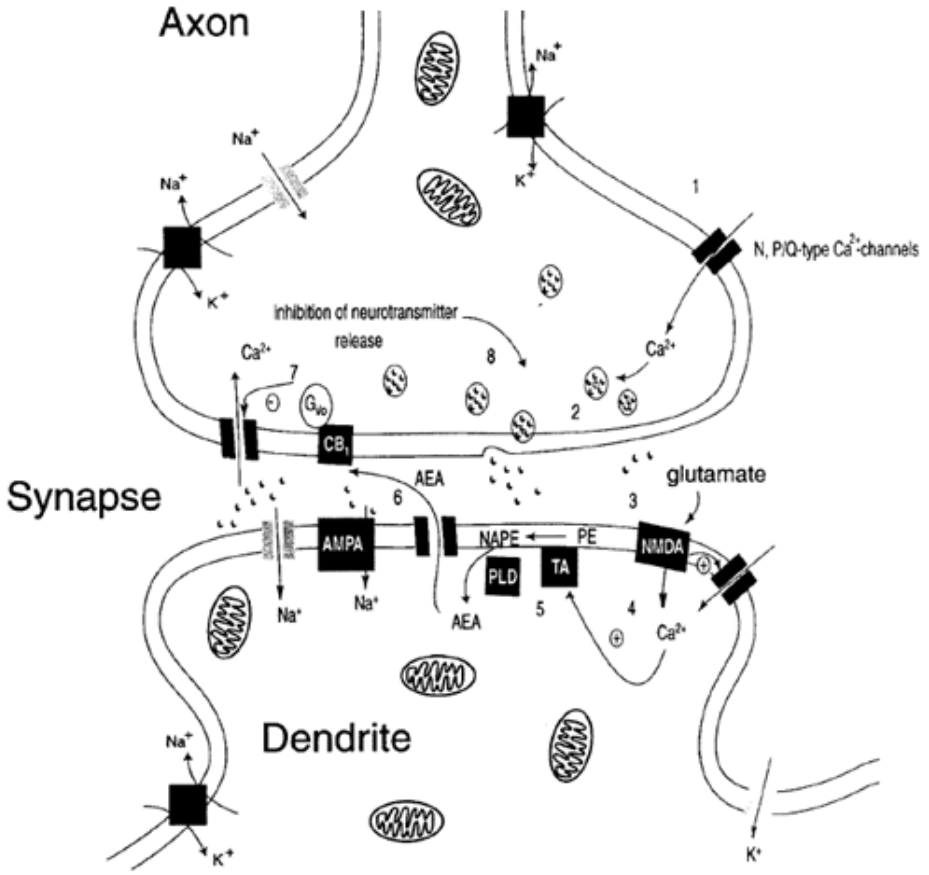

Fig 3.7 Model of retrograde AEA action

1. Presynaptic depolarization leads to calcium influx, which in turn activates glutamate exocytosis. 2. Glutamate diffuses through synaptic cleft and activates postsynaptic glutamate receptors. 3. Activation of NMDA, AMPA and other glutamate-subtype receptors leads to postsynaptic depolarization and calcium influx. 4. Elevated postsynaptic calcium levels activate a transacetylase (TA), which converts phosphatidylethanolamine (PE) into N-arachidonoyl phosphatidylethanolamine (NAPE). 5. NAPE is hydrolysed by a phospholipase D (PLD), which yields AEA. AEA is released from postsynaptic cell and diffuses back to presynaptic CB1 receptors. 6. Upon activation of CB1 receptors by AEA, Gi/o proteins are released, which inhibit N-, and P/Q-type voltage sensitive calcium channels. 7. Closing of voltage-sensitive calcium channels results in reduced release of neurotransmitters, such as glutamate or GABA (adapted from Van der Stelt et al., 2002).

Further studies indicated that, when interaction of the CB1R with $G_{i/o}$ is not possible, it can

interact with G_s proteins, known to have stimulatory effect on adenylate cyclase (Felder et al., 1998; Glass and Felder, 1997; Maneuf and Brotchie, 1997).

Studies from several laboratories have demonstrated that different cannabinoid ligands can promote interactions with different G proteins (Bonhaus et al., 1998; Glass and Northup, 1999; Griffin et al., 1998; Kearn et al., 1999; Mukhopadhyay et al., 2000; Selley et al., 1996). For example, one study on hamster ovary cells with PTX (pertussis toxin) pretreatment (to observe coupling with G_s) reports that different CB1R agonists had different potencies to stimulate or inhibit cAMP accumulation (Bonhaus et al., 1998).

3.3.5.2. Regulation of ion channels

During studies on endocannabinoid receptors it has been shown that they can affect several ion channels crucial for cellular excitability. N- and Q-type voltage-dependent calcium channels (VDCC) are inhibited by AEA (Mackie et al., 1995, 1993). Interestingly, inhibition produced by AEA is apparently not due to the cAMP pathway, but a direct interaction of the G subunits with those channel proteins (Ikeda, 1996; Mackie et al., 1993).

Currently a vast array of studies in different brain regions have shown that activation of the CB1Rs on axon terminals leads to decrease in the neurotransmitter release by inhibition of calcium channels (Brown et al., 2003; Castillo et al., 2012; Kreitzer and Regehr, 2001; Pertwee, 1997; Petrocellis et al., 2004; Wilson et al., 2001). This signalling is elicited in a retrograde fashion, which is in line with apresynaptic CB1R distribution and postsynaptic localization of the enzymes involved in metabolism of endocannabinoids (Di Marzo et al., 1994; Egertová et al., 1998; Elphick and Egertová, 2001; Stella et al., 1997). Retrograde signalling of endocannabinoids was demonstrated during studies on depolarization-induced suppression of inhibition or excitation (DSI or DSE) (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). In a study on hippocampal slices, Wilson and his colleagues demonstrated that endocannabinoids target specific subtypes of GABAergic interneurons and inhibit N- type VDCCs to decrease the release of GABA (Wilson and Nicoll, 2001). In the cerebellum, CB1R activation leads to inhibition of firing due to activation of K^+ current and, thus a decrease in the neurotransmitter release (Kreitzer et al., 2002). CB1Rs can also inhibit L-type calcium channels, as demonstrated in cat cerebral vascular smooth muscle cells (VSMC) (Gebremedhin et al., 1999). In the retinal slices cannabinoids both enhance (in rods) and inhibit (in cones) L-type calcium channel activity (Straiker and Sullivan,

2003). The enhancement of L-type calcium currents in rods is due to modulation of the cAMP pathway by CB1Rs. Moreover, in both rods and cones, potassium currents are also inhibited (Straiker and Sullivan, 2003).

Moreover, CB1Rs have been reported to influence calcium release from intracellular stores (Hampson et al., 1998, 2011; Netzeband et al., 1999). For example, it has been indicated that activation of CB1Rs at hippocampal glutamatergic synapses can modulate the ability of NMDA receptor-gated Ca^{2+}influx to potentiate calcium-sensitive endoplasmic reticulum ryanodine (RYR) receptors. Activation of the CB1Rs reduces the release of intracellular calcium most likely by facilitating phosphorylation of RYR receptors (Hampson et al., 2011).

In addition to those presented above, CB1Rs or even their ligands can modulate other signalling pathways. Effects of CB receptor dependent and independent actions are summarized in Tables 3.2. and 3.3.

Table 3.2. CB receptor-mediated signal transduction targets (acquired from Demuth and Mollean, 2006)

Receptor	**Signalling target**	**Suggested Effect**	**References**
CB1R	$G_{i/o}$	Activation	Bonhaus et al., 1998; Bouaboula et al., 1999
	G_s	Activation	Maneuf and Brotchie, 1997; Jarrahian et al., 2004
	Adenylate cyclase	Inhibition	Felder et al., 1993; Wade at al., 2004
		Activation	Felder at al., 1998; Busch et al., 2004
	p38 mitogen activated protein kinase	Activation	Derkinderen at al., 2001
	p42/p44 mitogen	Activation	Bouaboula et al., 1995b;

	activated protein kinase		Derkinderen et al., 2003
	phosphatidyl inositol-3-kinase/ protein kinase B	Activation	Sánchez et al., 1998; Galve-Roperh et al., 2002
	Ceramide	Activation	Sánchez et al., 1998; Galve-Roperh at al., 2000
	Voltage-activated Ca^{2+} channels:		
	N-type	Inhibition	Pan et al., 1996; Wilson et al., 2001
	P/Q-type	Inhibition	Twitchell et al., 1997; Hampson et al. 1998
	L-type	Inhibition	Gebremedhin et al., 1999; Straiker at al., 1999
	K^+ channels:		
	G-protein couples inwardly rectifying K^+ channel	Activation	McAllister at al., 1999; Robbe et al., 2001
	I_A	Activation	Hampson et al., 1995; Mu et al., 2000
	I_D	Inhibition	Mu et al., 1999
	I_M	Inhibition	Schewitzer, 2000

	N-methyl-D-aspartate receptor	Inhibition	Hampson et al., 1998; Netzeband et al., 1999
	$[Ca^{2+}]_i$	Activation	Fimiani et al., 1999; Begg et al., 2001; Demuth et al., 2004
	Arachidonic acid	Activation	Demuth et al., 2005; Shivachar et al., 1996
	Phospholipase C/ inositol 1,4,5-triphosphate	Activation	Sugiura et al., 1997; Netzeband et al., 1999
	Nitric oxide	Activation	Fimiani et al., 1999; Mombouli et al., 1999
CB2R	$G_{i/o}$	Activation	Beyewitch et al., 1995; Kobayashi et al., 2001
	Adenylate cyclase	Inhibition	Bayewitch et al., 1995; Slipetz et al., 1995
	p42/p44 mitogen activated protein kinase	Activation	Bouaboula et al., 1996; Kobayashi et al., 2001
	$[Ca^{2+}]_i$	Activation	Zoratti et al., 2003
	Phospholipase C/ inositol 1,4,5-triphosphate	Activation	Zoratti et al., 2003

Table 3.3. CB receptor-independent signal transduction pathways (from Demuth and Mollean, 2006)

Signalling Target	Cannabinoid	Suggested Effect	References
Transient receptor potential vanilloid 1 receptor	Anandamide	Activation	Smart et al., 2000; De Petrocellis et al., 2001
5-hydroxytryptamine receptor	Anandamide	Inhibition	Barann et al., 2002; Oz et al., 2002
	CP 55,940	Inhibition	Fan, 1995; Godlewski et al., 2003
	WIN 55,212-2	Inhibition	Barann et al., 2002; Godlewski et al., 2003
	Δ^9-THC	Inhibition	Barann et al., 2002
	LY 320135	Inhibition	Barann et al., 2002
Nicotinic acetylcholine receptor	Anandamide	Inhibition	Oz et al., 2003
	2-AG	Inhibition	Oz et al., 2004
	CP 55,940	Inhibition	Oz et al., 2004
N-methyl-D-aspartate receptor	Anandamide	Activation	Hampson et al., 1998
T-type Ca^{2+} channel	Anandamide	Inhibition	Chemin et al., 2001
TWIK-related acid-	Anandamide	Inhibition	Maingret et al., 2001

sensitive K^+ 1 (TASK-1) channel			
	CP 55,940	Inhibition	Maingret et al., 2001
	WIN 55,2122	Inhibition	Maingret et al., 2001
Na^+ channel	Anandamide	Inhibition	Nicholson et al., 2003
	WIN 55,212-2	Inhibition	Nicholson et al., 2003

3.3.6. Endocannabinoids in pathological conditions

A systematic description of the endocannabinoid system properties and function has led to the proposal that it may play a role in the pathological processes occurring in the central nervous system. Numerous studies have correlated the endocannabinoid (ECB) system with many various conditions including brain ischemia, traumatic brain injury, Huntington's and Alzheimer's diseases, excitotoxicity, multiple sclerosis and ALS (Bilsland and Greensmith, 2008; Croxford, 2003; Fernández-Ruiz, 2009; Hillard, 2008; Pacher et al., 2006; Pertwee, 2005; Scotter et al., 2010; Shouman et al., 2006).

3.3.6.1. Excitotoxicity

Excitotoxicity is taking part in many pathological conditions like brain ischemia, traumatic brain injury and also neurodegenerative diseases like Huntington's disease or ALS (Dong et al., 2009; Hillard, 2008; Van Den Bosch et al., 2006; Werner and Engelhard, 2007). Studies on the endocannabinoid system reveal that it can significantly influence the extent of damage caused by this type of insult. For example, CB1R activation protects cells in cultures of hippocampal neurons during toxicity induced by Mg^{2+} removal (Gilbert et al., 2007; Shen and Thayer, 1998). In those experiments, cell death is due to excessive Ca^{2+} influx via NMDA receptors which results in calcium spiking. CB1Rs most likely decrease the release of glutamate from synaptic terminals (Shen and Thayer, 1998). The endocannabinoid system seems to be a part of an endogenous neuroprotective mechanism recruited during the insult, as shown by studies with kainic acid on

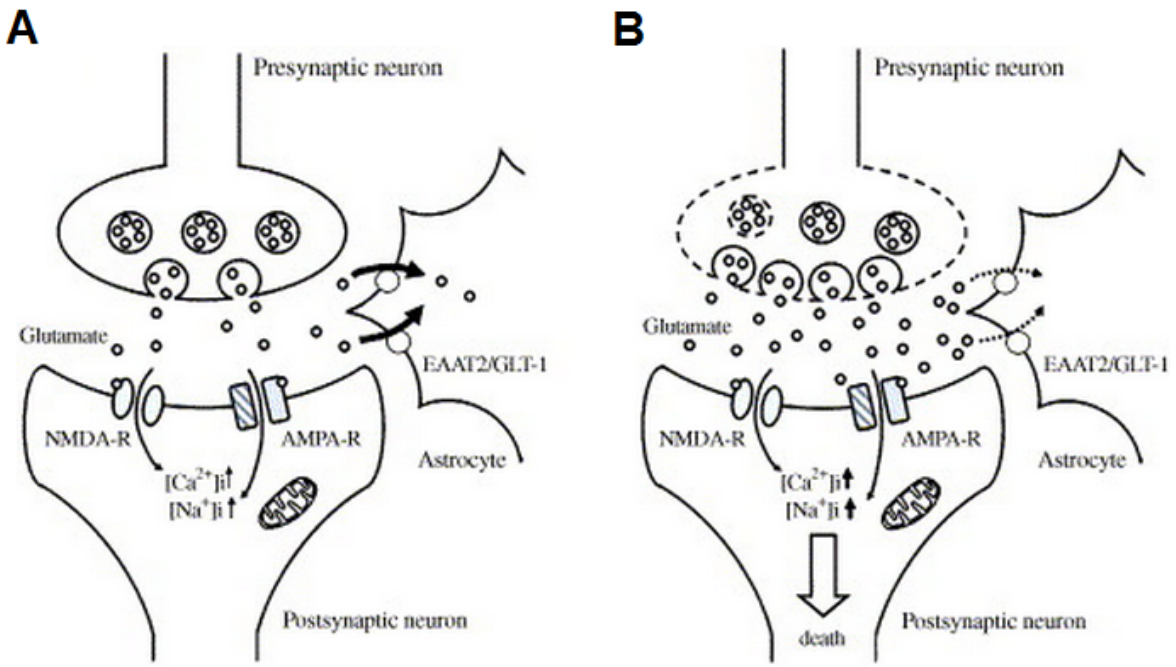

Fig 3.8 Glutamatergic neurotransmission and excitotoxicity

Under normal conditions, glutamate released from the presynaptic neuron activates the NMDA and AMPA receptors. This results in an influx of both Na^+ and Ca^{2+} ions, the depolarization of the postsynaptic neuron and ultimately in an action potential. (A) Excitotoxicity is induced by an elevation of the extracellular glutamate concentration. This can be caused by an increased release of glutamate or a deficient re-uptake of glutamate into the astrocytes by the EAAT2/GLT-1 transporter. The excessive stimulation of the glutamate receptors gives rise to an increased intracellular concentration of Na^+ and Ca^{2+} ions and this can result in neuronal death. The disintegration of neuronal cells causes a further increase of extracellular glutamate and amplifies the excitotoxic damage (B) (adapted from Van Den Bosch et al., 2006).

hippocampal cells (Khaspekov et al., 2004; Marsicano et al., 2003). Data from those studies prove that ablation of CB1Rs or co-application of a CB1R antagonist makes the cells more susceptible to damage caused by kainate application, for example by reducing the threshold for neuronal excitation (Marsicano et al., 2003). Also mice with deleted CB1Rs in glutamate neurons of the hippocampus and in the wild type mice treated with rimonabant, a CB1R antagonist, elicit more severe seizures induced by kainate, which indicate that CB1R signalling protects from excess excitability (Marsicano et al., 2003; Monory et al., 2006). Moreover, Marsicano and his colleagues detected an increase in the AEA levels in the wild-type hippocampal neurons after kainate application (Marsicano et al., 2003). Such up-regulation further supports the theory that cannabinoid signalling is triggered to fight against excess excitation.

3.3.6.2. Brain ischemia and traumatic brain injury

The most damaging consequences occurring during brain ischemia and traumatic brain injury are the generation of free radicals and increase in calcium and sodium intracellular levels (Leker and Shohami, 2002; Lipton, 1999). There are indications that the endocannabinoid system is activated during both pathological events. Experiments on rats confirmed that occlusion in the middle cerebral artery (MCA) resulted in a significant increase in AEA (Berger et al., 2004; Muthian et al., 2004), but not 2-AG (Muthian et al., 2004) concentrations. While transient MCA occlusion increases expression of CB1Rs (Jin et al., 2000), permanent occlusion does not (Sommer et al., 2006), thus indicating that increase in the CB1R staining is not due to ischemia per se, but probably tore-perfusion. In contrast to brain ischemia, a study on traumatic brain injury (TBI), using the closed head injury mouse model (CHI), detected elevated levels of 2-AG, but no change in the AEA levels (Panikashvili et al., 2001).

There are several studies supporting a protective effect of CB1R activation. For example, in rats exposed to global ischemia, pretreatment with the CB1R agonist WIN 55212-2 produces a dose-related increase in the number of surviving neurons (Nagayama et al., 1999). Furthermore, CB1R knock-out mice show increased mortality, increased infarct size and neurological deficits following transient MCA occlusion (Parmentier-Batteur et al., 2002). Another study has investigated if elevation of endocannabinoids during the ischemia is protective: application of a FAAH inhibitor significantly decreases infarct volume 24 hours after injury (Degn et al., 2007). CB1R activation can mediate protection in ischemic brain by several mechanisms. One of them is CB1R-mediated protection against glutamate-induced excitotoxicity because of inhibition of glutamate release from the presynaptic terminals (Hillard, 2008). In addition to anti-excitotoxic effects, activation of CB1Rs can lead to a decrease in body temperature (hypothermia) (Hillard et al., 1999), which has been shown to have beneficial effects in ischemia (Hoesch and Geocadin, 2007; Krieger and Yenari, 2004). For example, treatment with the synthetic cannabinoid HU 210 decreases body temperature (by nearly 6°C) and reduces infarct size in rats which underwent permanent occlusion of the MCA (Leker et al., 2003). This effect is abolished partially by application of a CB1R antagonist and completely by warming up the animals. Furthermore, CB1R activation is reported to reduce oedema, which commonly accompanies ischemia (Hillard, 2008).

In the CHI model, external application of 2-AG significantly reduces brain oedema, improves clinical recovery, reduces infarct volume and hippocampal cell death (Panikashvili et al., 2001). CB1R knock-out mice show less spontaneous recovery and no improvement in neurological

performance and oedema formation after administration of 2-AG (Panikashvili et al., 2005).

3.3.6.3. Neurodegenerative diseases

In Huntington disease (HD) loss of the CB1Rs from GABAergic efferent terminals and soma is one of the first signs of cellular dysfunction (Glass et al., 2000). Several studies on lesion models of HD, when excitotoxic drugs were used to evoke the insult, cannabinoid administration or up-regulation of their natural synthesis reduced the symptoms. For example, administration of an endocannabinoid uptake inhibitor attenuated the neurotransmitter deficits and improved motor function in animals treated with the excitotoxic 3-nitropropionic acid (de Lago et al., 2006; Lastres-Becker et al., 2002). Treatment with Δ^9-THC prevented development of lesions caused by this drug (Lastres-Becker et al., 2004). Ms

In Alzheimer's disease (AD) CB1R expression on neurons is reduced (Ramírez et al., 2005; Westlake et al., 1994). Studies in rodent models of this disease correlate elevation of endocannabinoid levels (AEA and 2-AG) with attenuation of the neuronal loss (van der Stelt et al., 2006).

Glial and some cells of the immune system expressing CB1 and CB2 receptors are reported to gather around plaques in multiple sclerosis (MS) (Benito et al., 2007). AEA is elevated in the cerebro-spinal fluid and plasma of the patients suffering from MS (Centonze et al., 2007a; Jean-Gilles et al., 2009). At the moment, several countries allow Δ^9-THC and cannabidiol therapy for the treatment of neuropathic pain and sleep disturbance correlated with MS (Rog et al., 2007, 2005; Wade et al., 2006).

3.3.6.4. ALS

Several studies of ALS have focused on the endocannabinoid system. In rodent models of ALS, both AEA and 2-AG accumulate in the lumbar spinal cord during disease progression (Bilsland et al., 2006; Witting et al., 2004). Another study reports increased levels of CB1R mRNA and protein in symptomatic ALS animals (Zhao et al., 2008). This may explain heightened control of both inhibitory and excitatory transmission by striatal CB1Rs in symptomatic SOD1 ALS mice

(Rossi et al., 2010). These data suggests that the endocannabinoid system is mobilised during disease development.

Administration of Δ^9-THC to SOD1 ALS animals modestly prolongs survival (by 5%) and delays symptom onset (by 6%) (Raman et al., 2004).A study with WIN55,212-2 administered after symptom onset has shown delayed disease progression, yet unchanged survival (Bilsland et al., 2006). The same study has shown that administration of cannabinoids in the late phase of the

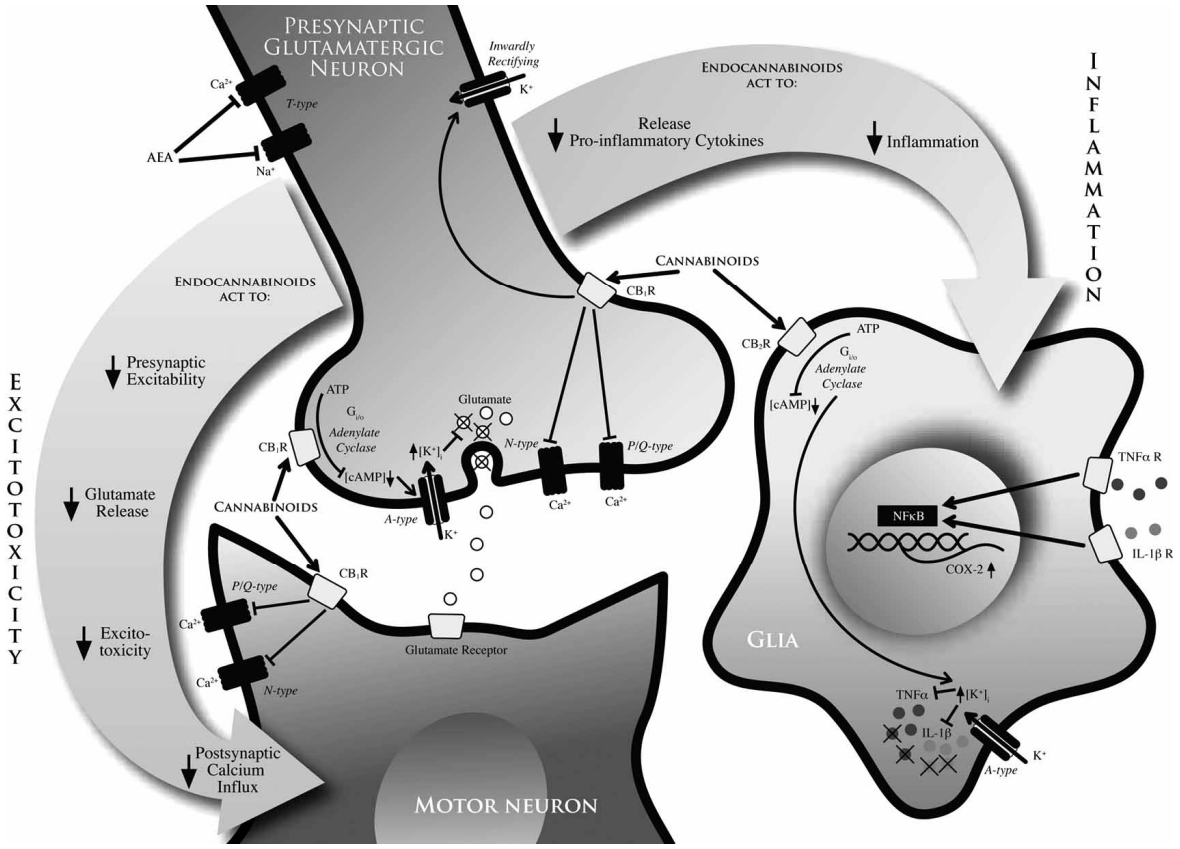

Fig 3.9 Summary of the potential neuroprotective mechanisms of the endocannabinoid system in ALS

Endocannabinoids may exert neuroprotective actions in ALS by targeting two main neurotoxic mechanisms; excitotoxicity and inflammation. This illustration gives a basic overview of the potential neuroprotective actions of endocannabinoids in ALS, and shows the actions of endocannabinoids against excitotoxicity on the left hand side and against inflammation on the right hand side. Activation of CB1 receptors by cannabinoids may alter the calcium and potassium permeability of the pre- and postsynaptic neuron reducing their excitability. This will result in the inhibition of glutamate release, causing a reduction in postsynaptic calcium influx, thereby minimising the effects of excitotoxicity. Meanwhile CB2 receptor activation will similarly act to reduce cellular excitability and the localisation of the CB2 receptor to glial cells will result in a reduction in the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), thereby inhibiting further propagation of the inflammatory response, so that for example COX-2 levels do not increase. The endocannabinoid system may also exert neuroprotective effects in a non-receptor mediated manner, although these mechanisms are not depicted in this diagram (adapted from Bilsland and Greensmith, 2008).

disease (120 days in ALS mouse model) has a beneficial effect on motor unit survival. Improvement in motor neuron survival could be also obtained by crossing ALS mice with FAAH knock-out animals (Bilsland et al., 2006).

Putative pathways by which endocannabinoids can influence pathological processes occurring during ALS are presented in Fig 3.9. Despite preventing excitotoxicity by glutamate release inhibition (via CB1Rs), in addition, they can target inflammation (via CB2Rs) (Bilsland and Greensmith, 2008).

4. Aims

Previous immunostaining studies have shown CB1R localization in the brainstem region and that hypoglossal motoneurons are stained by a CB1R antibody (Glass et al., 1997; Herkenham et al., 1991; Jin et al., 2014; Herkenham, 1990; Xing et al., 2011). In thepresent study we investigated if the endocannabinoid system is active and functional in this brainstem region, with special focus on the TBOA-evoked excitotoxic insult. We aimed at clarifying the following issues:

- Evaluate the effect of the endocannabinoid system modulation on hypoglossal motoneuron responses to glutamate uptake inhibition caused by TBOA.
- Investigate if endocannabinoid receptor modulation (either stimulation with agonist or block by antagonist) can influence spontaneous synaptic activity (both inhibitory and excitatory) of motoneurons of the XII nucleus of the rat brainstem.
- Analyse changes in the CB1R expression in the brainstem during excitotoxic stress caused by TBOA.
- Test if a CB1R agonist or antagonist can affect motoneuron survival following excitotoxic insult caused by TBOA.

5. Materials and methods

All experiments were carried out in accordance with the regulations of the Italian Animal Welfare act (DL 27/1/92 n.116) following the European Community directives no. 86/609 93/88 (Italian Ministry of Health authorization for the local animal care facility in Trieste D 69/98-B), and approved by the local authority veterinary service.

5.1. Electrophysiology

5.1.1 Slice preparation

Experiments were carried out on the brainstem slices isolated from Wistar rats (postnatal day 2-4). Animals were terminally anaesthetized with urethane and decapitated (Lamanauskas and Nistri, 2006; Quitadamo et al., 2005). The brainstem region was isolated in modified, continuously oxygenated (95% O_2 and 5% CO_2 mixture), ice-cold Krebs solution (see below). The lower medulla was pinned to an agar block for stabilization and cut inside a Vibratome (Vibratome 1000 S, Leica) chamber (filled with modified Krebs solution) into 270 µm slices. Slices were transferred into the incubation chamber filled

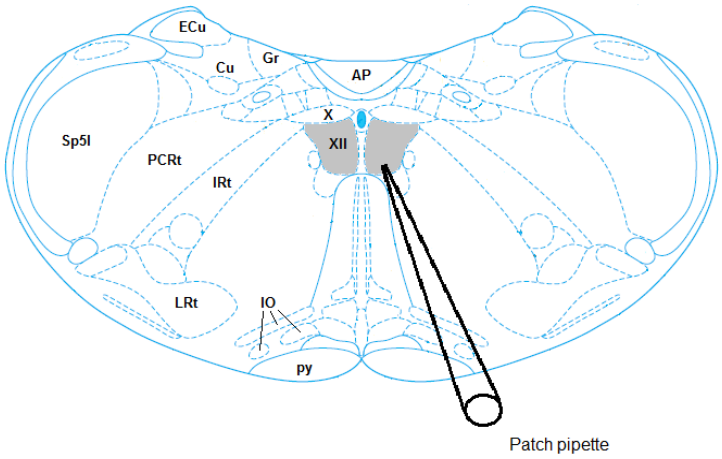

Fig 5.2 Hypoglossal nucleus identification within brainstem slice with some of the surrounding structures

ECu, external cuneate nucleus; Cu, cuneate nucleus; Gr, gracile nucleus; AP, area postrema; X, dorsal motor nucleus of vagus; XII, hypoglossal nucleus; Sp5I, spinal trigeminal nucleus, interpolar part; PCRt, parvicellular reticular nucleus; IRt, intermediate reticular nucleus; LRt, lateral reticular nucleus; IO, inferior olive; py, pyramidal tract (adapted from Paxinos and Watson, 2006).

with modified Krebs to recover for 20 min at 32°C under constant oxygenation. Later, the temperature was lowered to ambient level and samples were maintained in this condition for around 1 h before use. All the following procedures were performed at room temperature (23°C).

5.1.2 Electrophysiological recordings

Single slices were placed into a small recording chamber filled with oxygenated Krebs solution. They were held in place by fine nylon strands glued to a horseshoe-shaped platinum wire. The hypoglossal nucleus region was visualised with an infra-red video-camera. Motoneurons were identified on the basis of their size and shape. Voltage clamp experiments used borosilicate glass electrodes (pulled with a Narishige PC-10 puller; DC resistance of 2.5-4 MΩ) connected to a patch clamp L/M-EPC7 amplifier (List Medical). When advancing the patch pipette toward the cell, a positive pressure was applied to the electrode in order to prevent blockage. Offset potentials were cancelled with the DC offset control. Seal resistance was usually exceeding 2 GΩ. Upon seal rupture and obtaining voltage clamp, the holding potential (V_h) was kept at the value of -70 mV while series resistance (R_s; 5-25 MΩ) was routinely monitored and compensated. Data were discarded when changes in R_s exceeded 20% of the initial value. Voltage pulses and recordings were obtained with Clampex 9.2 software (Molecular Devices). Currents were filtered at 3-10 kHz and sampled at 10 kHz. All recordings were performed at room temperature.

5.1.3 Solution and drugs

For the dissection and subsequent incubation of the brainstem slices, a modified Krebs solution was used with the following salt composition (values in mM): NaCl 130, KCl 3, NaH_2PO_4 1.5, $MgCl_2$ 5, $CaCl_2$ 1, NaH_2CO_3 25, glucose 10 (osmolarity = 290-330 mOsm). During recording, slices were superfused with oxygenated Krebs solution (NaCl 130, KCl 3, NaH_2PO_4 1.5, $MgCl_2$ 1, $CaCl_2$ 1.5, NaH_2CO_3 25, glucose 10, osmolarity = 290-330 mOsm) at the speed of 2 ml/min. The patch pipette was filled with intracellular solution containing CsCl 130, NaCl 5, $MgCl_2$ 2, $CaCl_2$ 1, HEPES 10, EGTA 10 (ethylene glycol tetraacetic acid), ATP-Mg salt 2, glucose (osmolarity ranging between 290 and 330 mOsm). The solution pH was set at 7.2 with CsOH. The reason to use CsCl for the intracellular solution was to minimize the leak current of the recorded cell. All drugs were applied via the perfusion system for at least 10 min in order to reach equilibrium conditions. The following drugs were used during the experiments: DL-TBOA (Tocris), bicuculline methiodide

(Abcam), strychnine hydrochloride (Tocris), DNQX (Tocris), D-APV (Tocris), AEA (Tocris), AM251 (Tocris).

5.1.4 Data analysis

Cell R_{In} was calculated by measuring the current response to 10 mV hyperpolarizing steps from V_h. All electrophysiological data were analysed using Clampfit 10.0 software (Molecular Devices). Spontaneous post synaptic currents, both excitatory and inhibitory, were detected using the template search function of the Clampfit software. Burst parameters were calculated according to Fig 5.2, where A indicates period, B duration, C half-width and D amplitude of the burst. Burst fragmentation is defined as alteration in burst shape (for example double peaks, humps in rise or fall phase of the burst).

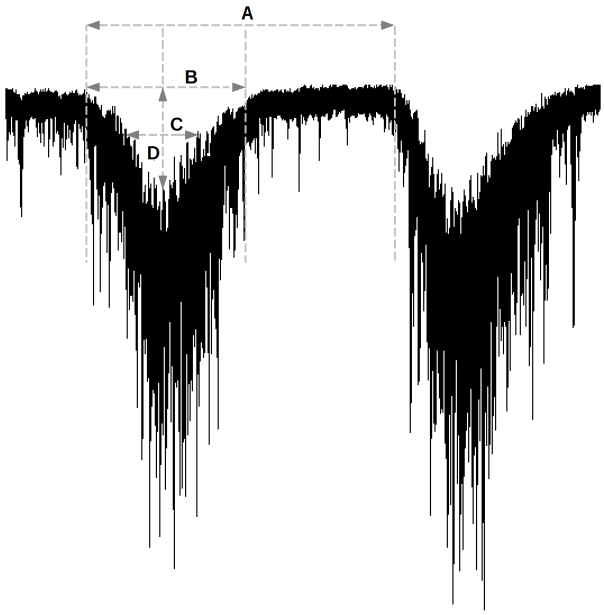

Fig 5.2 Burst parameters
A-period, B-duration, C-half-width and D-amplitude of the burst.

5.2. Western blot

5.2.1 Sample preparation

The brainstem was isolated as indicated in section 5.1.1 of this chapter. The medullary region containing the hypoglossal nuclei was cut out and incubated in a chamber filled with oxygenated, modified Krebs solution for 20 min at 32°C and then allowed to settle at room temperature for over 10 min before drug application. After test incubation, which lasted 4 h, samples were briefly rinsed in fresh Krebs solution, transferred to Eppendorf tubes and rapidly frozen in the liquid nitrogen and kept at -80°C until further processing.

After defrosting, brainstem regions were briefly rinsed in phosphate-buffered saline (PBS) buffer, transferred to 1.5 ml tubes containing lysis buffer and homogenized twice for 30 s with a T10 Basic ULTRA-TURRAX homogenizer (IKA). Samples were then centrifuged for 5 min at 4,000 rpm at 4°C and transferred to clean Eppendorf tubes. When necessary, protein isolates were additionally sonicated (with Sanyo sonicator Soniprep 150) in order to remove nuclear contamination. Protein levels were evaluated with the bicinchoninic acid (BCA) kit (Sigma-Aldrich) according to manufacturers' protocol. Their light absorbance was measured with a Multiscan FC spectrophotometer (Thermo Scientific).

5.2.2 Western Blot procedures

Proteins were separated via the standard SDS-PAGE technique. Samples were mixed with loading buffer and boiled at 95°C for 5 min. For each well, a sample of 50 μg protein was used. After separation on the gel, standard Western blot was performed in order to transfer proteins to the nitrocellulose membrane. Membranes were briefly washed in Tris-buffered saline and Tween 20 (TBST) solution and blocked with 1% milk diluted in TBST for approximately 1 h. For anti-CB1R antibody staining, membranes were incubated overnight at 4°C in the antibody solution (dilution 1:1000). For anti-β-actin staining, 1 h incubation at room temperature was used (dilution 1:10000). Bands were detected with the Amersham ECL WB detection reagents (GE Healthcare), and visualized via the Uvitec Cambridge system.

5.2.3 Solutions and drugs

For the brainstem isolation and incubation see section 5.1.3. Lysis buffer contained (in mM) HEPES 10 (pH 7.9), $MgCl_2$ 1.5, KCl 10, dithiothreitol (DTT) 1, and protease inhibitors (Sigma-Aldrich). The loading buffer used for SDS-PAGE was composed of 1 % sodium dodecyl sulfate (SDS), 5% 2-β-mercaptoethanol, 10% glycerol, 0.002% bromophenol blue, and 60 mM Tris HCl (pH 6.8). Anti-CB1R antibodies (Abcam) and anti-β-actin antibodies (Sigma-Aldrich) were respectively diluted in 1 % or 10 % dry milk (Delicat Gramm) dissolved in the TBST.

5.2.4 Data analysis

ImageJ software was used to analyse the WB results. Quantification and band density were calculated with a gels analysis plug-in. All protein levels were normalised to β-actin and compared to control sample (incubated simultaneously in Krebs with no additional drugs).

5.3. Viability assay

The brainstem region was isolated and cut into slices as described in section 5.1.1 of this chapter. Two slices from the same brainstem were paired with each other for the control and treatment. 250 μm thick slices were transferred to the incubation chamber filled with Krebs and allowed to settle for 10 min at room temperature under constant oxygenation. Drugs were then applied to the Krebs solution. At the end of the exposure time (4 h), slices were rinsed and transferred into a fresh Krebs solution. Propidium iodide (Sigma-Aldrich, final dilution 1:3000) (staining for non-viable cells) and Hoechst 33342 (Invitrogen, final dilution 1:1000) (cell-permeant nuclear stain) were applied via the Krebs solution and incubated for 45 min. Thereafter, slices were rinsed in Krebs solution, transferred to small Petri dishes and analyzed under a Nikon Eclipse T confocal microscope (x20) with NILS-Elements software (Nikon Instruments S.P.A.) without fixation. Data were processed with ImageJ 1.48 software (Wayne Rasband, National Institutes of Health, USA) using the cell counter plug-in. The number of PI and Hoechst 33342-positive cells was recorded and compared.

5.4. Statistical analysis

Results are presented as means±SEM and medians±SEMD as indicated in the figures description, which were calculated automatically using the following equations:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i \qquad SEM = \frac{s_X}{\sqrt{n}}$$

$$\tilde{X} = \begin{cases} X_{\left(\frac{n+1}{2}\right)} & if\ n\ is\ odd \\ \frac{1}{2}\left(X_{\left(\frac{n}{2}\right)} + X_{\left(\frac{n+1}{2}\right)}\right) & if\ n\ is\ even \end{cases} \qquad SEMD = \sqrt{\frac{\pi}{2}}\frac{s_X}{\sqrt{n}}$$

Where $\bar{X}$ represents mean, $\tilde{X}$ median, n sample size, X_i *the* i^{th} value of the sample and s_x sample standard deviation. The reason to use medians in some data presentation was due to a fact that this statistical parameter is less influenced by extreme values in compare to mean (Harding et al., 2014). Some of the data presented in this work were marked by significant data spread, particularly burst parameters in the electrophysiological recordings. This can markedly influence the value of the mean giving inaccurate impression of the observed parameters. In order to grant most reliable data presentation, some histograms in this work are presented as medians instead of more commonly used means.

In the Results section *n* refers to the number of cells/slices/brainstems used in the experimental procedures. Data were analysed with the GraphPad statistical online platform (GraphPad Software, Inc.) and SigmaStat 3.5 software (Systat Software). When samples passed the normality test, the Student's t-test was used (power of performed test with alpha = 0.050). Otherwise the Mann-Whitney test (for unpaired) or the Wilcoxon Signed Rank test (for paired) was used. For burst occurrence and fragmentation, the statistical analysis used the Chi-squared test without Yates correction. In all cases, statistical significance was considered only if $P \leq 0.05$.

6. Results

6.1 Anandamide action on hypoglossal motoneurons

6.1.1 Anandamide action on hypoglossal motoneurons during glutamate uptake inhibition

In the past years, our laboratory has studied responses of hypoglossal motoneurons of the neonatal rat to excitotoxic stress caused by glutamate uptake inhibition (Cifra et al., 2011a, 2009; Sharifullina and Nistri, 2006). Accumulation of glutamate at synaptic level not only increases the number of dead cells in the medullary slice, but it also elicits prolonged, repetitive depolarisations (bursting) in about 50% of the motoneuron population. This bursting process is exemplified in Fig 6.1. Furthermore, pharmacological agents which suppress bursting also prevent motoneuron death caused by DL-TBOA, a drug used to fully block excitatory amino acid transporters in the medullary slice (Cifra et al., 2011a, 2011b, 2009; Sharifullina and Nistri, 2006).

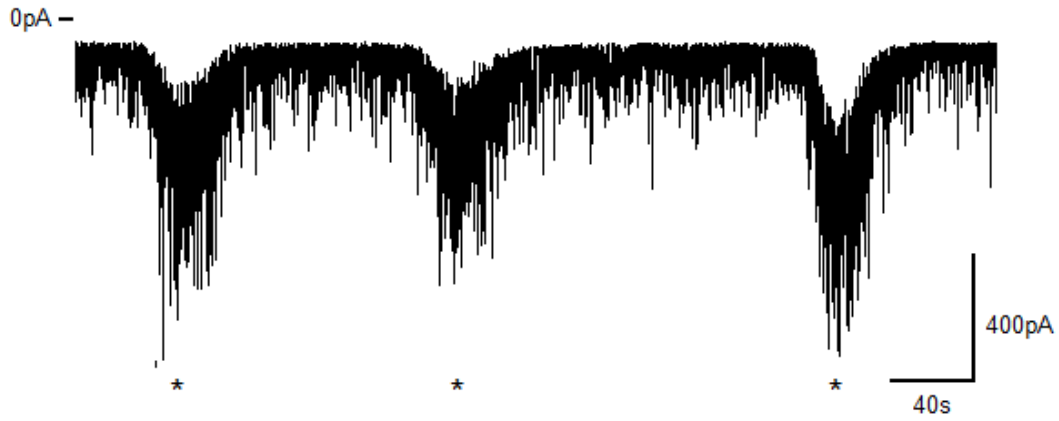

Fig 6.1 Example of TBOA-evoked bursts recorded in a hypoglossal motoneurone under voltage clamp (V_h =-70mV).

Application of DL-TBOA evoked long-lasting, large inward currents referred to as bursts characterized by fast superimposed oscillations and spikelets. These events reflect group bursting of motoneurons, originating within the premotoneuron network and are supported by a combination of gap junctions and glutamatergic synaptic transmission.

In the initial phases of my experiments I have gathered control responses from hypoglossal motoneurons whose electrophysiological properties were studied before and after application of TBOA alone. After patching cells and obtaining stabilization of the baseline current, I applied 50

µM DL-TBOA via the bathing solution. In these experiments I did not observe any significant change in the baseline inward current or input resistance (Fig 6.2 A and B). Despite lack of development of baseline currents, the majority of recorded cells generated strong bursting activity (around 70%) as shown in Fig 6.2 C.

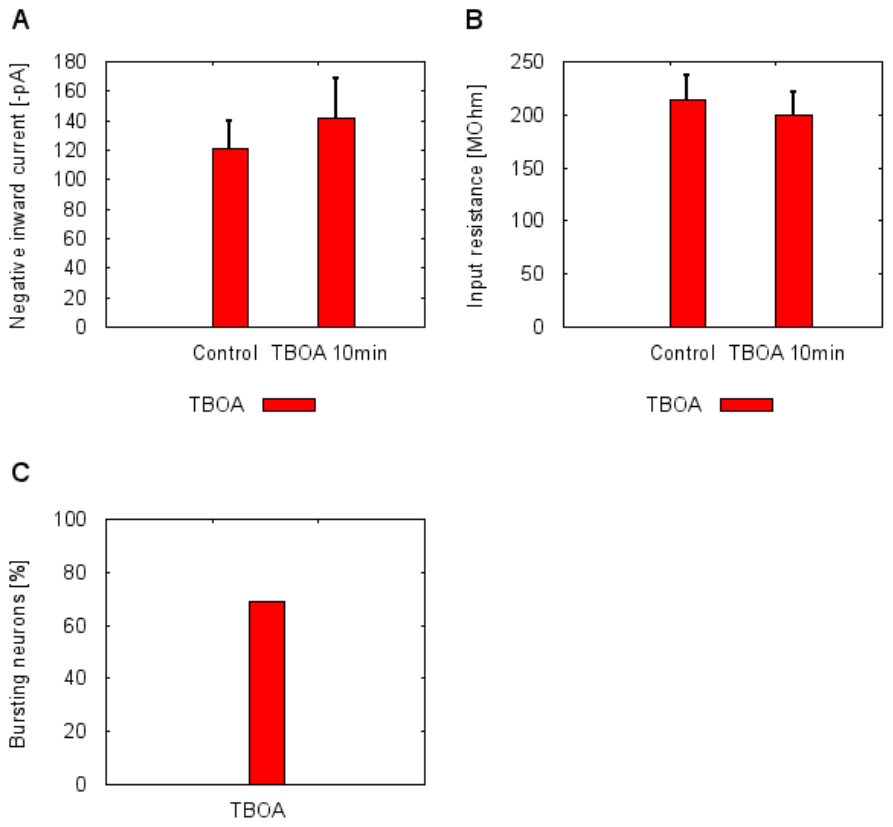

Fig 6.2 Parameters of TBOA- treated cells (n=15); V_h=-70mV.

Application DL-TBOA did not change significantly inward current (A) or input resistance (B) of the cells under study (Wilcoxon Signed Rank Test P>0.05). Application of TBOA elicited bursting in majority of the cells recorded (C). Data presented as means±SEM.

The recorded parameters were comparable to those previously reported (Cifra et al., 2011b; Sharifullina and Nistri, 2006). In order to better understand the properties of bursting, in the present study I divided bursts into early (first two) and late (third to fifth) ones. Usually, the number of bursts in a cell varied from three to five. Because cells displaying more than five bursts were not frequent, and previous reports have indicated that the phenomenon is connected to early cellular damage, I decided to limit my systematic analysis to the first five bursts.

I measured multiple burst parameters: amplitude, half-width, duration and frequency (see Fig 5.2 of Materials and methods section). I observed that, in the case of TBOA-evoked bursts, the

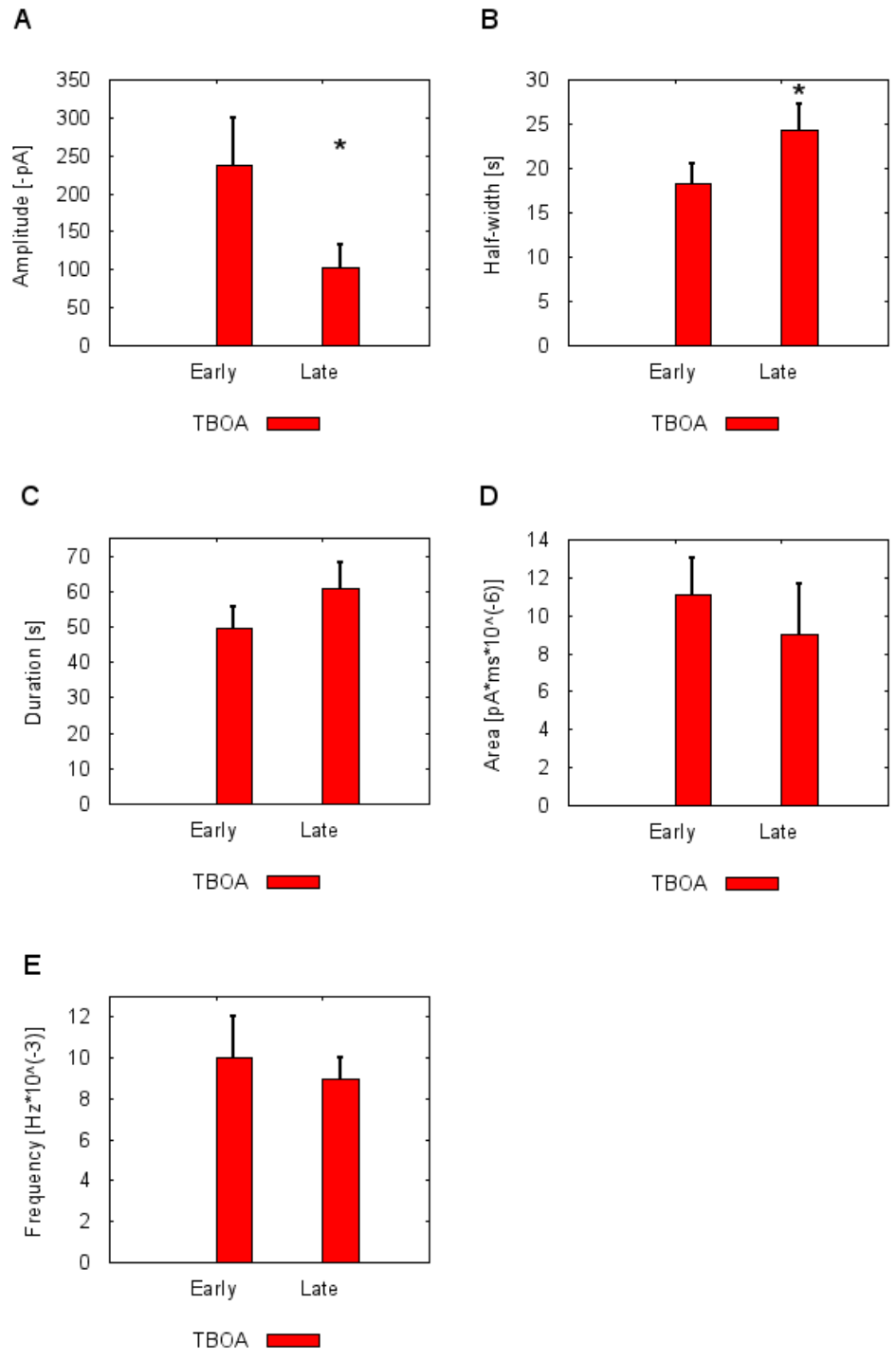

Fig 6.3 Parameters of bursts in TBOA- treated cells (n=11); V_h=-70mV.

Comparison of early versus late bursts revealed a decrease in amplitude (Mann-Whitney Rank Sum Test, P = 0.036; A) and increase in half-width with time (Students t-test, P =0.014; B). Duration (C), area (D), and frequency (E) however, remained comparable (Students t-test P>0.05). Data presented as medians±SEMD.

amplitude of early bursts was the largest (Rank Sum-Test, *P*=0.036; Fig 6.3 A), while the half-width of late bursts was wider (Students t-test, *P*=0.014; Fig 6.3 B). However, the total burst duration, despite an apparent increase, was not statistically changed (Fig 6.3 C). Area under the burst (Fig 6.3 D) and burst frequency (Fig 6.3 E) were similar in early and late bursts.

In order to investigate how AEA might affect bursting properties of HMs, I first applied 10 μM AEA for 10 min to reach equilibrium. Afterwards, it was co-applied with DL-TBOA (50 μM) as illustrated in the representative experiment shown in Fig 6.4 where the first arrow points to the start of AEA application.

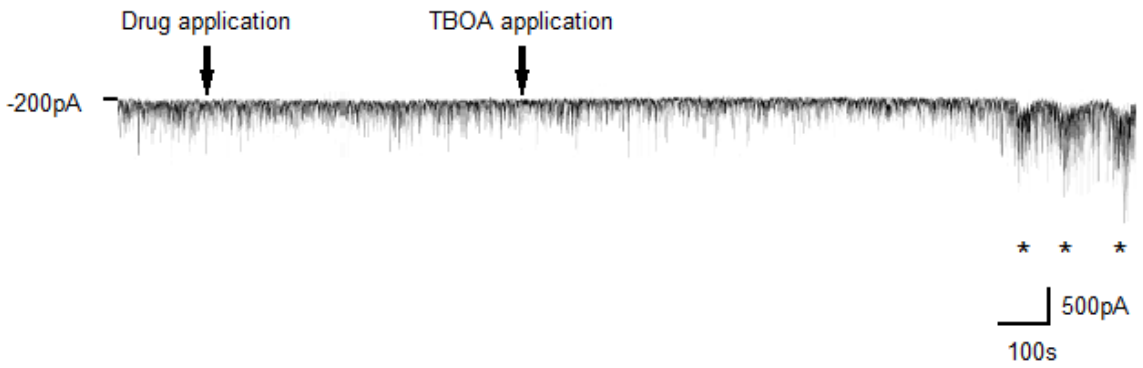

Fig 6.4 Example of time profile of the electrophysiological experiment with AEA and TBOA co-application (V_h =-70mV).

First arrow indicates start of drug application (AEA example presented here), second arrow shows start of TBOA co-application. Asterisks marks TBOA-evoked bursts.

Several reports have shown that high doses of AEA can negatively affect cells causing damage and even death (Fonseca et al., 2009; Movsesyan et al., 2004). Many reports (including electrophysiological studies) indicate that this AEA effect might be present even at low doses (ranging from tens of nM to few μM) in sensitive cells (Al Kury et al., 2014; Correa et al., 2008; Evans et al., 2004; Gebremedhin et al., 1999; Golovko et al., 2014; Howlett and Mukhopadhyay, 2000; Li et al., 2009; Oz et al., 2007; Spivak et al., 2007; Wacnik et al., 2008). In a study by Tree and his colleagues, who investigated modulation of the respiratory rhythm by this drug, the effect was evident only after application of 30 μM. Note that 15 μM concentration failed to produce significant change in electrophysiological recordings from the fourth cervical roots of the neonatal mouse preparation (Tree et al., 2010). Thus, I decided to use 10 μM AEA in the recording solution in order to minimize possible negative effects, while ensuring drug effectiveness.

Anandamide application did not caused significant change in baseline inward current (Student t-test *P*=0.080; Fig 6.5; A). Although in the majority of recordings I detected an increase in

input resistance, in some of them it did not change or it even declined, so that statistical significance was not reached (Student t-test *P*=0.113; Fig 6.5; B). Co-application of TBOA evoked outward current. Change was significant with respect to AEA alone, but not to control inward current before drug application (Student t-test *P*<0.005; Fig 6.5; A). There was no significant change in input resistance after TBOA co-application (Fig 6.5; B).

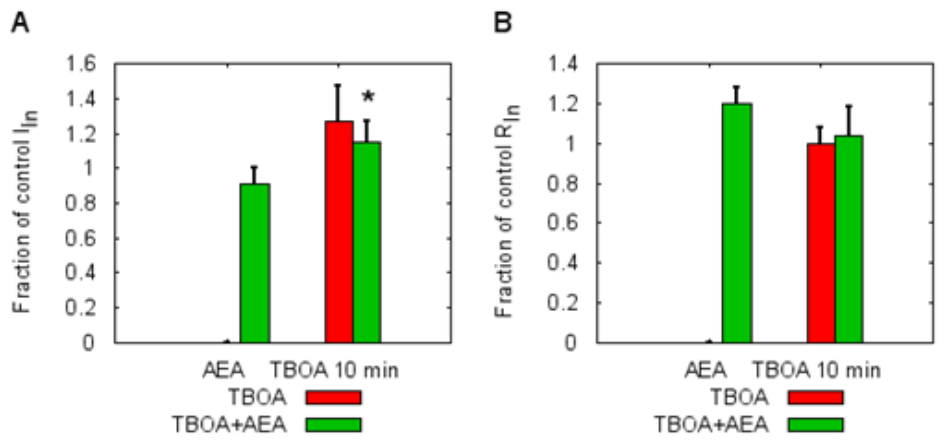

Fig 6.5 Parameters of AEA and TBOA co-treated cells and bursts (n=18); V_h=-70mV.

Application of AEA did not change significantly inward current of the HM. After co-application with TBOA I_{In} increased significantly in respect to AEA alone, but not to control current (Student t-test P=0.003; A). Despite a general decrease there were no significant changes in R_{In} (Student t-test P>0.05; B). Data presented as means±SEM.

One example of TBOA-evoked bursts in the presence of AEA can be seen in Fig 6.6 A. When I analysed burst parameters in cells co-treated with AEA, I observed a significant decrease in the occurrence of the bursting (Chi-squared test, *P*=0.0393; Fig 6.6 B). When I compared early and late bursts in TBOA and AEA co-treated cells, I did not find differences in their amplitude (Mann-Whitney Rank Sum Test, *P*>0.05; Fig 6.7 A). Half-width (Students t-test, *P*=0.021) and duration (Mann-Whitney Rank Sum Test, *P* = 0.032) both increased (Fig 6.7 B and C respectively) in late bursts in compare to early ones. There was no change in burst area or frequency (Paired Student t-test *P*>0.05; Fig 6.7 D and E respectively).

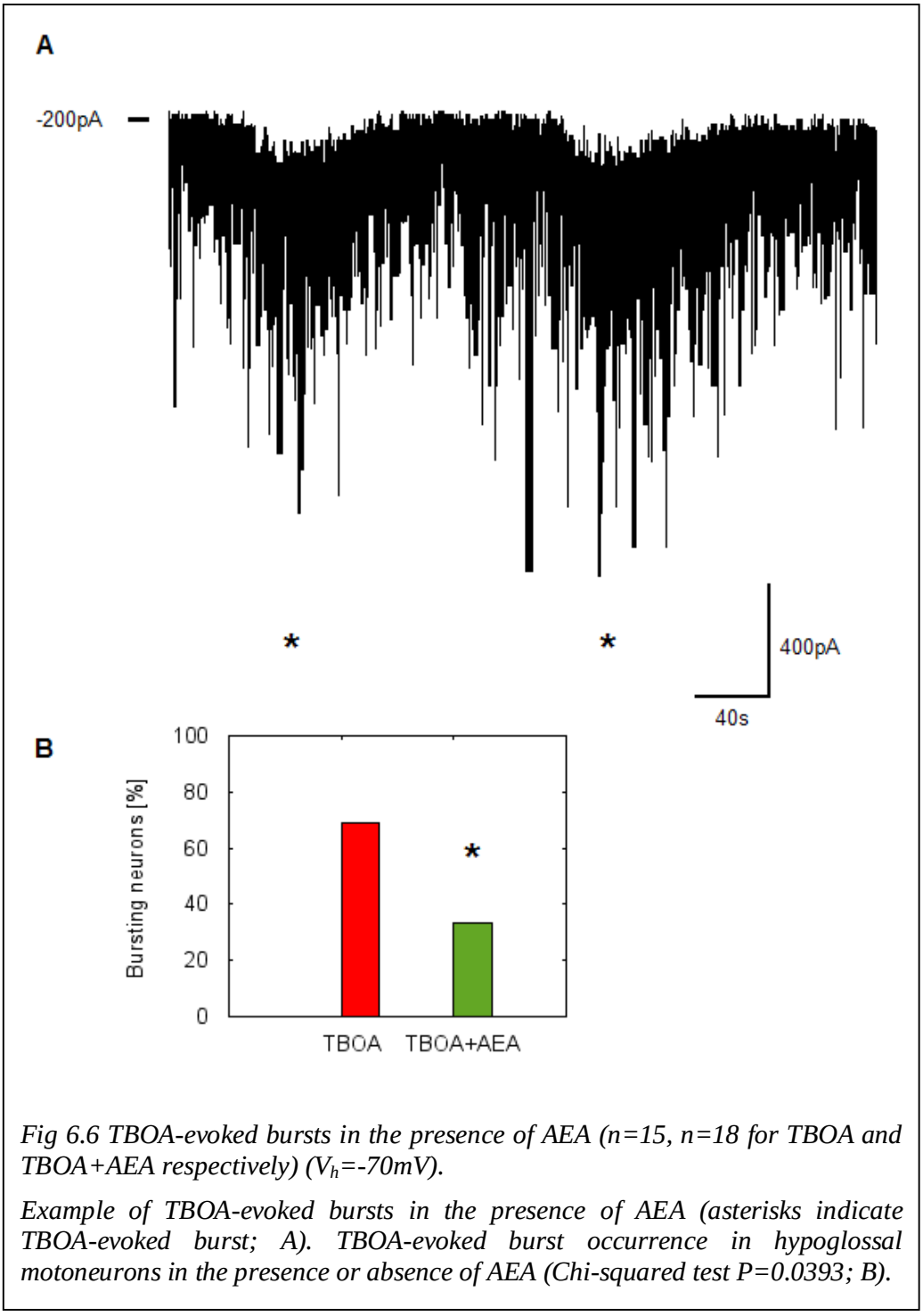

Fig 6.6 TBOA-evoked bursts in the presence of AEA (n=15, n=18 for TBOA and TBOA+AEA respectively) (V_h=-70mV).

Example of TBOA-evoked bursts in the presence of AEA (asterisks indicate TBOA-evoked burst; A). TBOA-evoked burst occurrence in hypoglossal motoneurons in the presence or absence of AEA (Chi-squared test P=0.0393; B).

When I compared burst characteristics between early and late bursting in TBOA alone and co-applied with AEA, no statistically significant difference was detected in amplitude, half width, duration, area or frequency (Student t-test P>0.05; Fig 6.7 A-E).

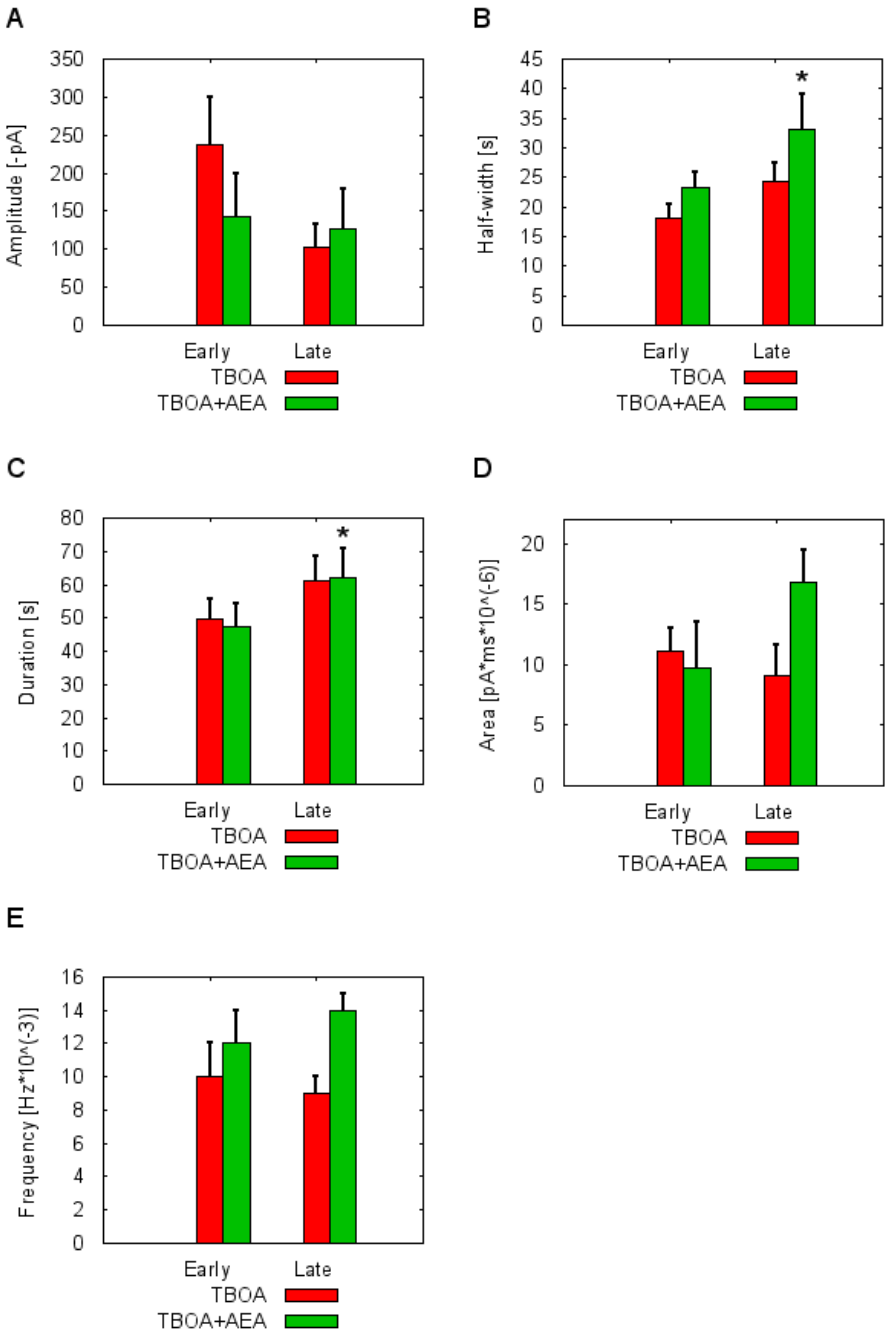

Fig 6.7 Parameters of bursts from AEA and TBOA co-treated cells (n=11 and n=6 for TBOA and TBOA+AEA respectively); V_h=-70mV.

Although AEA-treated samples in general had smaller amplitude than early TBOA-evoked bursts, statistical significance was not reached (Mann-Whitney Rank Sum Test P>0.05; A). Amplitude of early and late bursts in the presence of AEA remained comparable. Their half width (Students t-test, P = 0.021; B) and duration (Mann-Whitney Rank Sum Test, P = 0.032; C) increased in the case of AEA co-treated late bursts in comparison to early ones, but there was no statistical significance between late-TBOA and late-TBOA+AEA treatment. Despite general increase in burst area (E) and frequency (F) with respect to TBOA- alone treated cells, there was no statistical significance in any of those parameters (Students t-test P>0.05). Data presented as medians±SEMD.

6.1.2 Anandamide modulation of sPSCs of the hypoglossal motoneurons

Because TBOA-evoked bursting depends on the network synaptic activity impinging on motoneurons, I investigated if and how AEA might influence spontaneous postsynaptic activity of those cells.

Previous data have indicated that endocannabinoids can modulate glycinergic IPSCs in hypoglossal motoneurons and the release of synaptic vesicles at glutamatergic synapses (García-Morales et al., 2015; Lozovaya et al., 2011; Mukhtarov et al., 2005). Thus, I decided to perform two sets of experiment: investigating sEPSCs in the presence of bicuculline and strychnine (in order to block inhibitory synaptic transmission), and sIPSCs (in presence DNQX and D-APV in order to block glutamatergic synaptic transmission). The protocol of both experiments was analogous and is shown, as an example, for bicuculline and strychnine application in Fig 6.8.

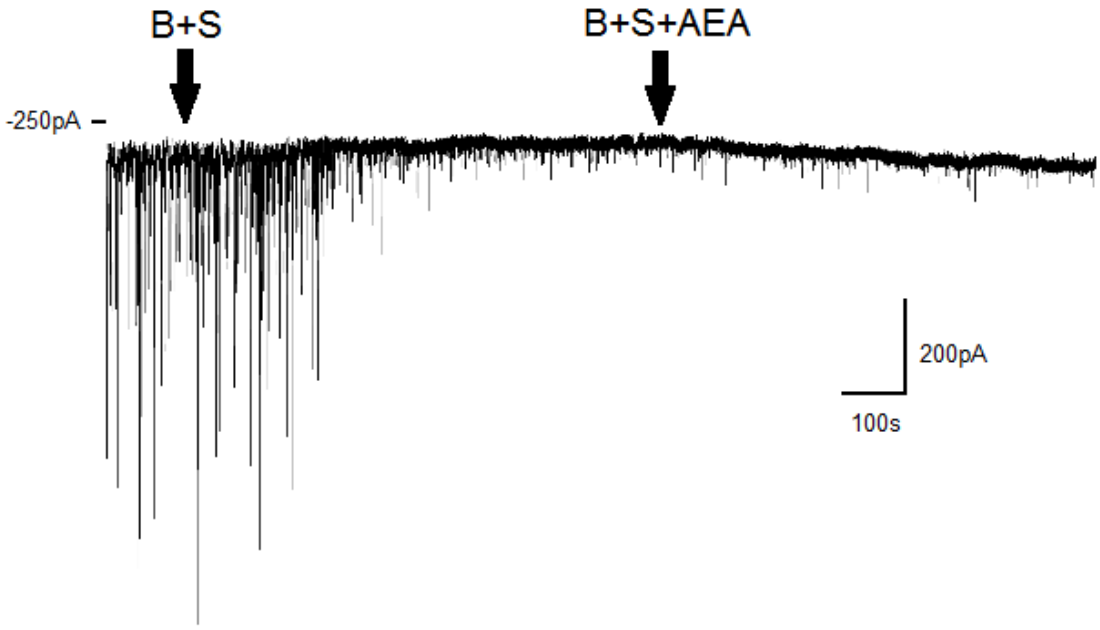

Fig 6.8 Time course of the experiment for investigating modulation of sEPSCs by AEA (V_h=-70mV).

First arrow indicates start of drug application (B-bicuculline and S-strychnine in the presented example), second arrow indicates AEA co-application.

Comparison of example traces during bicuculline and strychnine with those during bicuculline, strychnine and anandamide co-application can be seen in Fig 6.9 A. The frequency and amplitude of the sEPSCs of individual cells (dark narrow lines) and their means (red thick lines) are shown in Fig. 6.9. I observed a statistically significant decrease in the frequency of these events,

without changes in their amplitude (n=5; Paired Students t-test P<0.05; Fig 6.9 B and C respectively).

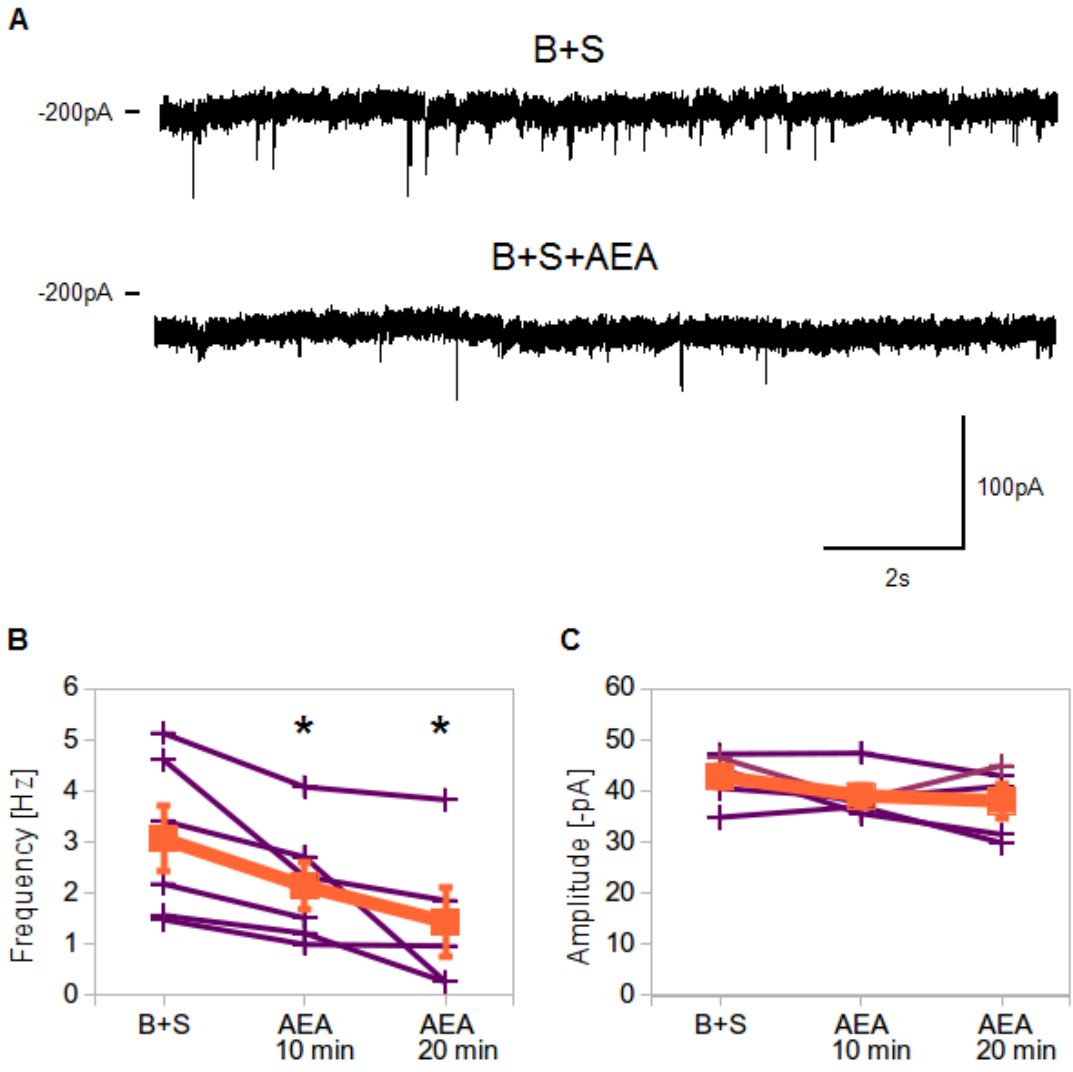

Fig 6.9 AEA modulation of sEPSCs (n=6); V_h=-70mV.

Examples of traces in the presence of bicuculline (indicated by B) and strychnine (indicated by S) at the concentrations of 10 μM and 0.4 μM respectively (top trace) and bicuculline, strychnine and AEA 10 μM (bottom trace) (A). Changes in the sEPSCs in single cells (dark lines) and means (red lines). Co-application of AEA significantly decreased frequency (Paired Students t-test P<0.05; B) but not amplitude (Paired t-test P>0.05; C) of the sEPSCs. Data presented as means±SEM.

For the co-application of DNXQ, D-APV and AEA, I collected four cells in total. In two of them, the frequency of sIPSCc slightly decreased after co-application of AEA. In the other two, I observed development of biphasic activity with periods of spontaneous activity comparable to those

before AEA application, with the irregular occurrence of high sIPSC frequency epochs when synaptic events were clustered together (Fig 6.10 A and B). The average amplitudes of sIPSCs did not significantly changed during AEA co-application (n=4, Paired t-test P>0.05; Fig 6.10 C).

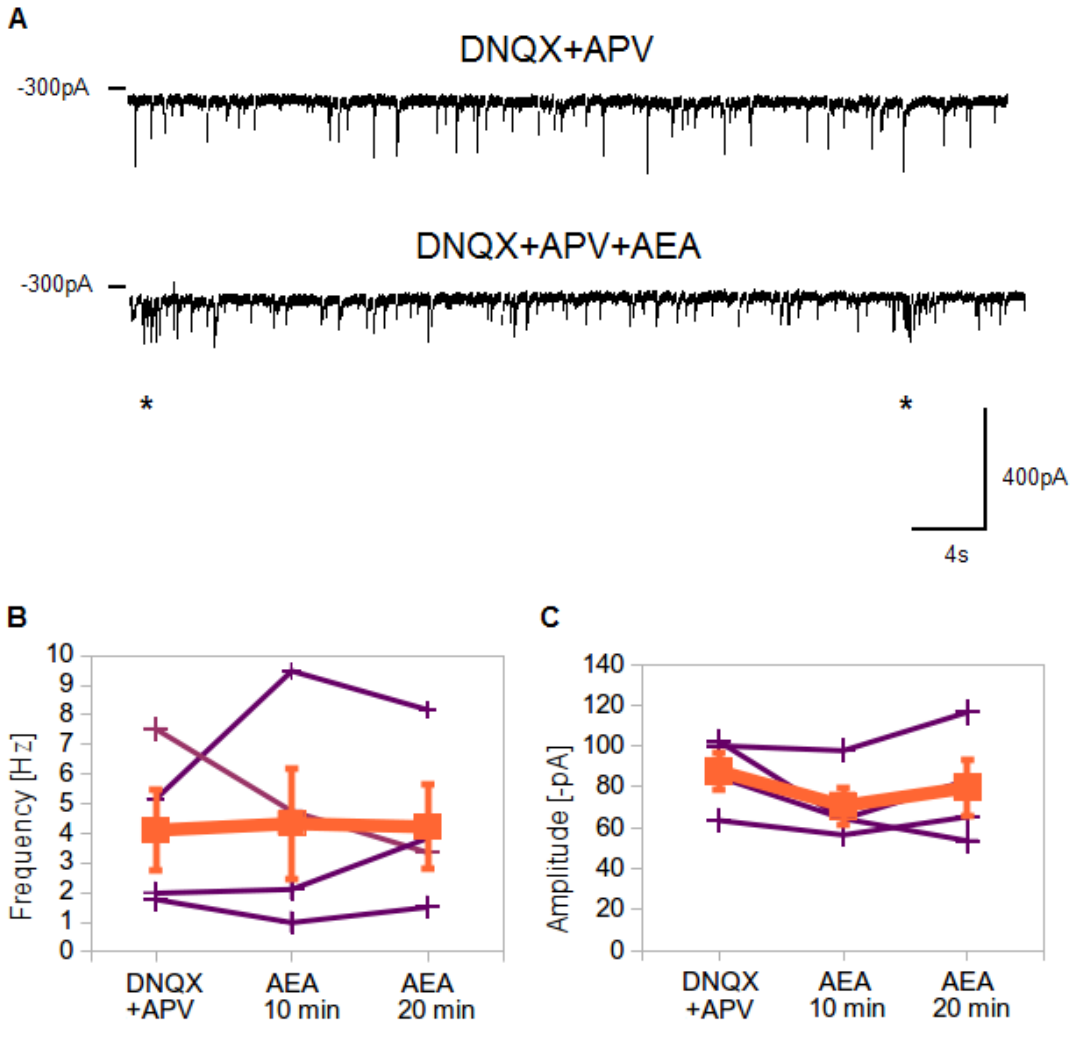

Fig 6.10 AEA modulation of sIPSCs in HMs (n=4); V_h=-70mV.

Examples of the traces in the presence of DNQX and APV (at the concentration of 10 μM and 50 μM respectively; top trace) and DNQX, APV and AEA (10 μM; bottom trace) (A). Asterisks mark regions of high frequency of sIPSC. After 10 min of AEA co-application I detected an increase in the frequency of spontaneous activity in two and a decrease in other two cells (thin dark lines). Mean value from all cells remained unchanged for 10 and 20 minutes of AEA co-application (thick red line; Paired t-test P>0.05; B). The average amplitudes of sIPSCs did not change significantly during AEA co-application (Paired t-test P >0.05; C). Data presented as means±SEM.

6.2. AM251 action on hypoglossal motoneurons

6.2.1 AM251 action on hypoglossal motoneurons during application of TBOA

My observations on the AEA effect on HMs led me to investigate what would be the effect of blocking CB1R signalling during glutamate uptake block. The experimental protocol was analogous to the one for the AEA experiments (Fig 6.4). First, I applied 10 μM AM251, a well-known CB1R inverse agonist/antagonist (Pertwee, 2006; Sink et al., 2010), and later co-applied it with 50 μM TBOA.

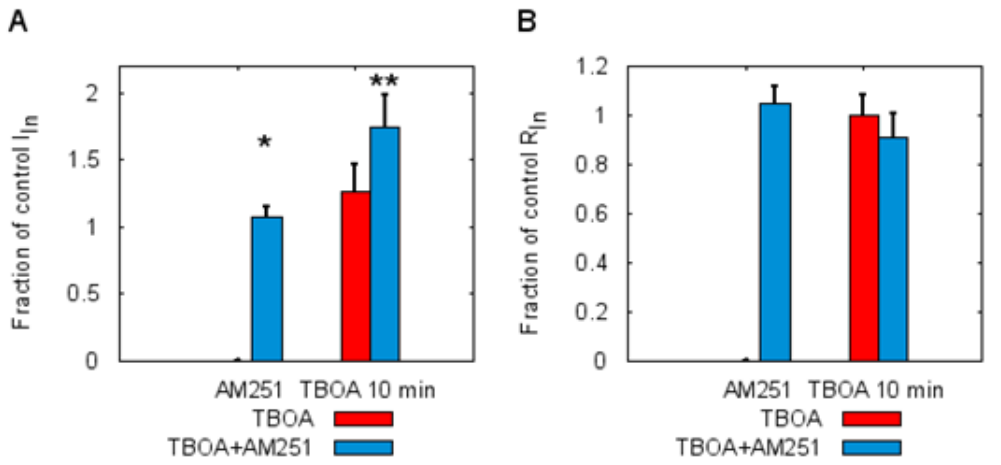

Fig 6.11 Parameters of AM251 and TBOA co-treated cells (n=15 and n=19 for TBOA and TBOA+AM251, respectively); V_h=-70mV.

Application of AM251 evoked small change in negative inward current I_{In} of the recorded cells. Co-application with TBOA evoked further increase in inward current (Paired t-test and Wilcoxon Signed Rank Test; P<0.05; A). There were no significant changes in R_{In} (Wilcoxon Signed Rank Test P>0.05; B). Data presented as means±SEM.

Application of AM251 evoked small inward current in the recorded cells (n=11; Paired t-test, P=0.038; Fig 6.11 A). Co-application of TBOA resulted in even larger inward current (Paired t-test and Wilcoxon Signed Rank Test; P<0.05). This increase in inward current was in general larger in comparison to TBOA alone, but the change was not statistically significant. No significant change in R_{In} was observed (Fig 6.11 B).

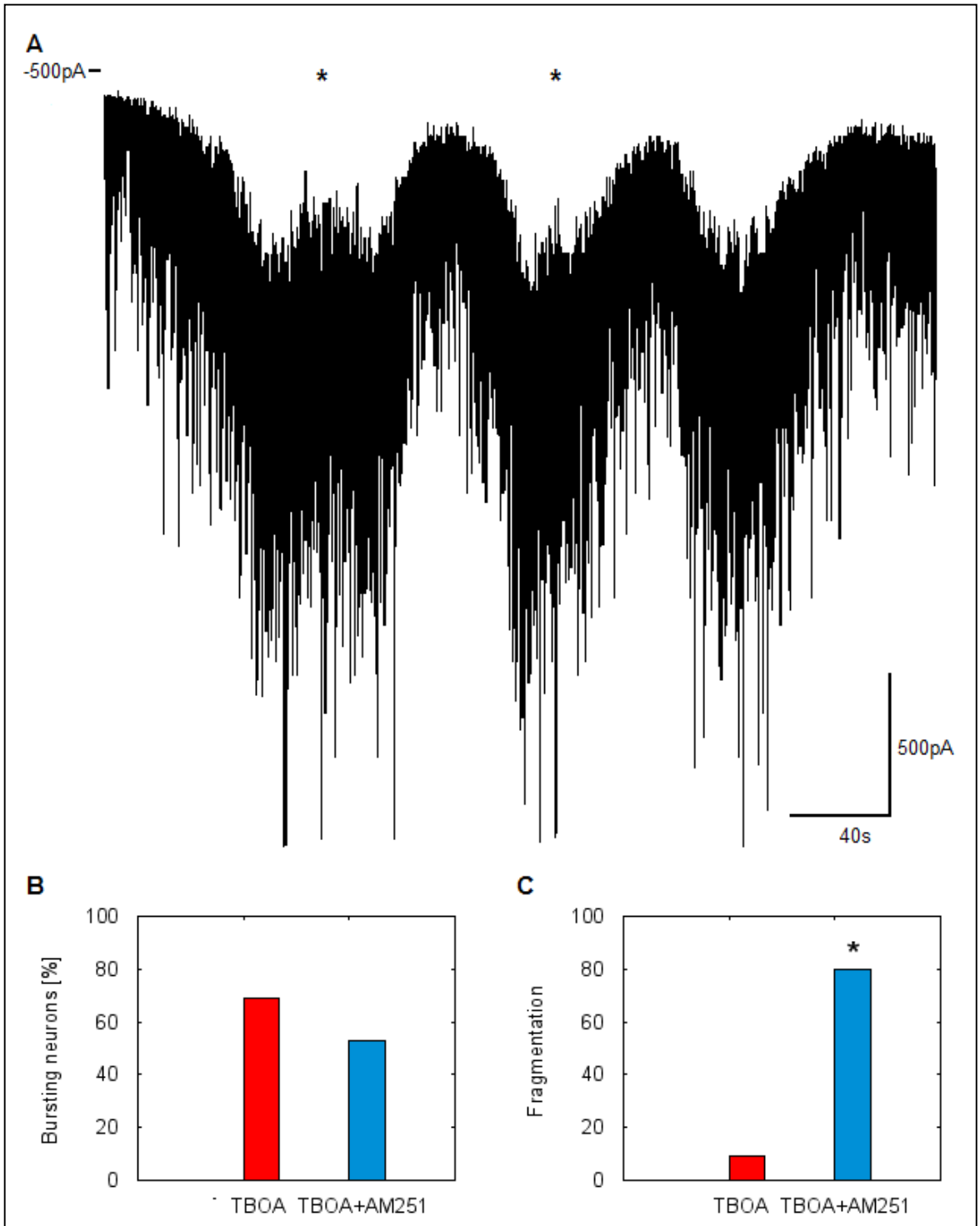

Fig 6.12 Example of TBOA- evoked bursts in the presence of AM251 (n=11, n=10 for TBOA and TBOA+AM251 respectively); V_h=-70mV.

Some of the TBOA-evoked bursts in the presence of AM251 became fragmented (fragmented bursts indicated by asterisks; A). During co-application of AM251 with TBOA I observed a slight, but statistically insignificant drop in burst occurrence (Chi-squared test P>0.05; B). The fragmentation of the bursts occurred in the majority of the bursting cells (Chi-squared test P=0.001; C).

An example of TBOA-evoked bursting in the presence of AM251 is shown in Fig 6.12 A. For TBOA co-application with AM251, while there was no statistically significant decrease in burst occurrence (Fig 6.12. B). A striking change in some burst quality emerged as bursting cells showed burst fragmentation (Fig 6.12 C). Bursts with such alteration in shape were present in the majority of bursting neurons co-treated with the CB1R antagonist and the change was statistically significant in comparison to TBOA alone (Chi-squared test P=0.001; Fig 6.12 C).

When I compared burst parameters of TBOA with AM251 and TBOA alone, I observed that there was an increase in the burst amplitude in case of AM251 co-treatment, but this change was statistically significant only in the case of late bursts (n=10; Mann-Whitney Rank Sum Test, P = 0.006; Fig 6.13 A). There was no statistically significant change in half-width, duration or frequency (Fig 6.13 B, C and E, respectively). The area of the bursts increased significantly in the case of late bursts (Mann-Whitney Rank Sum Test, P = 0.013; Fig 6.13 D).

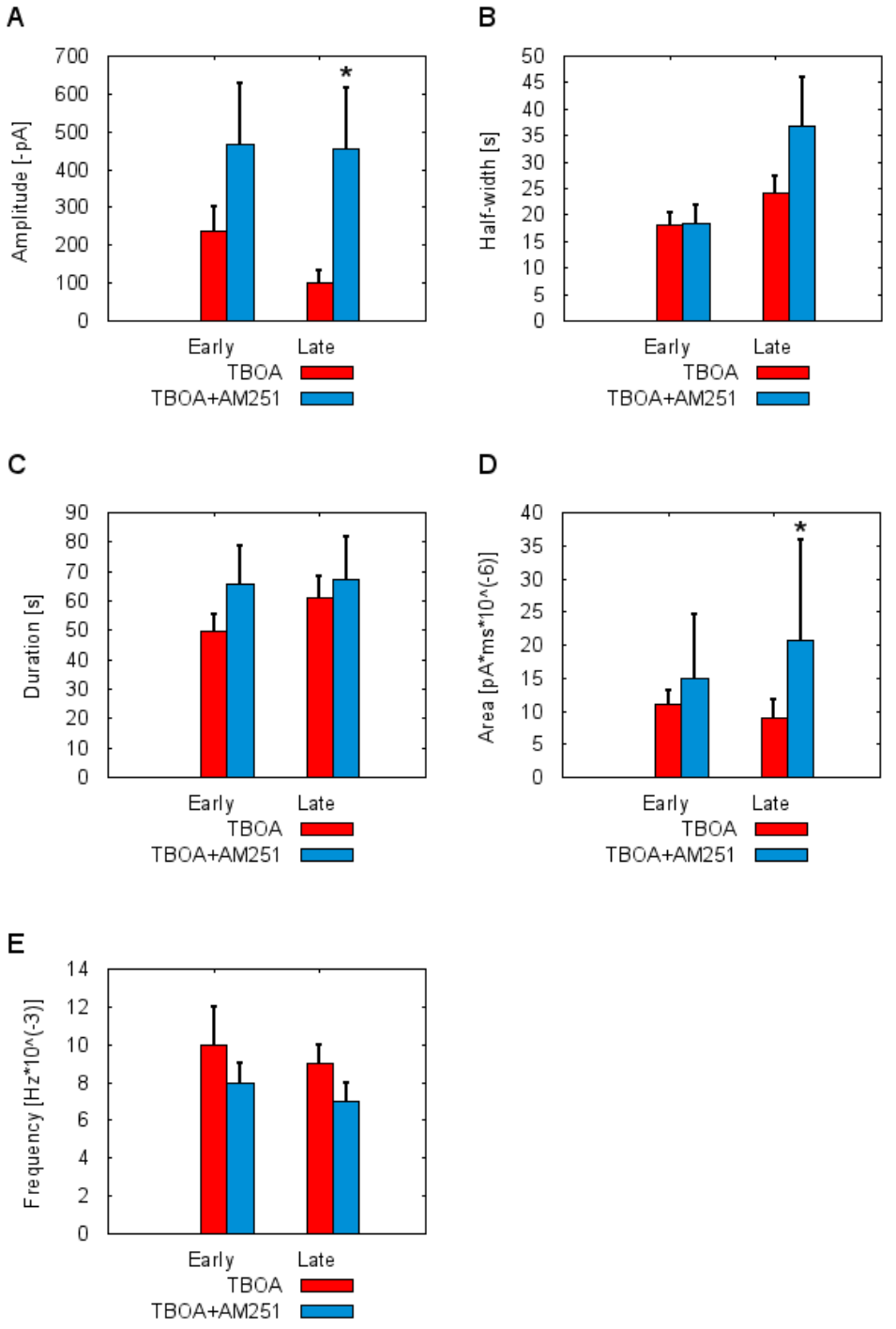

Fig 6.13 Parameters of bursts in AM251 and TBOA co-treated cells. (n=11, n=10 for TBOA and TBOA+AM251 respectively); V_h=-70mV.

The amplitude of TBOA-evoked bursts increased during AM251 co-application. Change in amplitude was significant only in case of late bursts (Mann-Whitney Rank Sum Test, P = 0.006; A). I did not observe any statistically significant change in case of the half-width (B) and duration (C) (Student t-test P>0.05). Area of the bursts significantly enlarged during AM251 co-application in the case of late bursts (Mann-Whitney Rank Sum Test, P = 0.022; D). There was no change in bursts frequency (Student t-test P>0.05; E). Data presented as medians±SEMD.

6.2.2 AM251 effect on sPSCs of hypoglossal motoneurons

I further investigated how CB1R inhibition might affect spontaneous synaptic activity. The protocol of the experiment was analogous to the one with AEA (Fig 6.8). Examples of sPSCs in the presence of DNQX and APV (top trace) and DNQX, APV and AM251 (bottom trace) are presented in Fig 6.14 A. Co-application of AM251 with DNQX and APV did not significantly change both amplitude and frequency (Paired t-test P>0.05; Fig 6.14 B and C, respectively) of sIPSCs.

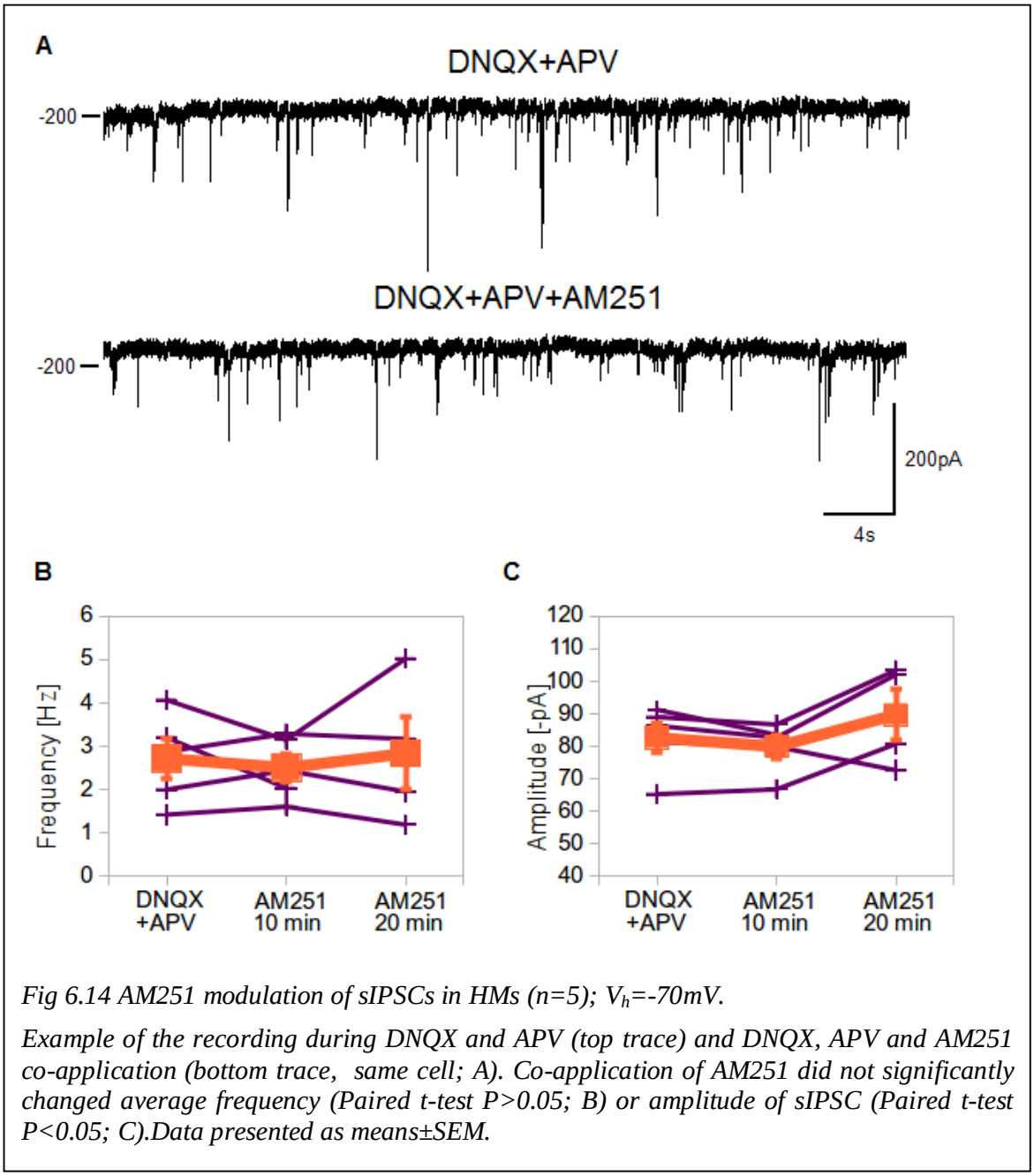

Fig 6.14 AM251 modulation of sIPSCs in HMs (n=5); V_h=-70mV.

Example of the recording during DNQX and APV (top trace) and DNQX, APV and AM251 co-application (bottom trace, same cell; A). Co-application of AM251 did not significantly changed average frequency (Paired t-test P>0.05; B) or amplitude of sIPSC (Paired t-test P<0.05; C).Data presented as means±SEM.

Lack of time prevented a systematic study of co-application of bicuculline and strychnine with AM251, however, the example illustrated here provided some interesting preliminary observations. Traces recorded in the presence of bicuculline (B) and strychnine (S) (top trace) and bicuculline, strychnine and AM251 (bottom trace, same cell) are illustrated in Fig 6.15 A. First of all, frequency and aplitude of sEPSCs after AM251 application increased vs. control in bicuculline and strychnine solution (Fig 6.15 B and C). Further experiments are needed to confirm if this

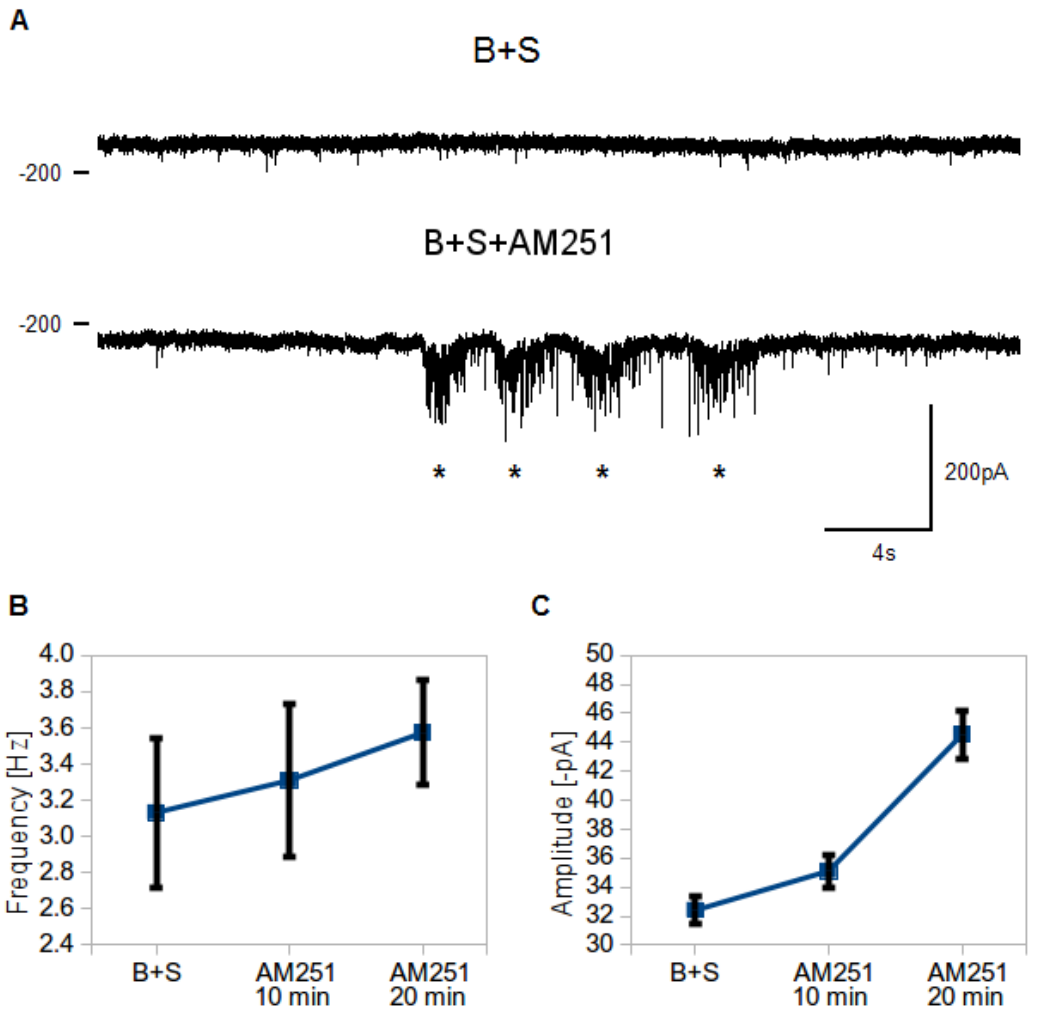

Fig 6.15 AM251 modulation of sEPSCs in HMs (n=1); V_h=-70mV.

Recording with bicuculline (B) and strychnine (S) application (top trace) later co-applied with AM251 (bottom trace). Under block of inhibitory transmission AM251 caused occurrence of small burst-like activity (marked by asterisks; A). After AM251 co-application I observed increase in both frequency (B) and amplitude (C) of sEPSCs. Data presented as means±SEM.

change is statistically significant in larger population of cells. After 10 min of AM251 co-application, a series of events resembling bursts occurred (Fig 6.15 A), although they were much smaller than TBOA-evoked bursts with amplitude of around -35 pA, and clustered in series of four to five events occurring one after another. The parameters of this phenomenon are presented in Table 6.1.

Table 6.1 Parameters of AM251 evoked bursts in the presence of bicuculline and strychnine

Amplitude	-35.63±3.977 pA
Duration	2.26±0.153 s
Area	0.11±0.005 10^(-6)*pA*ms

6.3. Bicuculline and strychnine co-application with TBOA

After investigating the responses of HMs in the presence of TBOA and CB1R drugs, it seemed interesting to find out if the observed changes in responses were due to inhibitory or excitatory transmission modulation. In order to answer this question, I performed a series of experiments in which I first co-applied bicuculline and strychnine together with CB1R agonist or antagonist (as in the protocol of the previous experiment represented in Fig 6.4) and then co-applied them with TBOA.

6.3.1 Effects of AEA on TBOA-evoked bursting in the presence of bicuculline and strychnine

In the case of bicuculline and strychnine co-application with AEA, I did not observe significant changes in inward current in comparison to bicuculline and strychnine alone. Co-application of TBOA evoked inward current only in the fraction of the cells, thus statistical significance has not been reached (Paired t-test $P>0.05$; Fig 6.16 A). In case of input resistance, AEA co-application with bicuculline and strychnine evoked a significant increase in this parameter (Paired t-test $P=0.036$; Fig 6.16 B), with respect to control value. There were no statistically significant differences during TBOA co-application with bicuculline, strychnine and AEA.

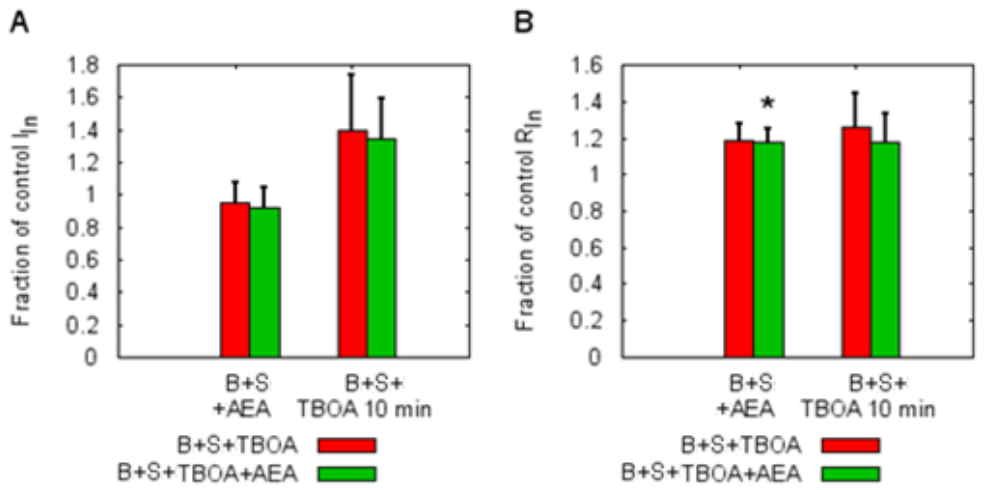

Fig 6.16 Bursting parameters of TBOA (n=8) and TBOA with anandamide- treated slices in the presence of bicuculline (B) and strychnine (S) (n=7); V_h=-70mV.

In case of I_{In} I did not observe significant change in the presence of AEA, bicuculline and strychnine when compared to bicuculline and strychnine alone (Paired t-test P>0.05; A). There was a significant increase in R_{In} with respect to control after application of bicuculline, strychnine and AEA (Paired t-test P = 0.036; B). No changes were observed in I_{In} and R_{In} in case of TBOA co-application with B+S (Wilcoxon Signed Rank Test P>0.05). Data presented as means±SEM.

One example of TBOA-evoked bursting in the presence of bicuculline and strychnine (top trace), and bicuculline, strychnine and AEA (bottom trace) is presented in Fig 6.17 A. Burst occurrence in the presence of bicuculline and strychnine did not change between TBOA and TBOA co-treated with AEA (Chi squared test *P*>0.05; Fig 6.17 B), but due to the small sample number, this issue should be verified with a larger number of experiments. Nonetheless, when I compared burst occurrence between TBOA and AEA (with 33.33% of bursting neurons; *n*=18) and TBOA, AEA, bicuculline and strychnine (with 100% of bursting neurons; *n*=7; Chi-squared test *P*= 0.0027; graph not shown), there was a statistical significance. In addition, bursts elicited by TBOA in the presence of bicuculline, strychnine and AEA became fragmented (Chi-squared test *P*=0.0384; Fig 6.17 A, C).

In terms of bursting properties during co-application of bicuculline, strychnine and AEA, TBOA evoked bursts did not change their amplitude, or half-width (Mann-Whitney Rank Sum Test *P*>0.05; Fig 6.18 A and B respectively). Late bursts in AEA co-treated cells were longer in compare to TBOA with bicuculline and strychnine alone (Mann-Whitney Rank Sum Test *P*=0.018; Fig 6.18 C). In terms of area and frequency, bursts were comparable to those in TBOA with bicuculline and

strychnine (Students t-test *P*>0.05; Fig 6.18 D and E, respectively).

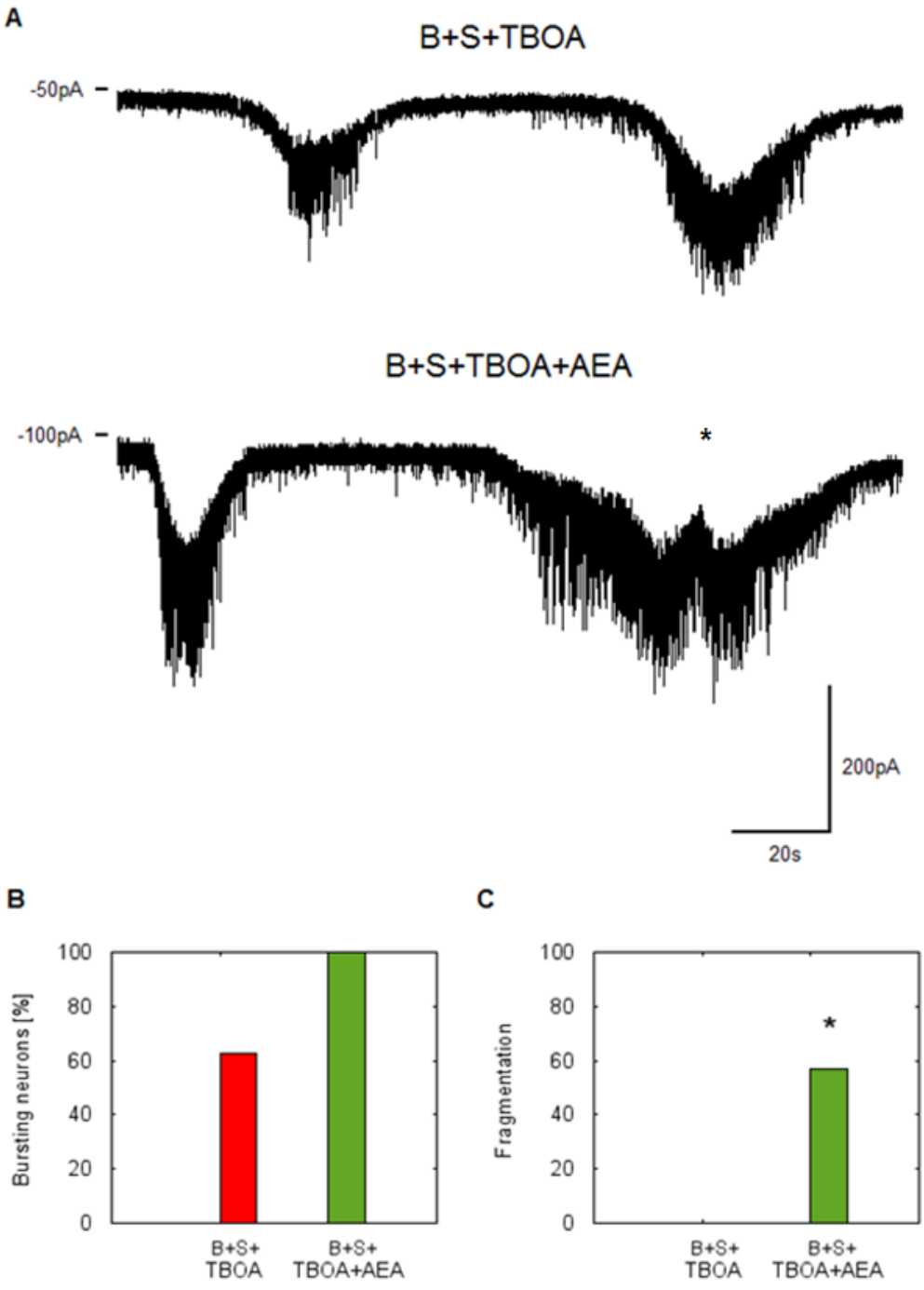

Fig 6.17 TBOA and TBOA+AEA bursts in the presence of bicuculine and strychnine (n=8, n=7 for TBOA+B+S and TBOA+B+S+AEA respectively); V_h=-70mV.

Examples of TBOA-evoked bursts in the presence of bicuculline and strychnine without (top trace) and with AEA (bottom trace, asterisk marks burst with fragmentation; A). There was no statistically significant difference between TBOA and TBOA with AEA for burst occurrence (Chi-squared test P>0.05; B). During co-application of AEA with TBOA and B+S many cells generated fragmented bursting (Chi-squared test P= 0.0384; C).

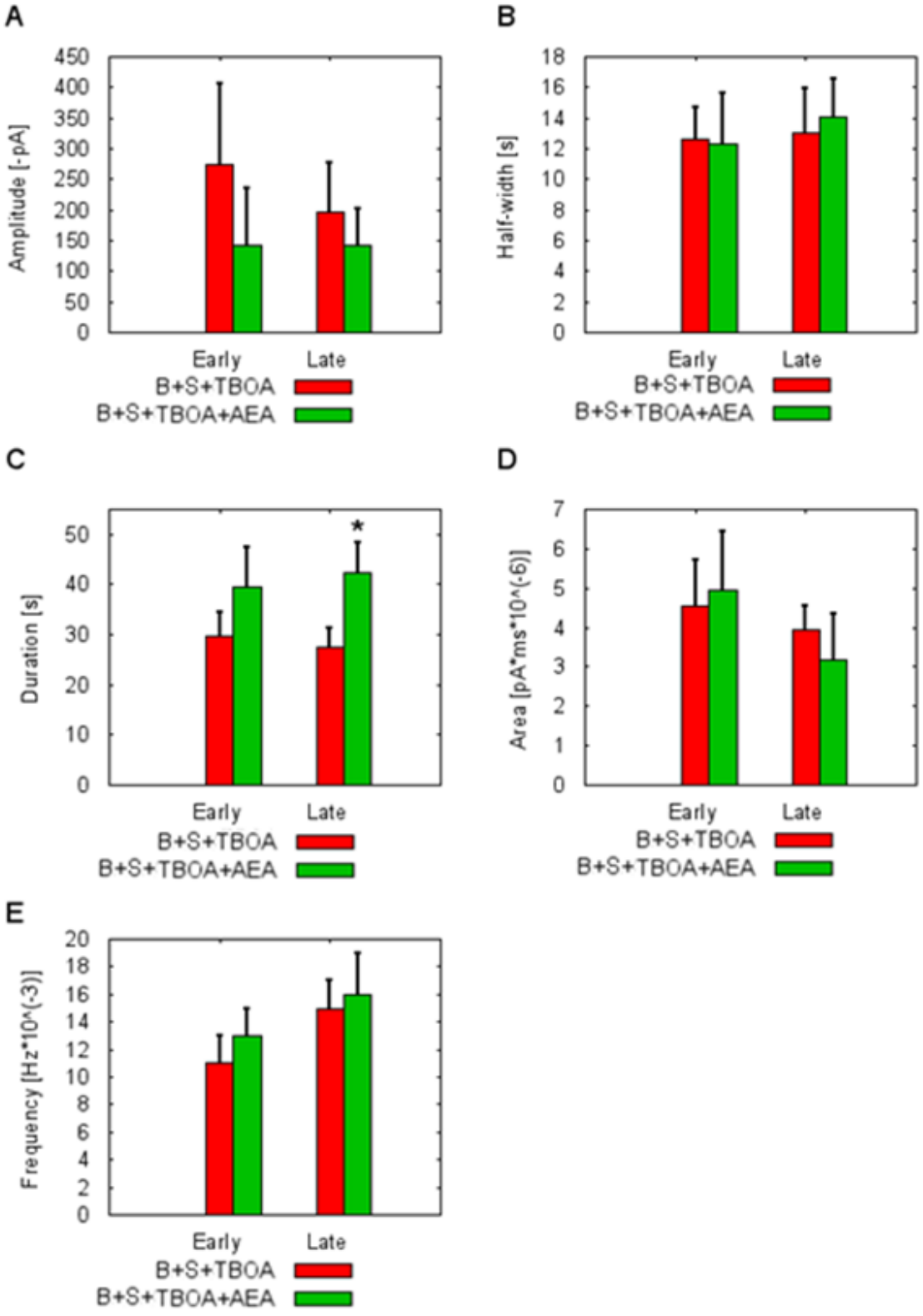

Fig.6.18 Bursting parameters of TBOA (n=5) and TBOA with anandamide (n=7) treated slices in the presence of bicuculline and strychnine (V_h=-70mV).

There were no significant changes in amplitude (A) and half width (B) of the bursts (Mann-Whitney Rank Sum Test P>0.05). In the presence of AEA, TBOA-evoked late bursts became longer (Mann-Whitney Rank Sum Test P= 0.018; C). Area (D) and frequency (E) of the bursts were not significantly changed (Students t-test P>0.05). Data presented as medians±SEMD.

6.3.2 Effects of AM251 on TBOA-evoked bursting in the presence of bicuculline and strychnine

Co-application of TBOA with bicuculline and strychnine or bicuculline, strychnine and AM251 evoked inward current, but this change after 10 min did not reach statistical significance (Fig 6.19 A). I did not observe any significant changes in input resistance (Paired t-test $P>0.05$; Fig 6.19 B).

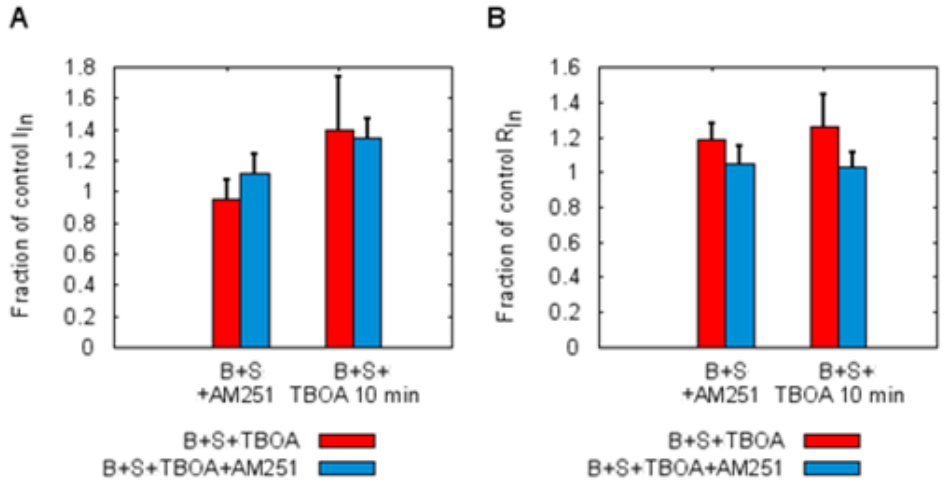

Fig 6.19 Bursting parameters of TBOA (n=8) and TBAO with AM251 (n=7) treated slices in the presence of bicuculline and strychnine (V_h=-70mV).

10 min co-application of TBOA evoked inward current, but the change was not statistically significant (Paired t-test P>0.05; A). There were no significant changes in R_{In} (Paired t-test P>0.05; B). Data presented as means±SEM.

Fig 6.20 A illustrates an example of TBOA-evoked burst in the presence of bicuculline, strychnine and AM251. I did not observe any significant changes in the TBOA-evoked burst occurrence after co-application of bicuculline, strychnine and AM251 (Fig 6.20 B), but I observed fragmentation of the bursts (Fig 6.20 C). Burst parameters during co-application of AM251 remained comparable to TBOA, bicuculline and strychnine co-treated cells (Mann-Whitney Rank Sum Test and Student t-test $P>0.05$; Fig 6.21 A-E).

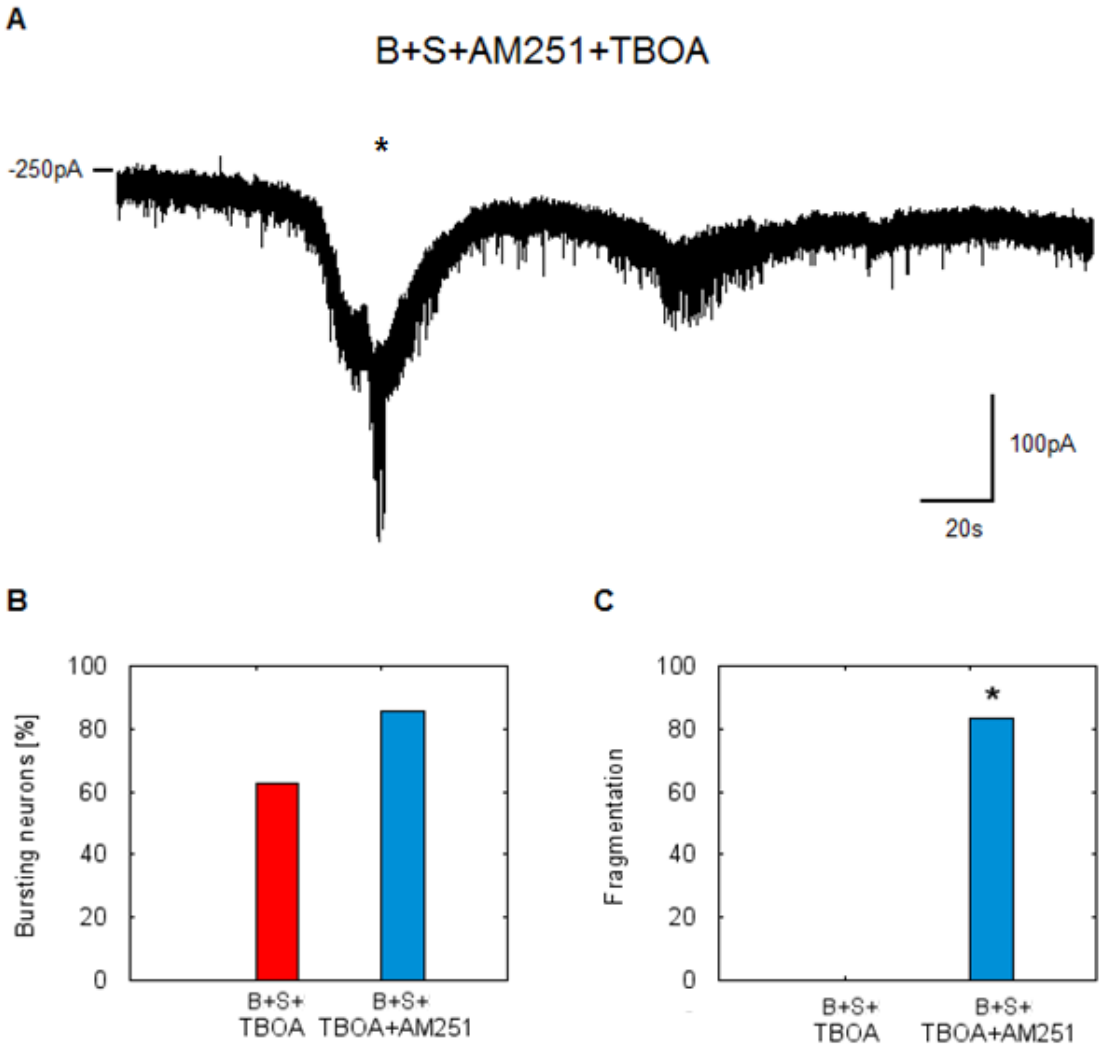

Fig 6.20 TBOA and TBOA with AM251 bursts in the presence of bicuculline and strychnine (n=8 and n=7 respectively); V_h=-70mV.

Example of the bursting evoked by TBOA in the presence of bicuculline, strychnine and AM251 (A). I did not observe any significant changes in the burst occurrence during AM251 co-application (Chi-square P>0.05; B). Fragmentation of the TBOA-evoked bursts in the presence of AM251 during bicuculline and strychnine co-applicatin was still present (Chi-square P=0.0057; C).

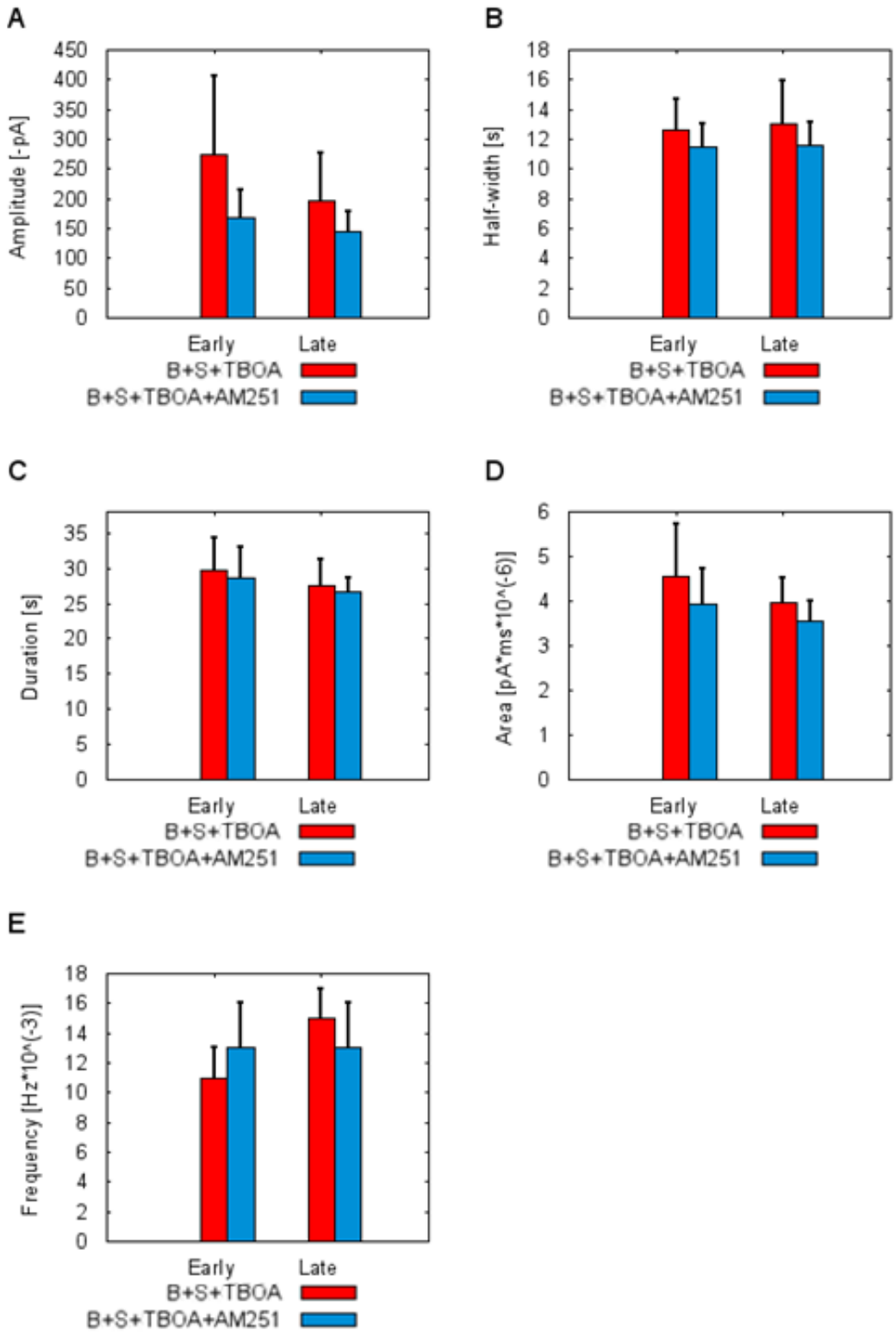

Fig 6.21 Bursting parameters of TBOA (n=5) and TBAO with AM251-treated slices in the presence of bicuculline and strychnine (n=6) (holding potential=-70mV).

There were no significant changes in burst parameters between TBOA+B+S and TBOA+B+S+AM251, including amplitude (Mann-Whitney Rank Sum Test P>0.05; A), half width (Mann-Whitney Rank Sum Test P>0.05; B), duration (Mann-Whitney Rank Sum Test P>0.05; C), area (Student t-test P>0.05; D) and frequency (Mann-Whitney Rank Sum Test P>0.05; E). Data presented as medians±SEMD.

6.4. Change in CB1R expression in the brainstem region

After investigating the functional action of CB1R on HMs, I become interested if the exposure to excitotoxic conditions can alter the expression of these receptors in the brainstem region containing such motoneurons.

In the view of technical difficulties in obtaining one hypoglossal nucleus in isolation, I isolated the whole medullary region containing this area. After isolating samples from six rats, I incubated them in six different conditions in parallel. In particular, in addition to untreated controls, I incubated brainstems in TBOA, TBOA co-applied with either AEA or AM251, and AM251 or AEA alone. In the protocols of TBOA co-application with CB1R agonists or antagonists, the latter were pre-applied for 10 min in order to activate/deactivate the CB1Rs before the excitotoxic insult and then co-applied with TBOA.

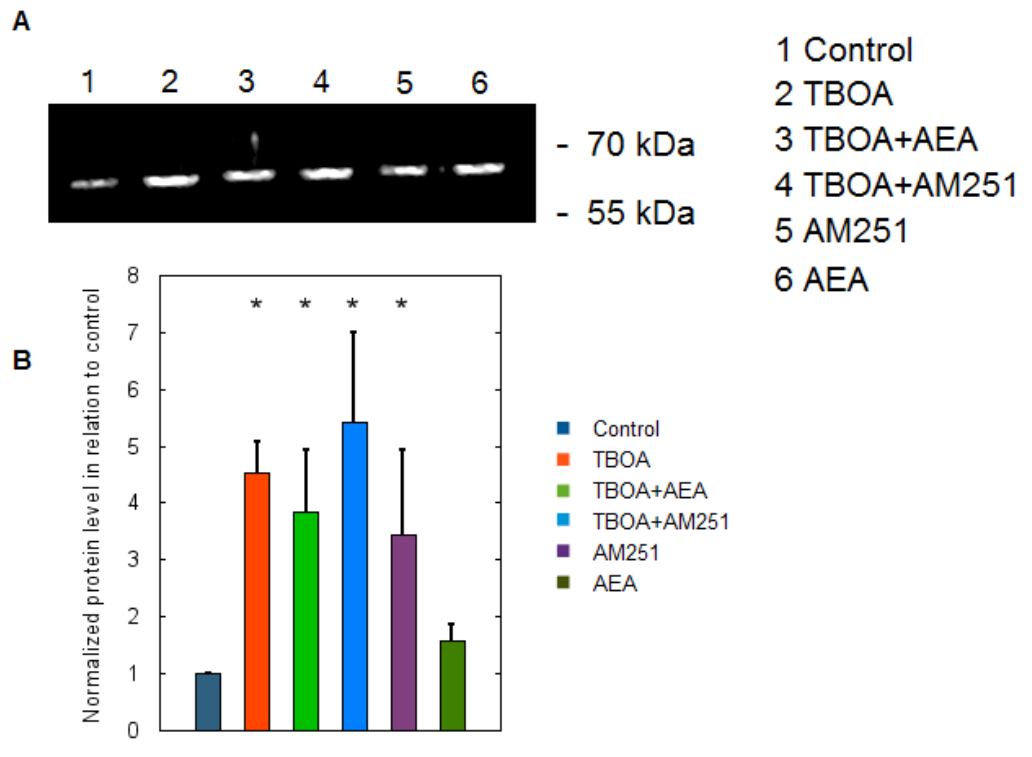

Fig 6.22 Changes in CB1R expression in the brainstem region of the rat during excitotoxic stress (n=4).

Comparison of CB1R expression levels during different treatments of the brainstem medullary regions (A). We have observed a clear CB1R protein level increase in the presence of TBOA in different drug co-application variants, and in AM251 alone (data normalized to β-actin; Mann-Whitney Rank Sum Test, P<0.05; B). Data presented as means±SEM.

Results from Western Blot analysis of these samples showed a significant increase in CB1R expression in the brainstems incubated with TBOA alone or co-incubated with AEA or AM251 (Mann-Whitney Rank Sum Test, $P<0.05$; Fig 6.22 A and B). Moreover, incubation with AM251 alone was sufficient to trigger a CB1R expression increase in a relatively short period of time (4 h) (Fig 6.22 B).

6.5. Viability of the hypoglossal motoneurons

Previous results from our laboratory reported that inhibition of glutamate uptake can cause cell damage and even death especially in those motoneurons with intense bursting (Cifra et al.,

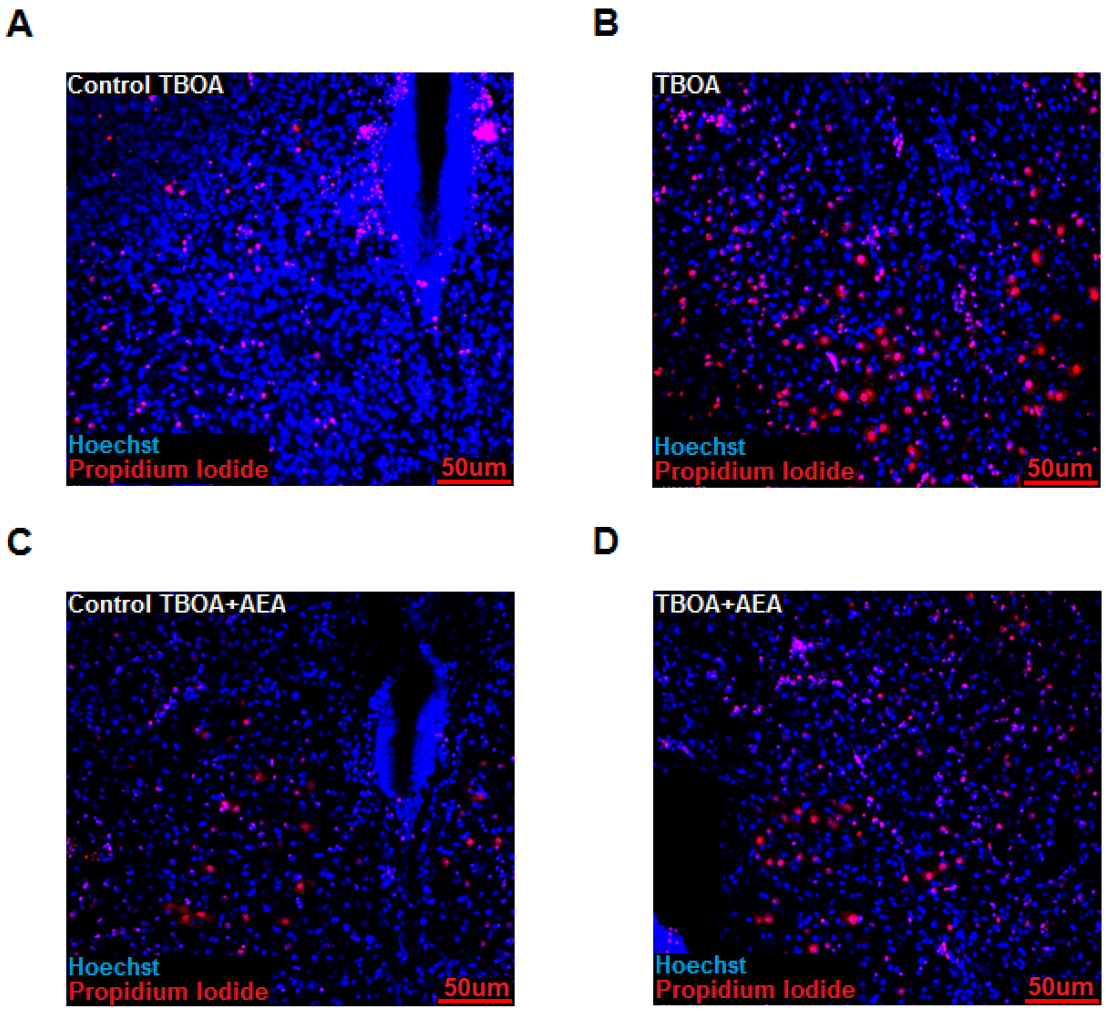

Fig 6.23 Comparison of cellular viability after TBOA and TBOA+AEA treatment

Comparison of PI staining during control for TBOA (A) and TBOA (B) and control for TBOA+AEA (C) and TBOA+AEA (D) incubation (4h).

2011a, 2009). Since I observed that modulation of endocannabinoid system has important effects on the excitotoxic stress responses in HM and enhanced CB1R protein expression in the medullary region containing the hypoglossal nuclei, I wondered if activation of CB1R by anandamide might protect cells from damage caused by TBOA over a relatively short time frame.

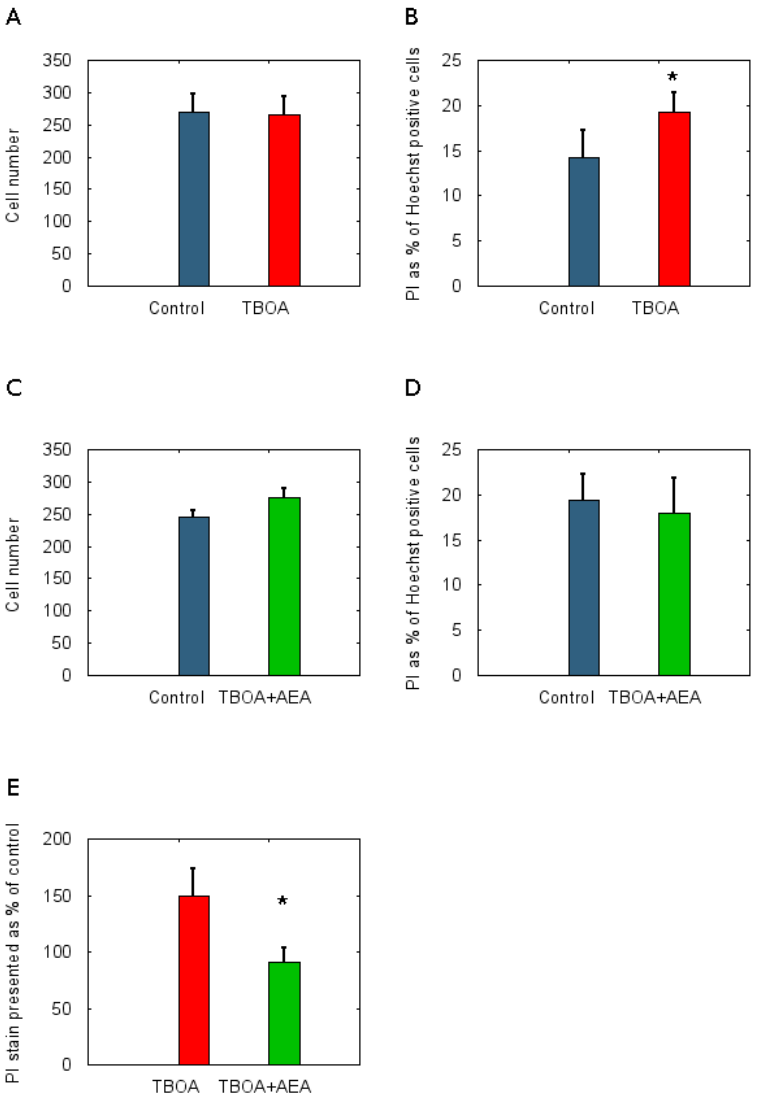

Fig 6.24 Quantification of cellular viability after TBOA or TBOA+AEA treatment (n=5; indicates number of slices).

I observed no change in total cell number in the treated slices, measured as Hoechst 33342-positive cells in TBOA alone or in TBOA+AEA (Paired t-test P>0.05; A and C). 4h of TBOA incubation significantly increased percentage of PI-positive cells expressed, as a percent of Hoechst-positive cells (Paired t-test P=0.010; B). During co-application of AEA and TBOA this effect was abolished (Paired t-test P>0.05; D). Difference between TBOA and TBOA+AEA treated slices (expressed as a percentage of control PI-positive cells) was significant (Students t-test P= 0.047; E). Data presented as means±SEM.

For this purpose, a viability assay was carried out by incubating brainstem slices in AEA (10 μM) for 10 min and subsequently with AEA and TBOA (50 μM) for further 4h. Other samples were kept in Krebs solution or just TBOA (50 μM) in Krebs for the same time period. Slices were stained with PI, to visualize damaged cell nuclei, and Hoechst 33342 for counter-staining. These samples were analysed with confocal microscopy (Fig 6.23 A-D). As expected, TBOA alone increased the fraction of PI stained nuclei without changing the average Hoechst positive cell number (n=5, where n is number of slices treated; Student t-test P=0.010; Fig 6.24 A and B). However, when AEA was co-applied with TBOA there was no significant change in either PI positive or Hoechst positive cells in comparison to control conditions (n=5; Fig 6.24 C and D). When I compared the differences between PI staining in TBOA and TBOA+AEA experiments, I observed significant decrease in PI positive cells during co-application with AEA (Student t-test P= 0.047; Fig 6.24 E) suggesting a protective effect.

6.7 A summary

A summary of the main experimental observations obtained is presented below in Table 6.2.

Table 6.2 Main observations on the role of endocannabinoids on TBOA-induced busting and excitotoxicity.

Electrophysiology - Drug treatment	***Endocannabinoid agents***	***Burst electrophysiological characteristics***
TBOA	AEA	Decrease in burst occurrence
		Abolished difference in the amplitude between early and late TBOA-evoked bursts
		Increase in the half-width and duration of the late bursts
	AM251	Occurrence of the burst

		fragmentation
		Increase in the amplitude and area of the late bursts
TBOA+bicuculline+strychnine	AEA	Occurrence of the burst fragmentation
		Increase in the late burst duration
Drug treatment	***Endocannabinoid agents***	***Synaptic events***
bicuculline+strychnine	AEA	Decrease in the frequency of sEPSCs
	AM251	Emergence of burst-like activity
DNXQ+APV	AEA	Emergence of biphasic activity (not all cells)
Molecular biology: Western blot – Drug treatment	***Endocannabinoid agents***	***Main observations***
TBOA		Increase in CB1 receptor expression
	AEA	Increase in CB1 receptor expression
	AM251	Increase in CB1 receptor expression
No TBOA	AEA	No change in CB1 receptor expression
No TBOA	AM251	Increase in CB1 receptor

		expression
Cell viability assay	***Endocannabinoid agents***	***Main observations***
TBOA		Increase in PI staining
	AEA	Decrease in PI staining vs. TBOA treatment

7. Discussion

7.1 Hypoglossal motoneurons as a model of excitotoxic stress

Coronal medullary slices offer a valuable system for studying pathological processes which can lead to the development of life-threatening diseases like ALS. This experimental preparation is relatively easy to obtain. Its small size allows for tight control of experimental parameters and rapid change of drug and extracellular solution composition, while preserving most of the dendritic tree of the neuronal networks (see section 3.2) (Sharifullinaand Nistri, 2006; Nüñez-Abades et al., 1994). Hypoglossal motoneurons (HMs) are of particular interest for studies on the early mechanisms involved in the development of ALS. These cells are severely damaged during the course of the disease leading to major problems in swallowing, talking and breathing (Atsumi and Miyatake, 1987; DePaul et al., 1988, 1988; Janzen et al., 1988; Langmore and Lehman, 1994; Rosen, 1978). Specific properties of HMs like their calcium permeable AMPA receptors (Essin et al., 2002; Shaw et al., 1999; Van Damme et al., 2002) and diminished calcium buffering capacity (Alexianu et al., 1994; Appel et al., 2001; Lips and Keller, 1998; Palecek et al., 1999) make them particularly vulnerable to excitotoxic damage, one of the proposed primary sources of pathological insult occurring during the early onset of ALS (Redler and Dokholyan, 2012). One critical disadvantage of this preparation is its survival for a short time frame (a few hours). Afterwards, the spontaneously occurring deterioration of the cells within the nucleus makes them unsuitable for scientific investigations and, thus, limits the possibility for performing long experiments (Cifra et al., 2011a, 2011b).

One of the possible protective mechanisms against excitotoxicity in hypoglossal motoneurons may be endocannabinoid signalling. Numerous studies have connected the endocannabinoid system with modulation of neuronal signalling, especially with its effect on neurotransmitter release from the nerve terminals (Di Marzo et al., 1998; Köfalvi et al., 2005; Pertwee and Ross, 2002; Schlicker and Kathmann, 2001). The well-known mode of action elicited by endocannabinoids is inhibition of cAMP production via activation of the CB1 receptor (Chen et al., 2010; Chevaleyre et al., 2007; Dalton et al., 2009; Howlett et al., 2010) and modulation of ionic channel activity (in particular Ca^{2+} and K^{+} channels; see section 3.3.7), which depress the release of neurotransmitters from presynaptic sites. Earlier reports pointed out that the endocannabinoid system elicits neuroprotection during pathological insults like traumatic brain injury or brain ischemia (Mechoulam et al., 2002a, 2002b; van der Stelt et al., 2002; Veldhuis et al., 2003;

Zogopoulos et al., 2013). Therefore, it seemed worth investigating whether this signalling pathway could function as a protective mechanism during excitotoxic stress in the hypoglossal nucleus. The principal finding of the work presented here is a characterization of how the endocannabinoid system influences HMs in a pathological model caused by glutamate uptake inhibition induced by TBOA.

7.2 Endocannabinoid system moderate responses of hypoglossal motoneurons to TBOA

Very modest changes in passive properties of the recorded cells (baseline current level and input resistance) found during stimulation or inhibition of CB1Rs in our preparation might suggest that any tonic activity exerted by CB1R signalling on HMs is slight, at least in the reduced slice preparation. This is in accordance with the proposed 'on demand' mode of action of the endocannabinoid system in the CNS. Earlier studies of endocannabinoid signalling indicate that the primary target of CB1Rs are presynaptic terminals (Alger, 2002; Freund et al., 2003; Hoffman and Lupica, 2000; Kreitzer and Regehr, 2002; Mackie, 2006; Wilson and Nicoll, 2001). In such a situation, cells affected by cannabinoid signalling should be premotoneurons innervating HMs, so that minimal changes in passive properties of HMs would be expected.

Previous experiments done in our laboratory suggest that the occurrence of strong bursting is a predictor of subsequent cell damage (Cifra et al., 2009; Sharifullina and Nistri, 2006). Moreover, drugs which suppress bursting facilitate cell survival in viability assays (Cifra et al., 2011a, 2011b, 2009; Sharifullina and Nistri, 2006). Results from the aforementioned studies indicate that bursting can be treated as an *in vitro* readout for testing the efficiency of neuroprotective drugs to inhibit excitotoxicity and pathological discharges of brainstem motoneurons (Cifra et al., 2011a, 2011b). In my work, I observed that co-application of AEA together with TBOA significantly decreased the occurrence of bursting in HMs. One important indication of the role of endocannabinoid system during bursting in HMs came from comparing early and late bursts. In fact, in the case of bursting produced by applying TBOA alone, I observed a significant drop in the amplitude of late bursts versus early ones. This difference was abolished when AEA was co-applied with TBOA and the median of the amplitude of early bursts was visibly lower in comparison to those in TBOA alone, although we did not reach statistical significance. In contrast, AM251, a CB1R antagonist, when co-applied with TBOA, significantly *increased* the median burst amplitude of late bursts in comparison to TBOA alone. In case of early bursts median amplitude was elevated, but statistical significance was not reached.

As mentioned in the Introduction (see section 3.3.2), the synthesis of endocannabinoids is triggered by postsynaptic depolarization and rise in the intracellular calcium that accompany this event. Large, prolonged depolarizations like the bursts evoked by TBOA might potentially stimulate endocannabinoid production and release from postsynaptic neurons in a time-dependent fashion. This is supported by my observation that early bursts evoked by TBOA alone were generally larger than late ones. This change was probably due to activation by endogenous endocannabinoids of presynaptically localized CB1Rs which negatively moderated late burst amplitude. In contrast, when we flooded the slice with *AEA prior to* TBOA application, the difference between early and late bursts was abolished, presumably due to pre-activation of CB1Rs. Moreover, inhibition of CB1R signalling with the antagonist AM251 *increased* burst amplitude, thus confirming that CB1R activation was necessary to obtain the time-related reduction in the burst amplitude detected in the recordings with TBOA alone.

7.3 Modulation of endocannabinoid signalling can lead to disturbance in the network which may result in burst fragmentation

An interesting observation was that, during co-application of AM251 and TBOA, some bursts showed alterations in shape, which in this work we called 'fragmentation'. The fragmentation was not present in all bursts in the individual cells, but rather concerned one or two bursts in the recording series. A similar effect was present during co-application of bicuculline and strychnine with TBOA and AM251, suggesting it was not the result of mere disruption of the network inhibition. Moreover, during co-application of AEA with bicuculline, strychnine and TBOA, burst fragmentation could also be observed. This bursting feature could arise for several reasons. The fragmentation might have resulted from deconstruction of an individual burst or from the coalescence of two independent events. Fragmented bursts are longer in comparison to normal ones, but the ones that may result from coalescence are expected to be even longer because they should represent the summation of the length of two bursts.

We know from previous studies that the occurrence of bursting evoked by TBOA requires coordination of several different ion channel activities and drive from the network of interconnected neurons (Sharifullina and Nistri, 2006). Thus, the observed alterations in burst shape may result from uncoupling among neurons connected via gap junctions, desynchronization of the network due to modulation of neurotransmitter release, modulation of intrinsic properties of hypoglossal motoneurons, or other yet unknown mechanism. A previous study on the properties of TBOA-

evoked responses in the HMs has concluded that the role of GABA and glycine neurotransmission is to constrain bursting (Sharifullina and Nistri, 2006). The observed fragmented shape in TBOA-evoked bursts obtained during AEA or AM251 co-application with concomitant inhibition of GABA and glycine receptors indicate that increased network excitability originating from bicuculline and strychnine application was further exacerbated by any endocannabinoid system perturbation. This combination of altered signalling could further impair the robustness of the network to prevent bursting and led to their fragmentation. It is also noteworthy that when we applied exogenous AEA, we flooded the slice with this agent and lost any pulsatile release of AEA related to rhythmic neuronal depolarization and network firing, which may have contributed to synchronization of the network. In addition, in AEA, TBOA, bicuculline and strychnine solution, all recorded neurons produced bursting thus indicating that concomitant disruption of the inhibitory signalling is abolishing AEA-evoked decrease in the burst iccurence.

7.4 Endocannabinoid system modulates sPSCs in hypoglossal motoneurons

In order to further understand the effect of endocannabinoid system modulation on HMs in the condition of elevated glutamate level, I have analysed the effects of AEA or AM251 application on spontaneous postsynaptic currents (sPSCs). Available studies indicate that both inhibitory (Mukhtarov et al., 2005) and excitatory (García-Morales et al., 2015) neurotransmission can be attenuated by application of endocannabinoids. My results confirm those data. AEA application resulted in a decrease in spontaneous excitatory postsynaptic current (sEPSC) frequency, which could be correlated with the decrease in burst occurrence during co-application of AEA with TBOA. Preliminary data on the AM251 effect on glutamatergic neurotransmission in the HMs revealed a very interesting phenomenon. Co-application of AM251 after initial suppression of inhibitory neurotransmission resulted in occurrence of burst-like activity, accompanied by increase in sEPSC amplitude and frequency. The AM251-evoked bursts had smaller amplitude in comparison to TBOA-evoked ones and were clustered in four to five events. AM251-evoked bursts were not detected in previous experiments in which we first co-applied AM251 with bicuculline and strychnine for 10 min and then co-applied TBOA. This may be due to longer time frame necessary to observe those events, which overlapped with TBOA co-application. This bursting activity appearance after co-application of AM251 with bicuculline and strychnine further supports the notion that the role of the endocannabinoid system is to decrease network excitation.

Inhibitory neurotransmission has been previously reported to be a factor, which diminishes

bursting probability (Sharifullina and Nistri, 2006). Previous studies have shown that activation of endocannabinoid signalling decreases both amplitude and frequency of glycinergic spontaneous events (Mukhtarov et al., 2005). In my recordings I observed that amplitude of sIPSC in two of the recorded cells had decreased after 10 min of AEA co-application with DNQX and APV (one of them regenerated after 20 min). In the other two cells changes were not observed thus statistical significance was not reached. The frequency of these synaptic events was increased in some cells, and had a bimodal change in others. In the presence of AM251 the mean value of both amplitude and frequency in the recorded cells of sIPSC did not change. Nonetheless frequency during AM251 application decreased after first 10 minutes in case of two cells. After 20 min of co-application of AM251 amplitude in three out of four cells (1 cell lost after around 10 min) amplitude increased, therefore it cannot be excluded that increase in the number of recordings would result in statistical significance in this value.

It is important to emphasise that in our experiments, spontaneous activity was network dependent, thus we cannot exclude that modulation of certain elements of the inhibitory network could have potentiated others, giving rise to complex, time related changes in sIPSCs. Enhancement of certain inhibitory mechanisms through inhibition of other upstream ones could also contribute to AEA-elicited decrease in burst occurrence observed during AEA and TBOA co-application. It is an interesting possibility, especially in the view that GABAergic transmission in HMs mainly depends on network activity and thus may be a source of complex pattern of sIPSCs observed in my recordings (Donato and Nistri, 2000).

Further experiments on miniature inhibitory (and excitatory) postsynaptic currents (mIPSCs, mEPSCs), in the presence of tetrodoxin (to block network activity), are necessary to clarify the effect of AEA or AM251 on IPSCs (and EPSCs). Such experiments would give us information on the possible effects of cannabinoid system modulation on *true* spontaneous release of neurotransmitter in isolation from the network and allow for better understanding of the processes occurring in our model.

7.5 TBOA evoked changes in CB1R expression in medullary brainstem

Due to the very small size of the tissue samples, we were not able to investigate hypoglossal nuclei alone for CB1R expression. Nonetheless, we isolated the medullary area, which contains the region of our interest and performed Western blot analysis on whole lysates from those samples. Due to the fact that hypoglossal motoneurons receive inputs from other areas in the brainstem

(Borke et al., 1983; Peever et al., 2002; Smith et al., 1991), this analysis is still valuable in order to understand possible delayed mechanisms activated after excitotoxic stress. Our data showed a significant increase in CB1R expression after TBOA-treatment. The fast triggering of CB1R over-expression during excitotoxic insult (4h) supports the early impact of endocannabinoid signalling. This is in accordance with data from other experimental systems indicating that some insults, like for example stroke, can also increase CB1R expression (Jin et al., 2000). The increase in CB1R protein in such a short time advocates against canonical neosynthesis. One possibility is the existence of a pre-assembled CB1R pool which is not detected by Western blot because it exists in the form of a tightly bound complex, but it might be mobilized by excitotoxic insult. Nonetheless, studies on CB1R degradation rates suggest another possibility. A study on neuroblastoma cells expressing CB1Rs estimated that the vast majority (~70%) of newly synthesized CB1R protein is degraded rapidly with a degradation rate half-time $t_{1/2}$=4.8 hours, with the remaining proteins most likely representing functional receptors (McIntosh et al., 1998). Another study has reported that in baby hamster kidney (BHK) cells expressing CB1Rs, these proteins are even more rapidly degraded with only ~10% remaining after 1 hour (Andersson et al., 2003). The same study has investigated the effect of inhibition of proteasome on CB1R expression, and has confirmed a significant receptor increase in less than one hour (Andersson et al., 2003). Thus, inhibition of CB1R degradation through intracellular signalling pathways or non-coding RNA regulation can perhaps result in the very rapid accumulation of CB1R protein observed in my experiments. Further detailed investigation is needed in order to understand if there are regional differences in the CB1R accumulation triggered by excitotoxicity and eventually evaluate their relation to signalling in the HMs.

7.6 Anandamide application protects cells in hypoglossal motoneuron nuclei from TBOA-induced damage

The present analysis of the effects of an endocannabinoids on hypoglossal motoneurons led us to explore an important question: does AEA actually protect HMs from TBOA-induced excitotoxicity. As previously mentioned, numerous studies indicate that endocannabinoid system activation has protective effect on cells (Mechoulam et al., 2002b; Veldhuis et al., 2003; Zogopoulos et al., 2013; Kano et al. 2009). For example, administration of the CB1R agonist WIN55, 212-2 decreases hippocampal neuronal loss during cerebral ischemia (Nagayama et al., 1999). In a mouse model of kainate-induced excitotoxicity it has also been demonstrated that CB1Rs on glutamatergic synapses of hippocampal neurons protect from kainate-induced seizures

(Monory et al., 2006).

Our laboratory has observed that TBOA increases the number of (damaged) propidium iodide (PI)-stained cells in the hypoglossal region of medullary slices (Cifra et al., 2009). At the same time, agents that suppress bursting, like riluzole, promote cell survival (Cifra et al. 2009, 2011a). Riluzole therapy is the only one currently available for ALS, but it prolongs the life of the patients for a few months only (Bensimon et al., 1994; Meininger et al., 2000; Miller et al., 1996). Thus, a search for alternative therapies for ALS treatment is urgently necessary. In my experiments, I have confirmed that TBOA increased the number of damaged cells identified with PI staining. Co-application of AEA with TBOA attenuated the number of PI-positive cells and thus, confirmed the protective effects of this endocannabinoid agonist. In analogy to the effect of riluzole on HMs (Cifra et al. 2011a), application of AEA produced burst inhibition, that correlated with an increased survival of the cells in the HM nuclei. The cellular protection was likely due to a decrease in glutamate release from presynaptic terminals, as indicated by my experiments on sEPSC modulation by AEA. This observation is in accordance with recent studies indicating that the administration of cannabinoids or modulation of the cannabinoid system can delay disease progression in SOD1 ALS transgenic animals, thus outlining a possible target for new therapies for this devastating disease (Bilsland et al., 2006; Raman et al., 2004; Weydt et al., 2005).

7.7 Summary

In summary, my work described the effect of modulating endocannabinoid signalling on the responses of hypoglossal motoneurons to excitotoxic stress induced by glutamate uptake inhibition. I provided a preliminary description of the modulation of inhibitory and excitatory synaptic events by agonism or antagonism of CB1Rs. My data indicated that CB1R expression could be up-regulated in a short time frame in response to the excitotoxic insult, which is in accordance with previous studies on CB1R expression during pathological events (Jin et al., 2000; Zhao et al., 2008). Moreover, I have confirmed the protective effects of AEA application on cell viability during excitotoxic stress caused by TBOA. The data presented here are, therefore, a useful contribution to the knowledge of the likely involvement of endocannabinoid signalling during excitotoxicity. In conclusion, my experiments provide a rationale for future work, especially *in vivo*, to target the endocannabinoid system as a potential neuroprotectant system against excitotoxicity in neurodegenerative diseases.

8. References:

Abel, E.L., 2007. Football increases the risk for Lou Gehrig's disease, amyotrophic lateral sclerosis. Percept. Mot. Skills 104, 1251–1254.

Ackerley, S., Thornhill, P., Grierson, A.J., Brownlees, J., Anderton, B.H., Leigh, P.N., Shaw, C.E., Miller, C.C.J., 2003. Neurofilament heavy chain side arm phosphorylation regulates axonal transport of neurofilaments. J. Cell Biol. 161, 489–495.

Al Kury, L.T., Voitychuk, O.I., Yang, K.-H.S., Thayyullathil, F.T., Doroshenko, P., Ramez, A.M., Shuba, Y.M., Galadari, S., Howarth, F.C., Oz, M., 2014. Effects of the endogenous cannabinoid anandamide on voltage-dependent sodium and calcium channels in rat ventricular myocytes. Br. J. Pharmacol. 171, 3485–3498. doi:10.1111/bph.12734

Aldes, L.D., Chronister, R.B., Marco, L.A., 1988. Distribution of glutamic acid decarboxylase and gamma-aminobutyric acid in the hypoglossal nucleus in the rat. J. Neurosci. Res. 19, 343–348. doi:10.1002/jnr.490190309

Alexianu, M.E., Ho, B.-K., Mohamed, A.H., La Bella, V., Smith, R.G., Appel, S.H., 1994. The role of calcium-binding proteins in selective motoneuron vulnerability in amyotrophic lateral sclerosis. Ann. Neurol. 36, 846–858.

Altschuler, S.M., Bao, X., Miselis, R.R., 1994. Dendritic architecture of hypoglossal motoneurons projecting to extrinsic tongue musculature in the rat. J. Comp. Neurol. 342, 538–550.

Amri, M., Lamkadem, M., Car, A., 1991. Effects of lingual nerve and chewing cortex stimulation upon activity of the swallowing neurons located in the region of the hypoglossal motor nucleus. Brain Res. 548, 149–155.

Anderson, C.M., Bridges, R.J., Chamberlin, A.R., Shimamoto, K., Yasuda-Kamatani, Y., Swanson, R.A., 2001. Differing effects of substrate and non-substrate transport inhibitors on glutamate uptake reversal. J. Neurochem. 79, 1207–1216.

Andersson, H., D'Antona, A.M., Kendall, D.A., Heijne, G.V., Chin, C.-N., 2003. Membrane Assembly of the Cannabinoid Receptor 1: Impact of a Long N-Terminal Tail. Mol. Pharmacol. 64, 570–577.

Appel, S.H., Beers, D., Siklos, L., Engelhardt, J.I., Mosier, D.R., 2001. Calcium: the Darth Vader of ALS. Amyotroph. Lateral Scler. Mot. Neuron Disord. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 2 Suppl 1, S47–54.

Armon, C., Nelson, L.M., 2012. Is head trauma a risk factor for amyotrophic lateral sclerosis? An evidence based review. Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 13, 351–356.

Atkin, J.D., Farg, M.A., Walker, A.K., McLean, C., Tomas, D., Horne, M.K., 2008. Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. Neurobiol. Dis. 30, 400–407.

Atsumi, T., Miyatake, T., 1987. Morphometry of the degenerative process in the hypoglossal nerves in amyotrophic lateral sclerosis. Acta Neuropathol. (Berl.) 73, 25–31.

Bae, J.S., Simon, N.G., Menon, P., Vucic, S., Kiernan, M.C., 2013. The puzzling case of hyperexcitability in amyotrophic lateral sclerosis. J. Clin. Neurol. Seoul Korea 9, 65–74.

Baekey, D.M., Morris, K.F., Gestreau, C., Li, Z., Lindsey, B.G., Shannon, R., 2001. Medullary respiratory neurones and control of laryngeal motoneurones during fictive eupnoea and cough in the cat. J. Physiol. 534, 565–581.

Bailey, E.F., Fregosi, R.F., 2004. Coordination of intrinsic and extrinsic tongue muscles during spontaneous breathing in the rat. J. Appl. Physiol. Bethesda Md 1985 96, 440–449.

Barann, M., Molderings, G., Brüss, M., Bönisch, H., Urban, B.W., Göthert, M., 2002. Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site. Br. J. Pharmacol. 137, 589–596.

Barnard, E.A., Sutherland, M., Zaman, S., Matsumoto, M., Nayeem, N., Green, T., Darlison, M.G., Bateson, A.N., 1993. Multiplicity, structure, and function in GABAA receptors. Ann. N. Y. Acad. Sci. 707, 116–125.

Bayewitch, M., Avidor-Reiss, T., Levy, R., Barg, J., Mechoulam, R., Vogel, Z., 1995. The peripheral cannabinoid receptor: adenylate cyclase inhibition and G protein coupling. FEBS Lett. 375, 143–147.

Bayliss, D.A., Viana, F., Bellingham, M.C., Berger, A.J., 1994. Characteristics and postnatal development of a hyperpolarization-activated inward current in rat hypoglossal motoneurons in vitro. J. Neurophysiol. 71, 119–128.

Beaulieu, J.M., Robertson, J., Julien, J.P., 1999. Interactions between peripherin and neurofilaments in cultured cells: disruption of peripherin assembly by the NF-M and NF-H subunits. Biochem. Cell Biol. Biochim. Biol. Cell. 77, 41–45.

Begg, M., Baydoun, A., Parsons, M.E., Molleman, A., 2001. Signal transduction of cannabinoid CB1 receptors in a smooth muscle cell line. J. Physiol. 531, 95–104.

Beierlein, M., Gibson, J.R., Connors, B.W., 2000. A network of electrically coupled interneurons drives synchronized inhibition in neocortex. Nat. Neurosci. 3, 904–910.

Beinfeld, M.C., Connolly, K., 2001. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. Neurosci. Lett. 301, 69–71.

Belli, S., Vanacore, N., 2005. Proportionate mortality of Italian soccer players: is amyotrophic lateral sclerosis an occupational disease? Eur. J. Epidemiol. 20, 237–242.

Beltramo, M., Piomelli, D., 2000. Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. Neuroreport 11, 1231–1235.

Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D., 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277, 1094–1097.

Bendotti, C., Atzori, C., Piva, R., Tortarolo, M., Strong, M.J., DeBiasi, S., Migheli, A., 2004. Activated p38MAPK is a novel component of the intracellular inclusions found in human amyotrophic lateral sclerosis and mutant SOD1 transgenic mice. J. Neuropathol. Exp. Neurol. 63, 113–119.

Bendotti, C., Calvaresi, N., Chiveri, L., Prelle, A., Moggio, M., Braga, M., Silani, V., De Biasi, S., 2001. Early vacuolization and mitochondrial damage in motor neurons of FALS mice are not associated with apoptosis or with changes in cytochrome oxidase histochemical reactivity. J. Neurol. Sci. 191, 25–33.

Benito, C., Romero, J.P., Tolón, R.M., Clemente, D., Docagne, F., Hillard, C.J., Guaza, C., Romero, J., 2007. Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. J. Neurosci. Off. J. Soc. Neurosci. 27, 2396–2402.

Bensimon, G., Lacomblez, L., Meininger, V., 1994. A Controlled Trial of Riluzole in Amyotrophic Lateral Sclerosis. N. Engl. J. Med. 330, 585–591.

Berger, A.J., 2000. Determinants of respiratory motoneuron output. Respir. Physiol. 122, 259–269.

Berger, A.J., Bayliss, D.A., Viana, F., 1996. Development of hypoglossal motoneurons. J. Appl. Physiol. Bethesda Md 1985 81, 1039–1048.

Berger, A.J., Dieudonné, S., Ascher, P., 1998. Glycine uptake governs glycine site occupancy at NMDA receptors of excitatory synapses. J. Neurophysiol. 80, 3336–3340.

Berger, C., Schmid, P.C., Schabitz, W.-R., Wolf, M., Schwab, S., Schmid, H.H.O., 2004. Massive accumulation of N-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia? J. Neurochem. 88, 1159–1167.

Bilsland, L.G., Dick, J.R.T., Pryce, G., Petrosino, S., Di Marzo, V., Baker, D., Greensmith, L., 2006. Increasing cannabinoid levels by pharmacological and genetic manipulation delay disease progression in SOD1 mice. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 20, 1003–1005.

Bilsland, L.G., Greensmith, L., 2008. The endocannabinoid system in amyotrophic lateral sclerosis. Curr. Pharm. Des. 14, 2306–2316.

Bisogno, T., Howell, F., Williams, G., Minassi, A., Cascio, M.G., Ligresti, A., Matias, I., Schiano-Moriello, A., Paul, P., Williams, E.-J., Gangadharan, U., Hobbs, C., Di Marzo, V., Doherty, P., 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J. Cell Biol. 163, 463–468.

Bisogno, T., MacCarrone, M., De Petrocellis, L., Jarrahian, A., Finazzi-Agrò, A., Hillard, C., Di Marzo, V., 2001. The uptake by cells of 2-arachidonoylglycerol, an endogenous agonist of cannabinoid receptors. Eur. J. Biochem. FEBS 268, 1982–1989.

Bisogno, T., Melck, D., De Petrocellis, L., Di Marzo, V., 1999. Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. J. Neurochem. 72, 2113–2119.

Bogdanov, M., Brown, R.H., Matson, W., Smart, R., Hayden, D., O'Donnell, H., Flint Beal, M., Cudkowicz, M., 2000. Increased oxidative damage to DNA in ALS patients. Free Radic. Biol. Med. 29, 652–658.

Bonhaus, D.W., Chang, L.K., Kwan, J., Martin, G.R., 1998. Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. J. Pharmacol. Exp. Ther. 287, 884–888.

Bonin, R.P., Martin, L.J., MacDonald, J.F., Orser, B.A., 2007. Alpha5GABAA receptors regulate the intrinsic excitability of mouse hippocampal pyramidal neurons. J. Neurophysiol. 98, 2244–2254.

Boone, T.B., Aldes, L.D., 1984. The ultrastructure of two distinct neuron populations in the hypoglossal nucleus of the rat. Exp. Brain Res. 54, 321–326.

Borke, R.C., Nau, M.E., Ringler, R.L., 1983. Brain stem afferents of hypoglossal neurons in the rat. Brain Res. 269, 47–55.

Borthwick, G.M., Johnson, M.A., Ince, P.G., Shaw, P.J., Turnbull, D.M., 1999. Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. Ann. Neurol. 46, 787–790.

Boston-Howes, W., Gibb, S.L., Williams, E.O., Pasinelli, P., Brown, R.H., Trotti, D., 2006. Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. J. Biol. Chem. 281, 14076–14084.

Bouaboula, M., Bianchini, L., McKenzie, F.R., Pouyssegur, J., Casellas, P., 1999. Cannabinoid receptor CB1 activates the Na+/H+ exchanger NHE-1 isoform via Gi-mediated mitogen activated protein kinase signaling transduction pathways. FEBS Lett. 449, 61–65.

Bouaboula, M., Poinot-Chazel, C., Bourrié, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., Casellas, P., 1995. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. Biochem. J. 312 (Pt 2), 637–641.

Bouaboula, M., Poinot-Chazel, C., Marchand, J., Canat, X., Bourrié, B., Rinaldi-Carmona, M., Calandra, B., Le Fur, G., Casellas, P., 1996. Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. Eur. J. Biochem. FEBS 237, 704–711.

Brasnjo, G., Otis, T.S., 2001. Neuronal Glutamate Transporters Control Activation of Postsynaptic Metabotropic Glutamate Receptors and Influence Cerebellar Long-Term Depression. Neuron 31, 607–616.

Bright, D.P., Aller, M.I., Brickley, S.G., 2007. Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J. Neurosci. Off. J. Soc. Neurosci. 27, 2560–2569.

Brown, S.P., Brenowitz, S.D., Regehr, W.G., 2003. Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat. Neurosci. 6, 1048–1057.

Bruijn, L.I., Becher, M.W., Lee, M.K., Anderson, K.L., Jenkins, N.A., Copeland, N.G., Sisodia, S.S., Rothstein, J.D., Borchelt, D.R., Price, D.L., Cleveland, D.W., 1997. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron 18, 327–338.

Bruijn, L.I., Miller, T.M., Cleveland, D.W., 2004. Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu. Rev. Neurosci. 27, 723–749. doi:10.1146/annurev.neuro.27.070203.144244

Burnashev, N., Khodorova, A., Jonas, P., Helm, P.J., Wisden, W., Monyer, H., Seeburg, P.H., Sakmann, B., 1992a. Calcium-permeable AMPA-kainate receptors in fusiform cerebellar glial cells. Science 256, 1566–1570.

Burnashev, N., Monyer, H., Seeburg, P.H., Sakmann, B., 1992b. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron 8, 189–198.

Busch, L., Sterin-Borda, L., Borda, E., 2004. Expression and biological effects of CB1 cannabinoid receptor in rat parotid gland. Biochem. Pharmacol. 68, 1767–1774.

Cadas, H., di Tomaso, E., Piomelli, D., 1997. Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. J. Neurosci. Off. J. Soc. Neurosci. 17, 1226–1242.

Cadogan, A.K., Alexander, S.P., Boyd, E.A., Kendall, D.A., 1997. Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. J. Neurochem. 69, 1131–1137.

Callaghan, B., Feldman, D., Gruis, K., Feldman, E., 2011. The association of exposure to lead, mercury, and selenium and the development of amyotrophic lateral sclerosis and the epigenetic implications. Neurodegener. Dis. 8, 1–8.

Campbell, S.L., Hablitz, J.J., 2004. Glutamate transporters regulate excitability in local networks in rat neocortex. Neuroscience 127, 625–635.

Carpenter, S., 1968. Proximal axonal enlargement in motor neuron disease. Neurology 18, 841–851.

Carrì, M.T., Valle, C., Bozzo, F., Cozzolino, M., 2015. Oxidative stress and mitochondrial damage: importance in non-SOD1 ALS. Front. Cell. Neurosci. 9.

Carrier, E.J., Kearn, C.S., Barkmeier, A.J., Breese, N.M., Yang, W., Nithipatikom, K., Pfister, S.L., Campbell, W.B., Hillard, C.J., 2004. Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. Mol. Pharmacol. 65, 999–1007.

Carter, B.J., Anklesaria, P., Choi, S., Engelhardt, J.F., 2009. Redox modifier genes and pathways in amyotrophic lateral sclerosis. Antioxid. Redox Signal. 11, 1569–1586.

Casas, C., Herrando-Grabulosa, M., Manzano, R., Mancuso, R., Osta, R., Navarro, X., 2013. Early presymptomatic cholinergic dysfunction in a murine model of amyotrophic lateral sclerosis. Brain Behav. 3, 145–158.

Castillo, P.E., Younts, T.J., Chávez, A.E., Hashimotodani, Y., 2012. Endocannabinoid signaling and synaptic function. Neuron 76, 70–81. doi:10.1016/j.neuron.2012.09.020

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Centonze, D., Bari, M., Rossi, S., Prosperetti, C., Furlan, R., Fezza, F., De Chiara, V., Battistini, L., Bernardi, G., Bernardini, S., Martino, G., Maccarrone, M., 2007a. The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. Brain J. Neurol. 130, 2543–2553.

Chakrabarti, A., Onaivi, E.S., Chaudhuri, G., 1995. Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. DNA Seq. J. DNA Seq. Mapp. 5, 385–388.

Chamberlin, N.L., Eikermann, M., Fassbender, P., White, D.P., Malhotra, A., 2007. Genioglossus premotoneurons and the negative pressure reflex in rats. J. Physiol. 579, 515–526.

Chemin, J., Monteil, A., Perez-Reyes, E., Nargeot, J., Lory, P., 2001. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J. 20, 7033–7040.

Chen, X., Yang, W., Fan, Y., Luo, J., Hong, K., Wang, Z., Yan, J., Chen, X., Lu, J., Benovic, J., Zhou, N., 2010. Structural determinants in the second intracellular loop of the human cannabinoid CB1 receptor mediate selective coupling to Gs and Gi. Br. J. Pharmacol. 161, 1817–1834.

Cheroni, C., Marino, M., Tortarolo, M., Veglianese, P., De Biasi, S., Fontana, E., Zuccarello, L.V., Maynard, C.J., Dantuma, N.P., Bendotti, C., 2009. Functional alterations of the ubiquitin-proteasome system in motor neurons of a mouse model of familial amyotrophic lateral sclerosis. Hum. Mol. Genet. 18, 82–96.

Chevaleyre, V., Heifets, B.D., Kaeser, P.S., Südhof, T.C., Castillo, P.E., 2007. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1α. Neuron 54, 801–812.

Chiò, A., Benzi, G., Dossena, M., Mutani, R., Mora, G., 2005. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. Brain J. Neurol. 128, 472–476.

Chitravanshi, V.C., Sapru, H.N., 1996. NMDA as well as non-NMDA receptors mediate the neurotransmission of inspiratory drive to phrenic motoneurons in the adult rat. Brain Res. 715, 104–112.

Choi, D.W., 1988. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1, 623–634.

Cifra, A., Nani, F., Nistri, A., 2011a. Riluzole is a potent drug to protect neonatal rat hypoglossal motoneurons in vitro from excitotoxicity due to glutamate uptake block. Eur. J. Neurosci. 33, 899–913.

Cifra, A., Nani, F., Nistri, A., 2011b. Respiratory motoneurons and pathological conditions: lessons from hypoglossal motoneurons challenged by excitotoxic or oxidative stress. Respir. Physiol. Neurobiol. 179, 89–96.

Cifra, A., Nani, F., Sharifullina, E., Nistri, A., 2009. A repertoire of rhythmic bursting produced by hypoglossal motoneurons in physiological and pathological conditions. Philos. Trans. R. Soc. B Biol. Sci. 364, 2493–2500.

Clapham, D.E., 2007. Calcium signaling. Cell 131, 1047–1058.

Cobb, S.R., Bulters, D.O., Davies, C.H., 2000. Coincident activation of mGluRs and mAChRs imposes theta frequency patterning on synchronised network activity in the hippocampal CA3 region. Neuropharmacology 39, 1933–1942.

Coffman, C.J., Horner, R.D., Grambow, S.C., Lindquist, J., VA Cooperative Studies Program Project #500, 2005. Estimating the occurrence of amyotrophic lateral sclerosis among Gulf War (1990-1991) veterans using capture-recapture methods. Neuroepidemiology 24, 141–150.

Collard, J.F., Côté, F., Julien, J.P., 1995. Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis. Nature 375, 61–64.

Correa, F., Docagne, F., Clemente, D., Mestre, L., Becker, C., Guaza, C., 2008. Anandamide inhibits IL-12p40 production by acting on the promoter repressor element GA-12: possible involvement of the COX-2 metabolite prostamide E(2). Biochem. J. 409, 761–770.

Côté, F., Collard, J.F., Julien, J.P., 1993. Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. Cell 73, 35–46.

Coyle, J.T., Puttfarcken, P., 1993. Oxidative stress, glutamate, and neurodegenerative disorders. Science 262, 689–695.

Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B., 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384, 83–87.

Croxford, J.L., 2003. Therapeutic potential of cannabinoids in CNS disease. CNS Drugs 17, 179–202.

Cunningham, E.T., Sawchenko, P.E., 2000. Dorsal medullary pathways subserving oromotor reflexes in the rat: implications for the central neural control of swallowing. J. Comp. Neurol. 417, 448–466.

D'Amico, E., Factor-Litvak, P., Santella, R.M., Mitsumoto, H., 2013. Clinical Perspective of Oxidative Stress in Sporadic ALS. Free Radic. Biol. Med. 65.

Dalton, G.D., Bass, C.E., Van Horn, C., Howlett, A.C., 2009. Signal Transduction via Cannabinoid Receptors. CNS Neurol. Disord. Drug Targets 8, 422–431.

Damiano, M., Starkov, A.A., Petri, S., Kipiani, K., Kiaei, M., Mattiazzi, M., Flint Beal, M., Manfredi, G., 2006. Neural mitochondrial Ca2+ capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. J. Neurochem. 96, 1349–1361.

De Grey, A.D.N.J., 2004. Mitochondrial mutations in mammalian aging: an over-hasty about-turn? Rejuvenation Res. 7, 171–174.

De Lago, E., Fernández-Ruiz, J., Ortega-Gutiérrez, S., Cabranes, A., Pryce, G., Baker, D., López-Rodríguez, M., Ramos, J.A., 2006. UCM707, an inhibitor of the anandamide uptake, behaves as a symptom control agent in models of Huntington's disease and multiple sclerosis, but fails to delay/arrest the progression of different motor-related disorders. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 16, 7–18.

De Petrocellis, L., Bisogno, T., Maccarrone, M., Davis, J.B., Finazzi-Agro, A., Di Marzo, V., 2001. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J. Biol. Chem. 276, 12856–12863.

De Vos, K.J., Chapman, A.L., Tennant, M.E., Manser, C., Tudor, E.L., Lau, K.-F., Brownlees, J., Ackerley, S., Shaw, P.J., McLoughlin, D.M., Shaw, C.E., Leigh, P.N., Miller, C.C.J., Grierson, A.J., 2007. Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. Hum. Mol. Genet. 16, 2720–2728.

Degn, M., Lambertsen, K.L., Petersen, G., Meldgaard, M., Artmann, A., Clausen, B.H., Hansen, S.H., Finsen, B., Hansen, H.S., Lund, T.M., 2007. Changes in brain levels of N-acylethanolamines and 2-arachidonoylglycerol in focal cerebral ischemia in mice. J. Neurochem. 103, 1907–1916.

Del Negro, C.A., Morgado-Valle, C., Hayes, J.A., Mackay, D.D., Pace, R.W., Crowder, E.A., Feldman, J.L., 2005. Sodium and calcium current-mediated pacemaker neurons and respiratory rhythm generation. J. Neurosci. Off. J. Soc. Neurosci. 25, 446–453.

Demuth, D.G., Gkoumassi, E., Dröge, M.J., Dekkers, B.G.J., Esselink, H.J., van Ree, R.M., Parsons, M.E., Zaagsma, J., Molleman, A., Nelemans, S.A., 2005. Arachidonic acid mediates non-capacitative calcium entry evoked by CB1-cannabinoid receptor activation in DDT1 MF-2 smooth muscle cells. J. Cell. Physiol. 205, 58–67.

Demuth, D.G., Molleman, A., 2006. Cannabinoid signalling. Life Sci. 78, 549–563.

DePaul, R., Abbs, J.H., Caligiuri, M., Gracco, V.L., Brooks, B.R., 1988. Hypoglossal, trigeminal, and facial motoneuron involvement in amyotrophic lateral sclerosis. Neurology 38, 281–283.

Derkinderen, P., Ledent, C., Parmentier, M., Girault, J.A., 2001. Cannabinoids activate p38 mitogen-activated protein kinases through CB1 receptors in hippocampus. J. Neurochem. 77, 957–960.

Derkinderen, P., Toutant, M., Burgaya, F., Le Bert, M., Siciliano, J.C., de Franciscis, V., Gelman, M., Girault, J.A., 1996. Regulation of a neuronal form of focal adhesion kinase by anandamide. Science 273, 1719–1722.

Derkinderen, P., Valjent, E., Toutant, M., Corvol, J.-C., Enslen, H., Ledent, C., Trzaskos, J., Caboche, J., Girault, J.-A., 2003. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. J. Neurosci. Off. J. Soc. Neurosci. 23, 2371–2382.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Di Marzo, V., 1998. "Endocannabinoids" and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. Biochim. Biophys. Acta 1392, 153–175.

Di Marzo, V., Bisogno, T., De Petrocellis, L., 2001. Anandamide: some like it hot. Trends Pharmacol. Sci. 22, 346–349.

Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., Piomelli, D., 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372, 686–691.

Di Marzo, V., Matias, I., 2005. Endocannabinoid control of food intake and energy balance. Nat. Neurosci. 8, 585–589.

Di, S., Boudaba, C., Popescu, I.R., Weng, F.-J., Harris, C., Marcheselli, V.L., Bazan, N.G., Tasker, J.G., 2005. Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. J. Physiol. 569, 751–760.

Dick, T.E., Oku, Y., Romaniuk, J.R., Cherniack, N.S., 1993. Interaction between central pattern generators for breathing and swallowing in the cat. J. Physiol. 465, 715–730.

Dinardo, L.A., Travers, J.B., 1994. Hypoglossal neural activity during ingestion and rejection in the awake rat. J. Neurophysiol. 72, 1181–1191.

Dinh, T.P., Carpenter, D., Leslie, F.M., Freund, T.F., Katona, I., Sensi, S.L., Kathuria, S., Piomelli, D., 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc. Natl. Acad. Sci. U. S. A. 99, 10819–10824.

Dinh, T.P., Kathuria, S., Piomelli, D., 2004. RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. Mol. Pharmacol. 66, 1260–1264.

Dobbins, E.G., Feldman, J.L., 1995. Differential innervation of protruder and retractor muscles of the tongue in rat. J. Comp. Neurol. 357, 376–394.

Doble, A., 1999. The role of excitotoxicity in neurodegenerative disease: implications for therapy. Pharmacol. Ther. 81, 163–221.

Donato, R., Lape, R., Nistri, A., 2003. Pre and postsynaptic effects of metabotropic glutamate receptor activation on neonatal rat hypoglossal motoneurons. Neurosci. Lett. 338, 9–12.

Donato, R., Nistri, A., 2000. Relative contribution by GABA or glycine to Cl(-)-mediated synaptic transmission on rat hypoglossal motoneurons in vitro. J. Neurophysiol. 84, 2715–2724.

Dong, X., Wang, Y., Qin, Z., 2009. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta Pharmacol. Sin. 30, 379–387.

E. Alger, B., 2002. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog. Neurobiol. 68, 247–286.

Egertová, M., Cravatt, B.F., Elphick, M.R., 2003. Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119, 481–496.

Egertová, M., Giang, D.K., Cravatt, B.F., Elphick, M.R., 1998. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc. Biol. Sci. 265, 2081–2085.

Eggan, S.M., Lewis, D.A., 2007. Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. Cereb. Cortex N. Y. N 1991 17, 175–191.

El Manira, A., Kyriakatos, A., 2010. The role of endocannabinoid signaling in motor control. Physiol. Bethesda Md 25, 230–238.

Elphick, M.R., Egertová, M., 2001. The neurobiology and evolution of cannabinoid signalling. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 356, 381–408.

Emerit, J., Edeas, M., Bricaire, F., 2004. Neurodegenerative diseases and oxidative stress. Biomed. Pharmacother. Bioméd. Pharmacothérapie 58, 39–46.

Essin, K., Nistri, A., Magazanik, L., 2002. Evaluation of GluR2 subunit involvement in AMPA receptor function of neonatal rat hypoglossal motoneurons. Eur. J. Neurosci. 15, 1899–1906.

Evans, R.M., Scott, R.H., Ross, R.A., 2004. Multiple actions of anandamide on neonatal rat cultured sensory neurones. Br. J. Pharmacol. 141, 1223–1233.

Fan, P., 1995. Cannabinoid agonists inhibit the activation of 5-HT3 receptors in rat nodose ganglion neurons. J. Neurophysiol. 73, 907–910.

Fegley, D., Kathuria, S., Mercier, R., Li, C., Goutopoulos, A., Makriyannis, A., Piomelli, D., 2004. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. Proc. Natl. Acad. Sci. U. S. A. 101, 8756–8761.

Felder, C.C., Briley, E.M., Axelrod, J., Simpson, J.T., Mackie, K., Devane, W.A., 1993. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. Proc. Natl. Acad. Sci. 90, 7656–7660.

Felder, C.C., Glass, M., 1998. Cannabinoid receptors and their endogenous agonists. Annu. Rev. Pharmacol. Toxicol. 38, 179–200. doi:10.1146/annurev.pharmtox.38.1.179

Felder, C.C., Joyce, K.E., Briley, E.M., Glass, M., Mackie, K.P., Fahey, K.J., Cullinan, G.J., Hunden, D.C., Johnson, D.W., Chaney, M.O., Koppel, G.A., Brownstein, M., 1998. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. J. Pharmacol. Exp. Ther. 284, 291–297.

Feldman, J.L., Del Negro, C.A., 2006. Looking for inspiration: new perspectives on respiratory rhythm. Nat. Rev. Neurosci. 7, 232–242.

Fernández-Ruiz, J., 2009. The endocannabinoid system as a target for the treatment of motor dysfunction. Br. J. Pharmacol. 156, 1029–1040.

Fimiani, C., Mattocks, D., Cavani, F., Salzet, M., Deutsch, D.G., Pryor, S., Bilfinger, T.V., Stefano, G.B., 1999. Morphine and anandamide stimulate intracellular calcium transients in human arterial endothelial cells: coupling to nitric oxide release. Cell. Signal. 11, 189–193.

Fischer, L.R., Culver, D.G., Tennant, P., Davis, A.A., Wang, M., Castellano-Sanchez, A., Khan, J., Polak, M.A., Glass, J.D., 2004. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp. Neurol. 185, 232–240.

Fonseca, B.M., Correia-da-Silva, G., Teixeira, N.A., 2009. Anandamide-induced cell death: dual effects in primary rat decidual cell cultures. Placenta 30, 686–692.

Fonseca, B.M., Costa, M.A., Almada, M., Correia-da-Silva, G., Teixeira, N.A., 2013. Endogenous cannabinoids revisited: a biochemistry perspective. Prostaglandins Other Lipid Mediat. 102-103, 13–30.

Fowler, C.J., Jacobsson, S.O.P., 2002. Cellular transport of anandamide, 2-arachidonoylglycerol and palmitoylethanolamide--targets for drug development? Prostaglandins Leukot. Essent. Fatty Acids 66, 193–200.

Fray, A.E., Ince, P.G., Banner, S.J., Milton, I.D., Usher, P.A., Cookson, M.R., Shaw, P.J., 1998. The expression of the glial glutamate transporter protein EAAT2 in motor neuron disease: an immunohistochemical study. Eur. J. Neurosci. 10, 2481–2489.

Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83, 1017–1066.

Funk, G.D., Smith, J.C., Feldman, J.L., 1993. Generation and transmission of respiratory oscillations in medullary slices: role of excitatory amino acids. J. Neurophysiol. 70, 1497–1515.

Furukawa, H., Gouaux, E., 2003. Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. EMBO J. 22, 2873–2885.

Galve-Roperh, I., Rueda, D., Gómez del Pulgar, T., Velasco, G., Guzmán, M., 2002. Mechanism of extracellular signal-regulated kinase activation by the CB(1) cannabinoid receptor. Mol. Pharmacol. 62, 1385–1392.

Galve-Roperh, I., Sánchez, C., Cortés, M.L., Gómez del Pulgar, T., Izquierdo, M., Guzmán, M., 2000. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat. Med. 6, 313–319.

Gaoni, Y., Mechoulam, R., 1964. Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. J. Am. Chem. Soc. 86, 1646–1647.

García Del Caño, G., Millán, L.M., Gerrikagoitia, I., Sarasa, M., Matute, C., 1999. Ionotropic glutamate receptor subunit distribution on hypoglossal motoneuronal pools in the rat. J. Neurocytol. 28, 455–468.

García-Morales, V., Montero, F., Moreno-López, B., 2015. Cannabinoid agonists rearrange synaptic vesicles at excitatory synapses and depress motoneuron activity in vivo. Neuropharmacology 92, 69–79.

Gebremedhin, D., Lange, A.R., Campbell, W.B., Hillard, C.J., Harder, D.R., 1999. Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca2+ channel current. Am. J. Physiol. 276, H2085–2093.

Gérard, C., Mollereau, C., Vassart, G., Parmentier, M., 1990. Nucleotide sequence of a human cannabinoid receptor cDNA. Nucleic Acids Res. 18, 7142.

Gestreau, C., Dutschmann, M., Obled, S., Bianchi, A.L., 2005. Activation of XII motoneurons and premotor neurons during various oropharyngeal behaviors. Respir. Physiol. Neurobiol. 147, 159–176.

Gestreau, C., Grélot, L., Bianchi, A.L., 2000. Activity of respiratory laryngeal motoneurons during fictive coughing and swallowing. Exp. Brain Res. 130, 27–34.

Gifford, A.N., Ashby, C.R., 1996. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. J. Pharmacol. Exp. Ther. 277, 1431–1436.

Gilbert, G.L., Kim, H.J., Waataja, J.J., Thayer, S.A., 2007. Delta9-tetrahydrocannabinol protects hippocampal neurons from excitotoxicity. Brain Res. 1128, 61–69.

Girotti, A.W., 1998. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J. Lipid Res. 39, 1529–1542.

Glaser, S.T., Abumrad, N.A., Fatade, F., Kaczocha, M., Studholme, K.M., Deutsch, D.G., 2003. Evidence against the presence of an anandamide transporter. Proc. Natl. Acad. Sci. U. S. A. 100, 4269–4274.

Glass, M., Dragunow, M., Faull, R.L., 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77, 299–318.

Glass, M., Dragunow, M., Faull, R.L., 2000. The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97, 505–519.

Glass, M., Felder, C.C., 1997. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. J. Neurosci. Off. J. Soc. Neurosci. 17, 5327–5333.

Glass, M., Northup, J.K., 1999. Agonist selective regulation of G proteins by cannabinoid CB(1) and CB(2) receptors. Mol. Pharmacol. 56, 1362–1369.

Godlewski, G., Göthert, M., Malinowska, B., 2003. Cannabinoid receptor-independent inhibition by cannabinoid agonists of the peripheral 5-HT3 receptor-mediated von Bezold-Jarisch reflex. Br. J. Pharmacol. 138, 767–774.

Golovko, T., Min, R., Lozovaya, N., Falconer, C., Yatsenko, N., Tsintsadze, T., Tsintsadze, V., Ledent, C., Harvey, R.J., Belelli, D., Lambert, J.J., Rozov, A., Burnashev, N., 2014. Control of Inhibition by the Direct Action of Cannabinoids on GABAA Receptors. Cereb. Cortex N. Y. N 1991.

Greer, J.J., Smith, J.C., Feldman, J.L., 1991. Role of excitatory amino acids in the generation and transmission of respiratory drive in neonatal rat. J. Physiol. 437, 727–749.

Griffin, G., Atkinson, P.J., Showalter, V.M., Martin, B.R., Abood, M.E., 1998. Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5'-O-(3-[35S]thio)-triphosphate binding assay in rat cerebellar membranes. J. Pharmacol. Exp. Ther. 285, 553–560.

Gurney, M.E., Pu, H., Chiu, A.Y., Dal Canto, M.C., Polchow, C.Y., Alexander, D.D., Caliendo, J., Hentati, A., Kwon, Y.W., Deng, H.X., 1994. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 264, 1772–1775.

Guzmán, M., Sánchez, C., 1999. Effects of cannabinoids on energy metabolism. Life Sci. 65, 657–664.

Haddad, G.G., Donnelly, D.F., Getting, P.A., 1990. Biophysical properties of hypoglossal neurons in vitro: intracellular studies in adult and neonatal rats. J. Appl. Physiol. Bethesda Md 1985 69, 1509–1517.

Hájos, N., Ledent, C., Freund, T.F., 2001. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience 106, 1–4.

Hampson, A.J., Bornheim, L.M., Scanziani, M., Yost, C.S., Gray, A.T., Hansen, B.M., Leonoudakis, D.J., Bickler, P.E., 1998. Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. J. Neurochem. 70, 671–676.

Hampson, R.E., Evans, G.J., Mu, J., Zhuang, S.Y., King, V.C., Childers, S.R., Deadwyler, S.A., 1995. Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of potassium "A-current" in cultured rat hippocampal neurons. Life Sci. 56, 2081–2088.

Hampson, R.E., Miller, F., Palchik, G., Deadwyler, S.A., 2011. Cannabinoid receptor activation modifies NMDA receptor mediated release of intracellular calcium: implications for endocannabinoid control of hippocampal neural plasticity. Neuropharmacology 60, 944–952.

Hanus, L., Abu-Lafi, S., Fride, E., Breuer, A., Vogel, Z., Shalev, D.E., Kustanovich, I., Mechoulam, R., 2001. 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 3662–3665.

Hanus, L., Gopher, A., Almog, S., Mechoulam, R., 1993. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J. Med. Chem. 36, 3032–3034.

Harding, B., Tremblay, C., Cousineau, D., 2014. Standard errors: A review and evaluation of standard error estimators using Monte Carlo simulations. TQMP. 10, 107-123.

Hardingham, G.E., Bading, H., 2003. The Yin and Yang of NMDA receptor signalling. Trends Neurosci. 26, 81–89.

Harkany, T., Dobszay, M.B., Cayetanot, F., Härtig, W., Siegemund, T., Aujard, F., Mackie, K., 2005. Redistribution of CB1 cannabinoid receptors during evolution of cholinergic basal forebrain territories and their cortical projection areas: a comparison between the gray mouse lemur (Microcebus murinus, primates) and rat. Neuroscience 135, 595–609.

Haverkamp, L.J., Appel, V., Appel, S.H., 1995. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. Brain J. Neurol. 118 (Pt 3), 707–719.

Hayashi, F., McCrimmon, D.R., 1996. Respiratory motor responses to cranial nerve afferent stimulation in rats. Am. J. Physiol. 271, R1054–1062.

Hayashi, H., Suga, M., Satake, M., Tsubaki, T., 1981. Reduced glycine receptor in the spinal cord in amyotrophic lateral sclerosis. Ann. Neurol. 9, 292–294.

Herkenham, A.B.L., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A. 87, 1932–6.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., Costa, B. de, Rice, K.C., 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A. 87, 1932–1936.

Hillard, C.J., 2000. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat. 61, 3–18.

Hillard, C.J., 2008. Role of cannabinoids and endocannabinoids in cerebral ischemia. Curr. Pharm. Des. 14, 2347–2361.

Hillard, C.J., Campbell, W.B., 1997. Biochemistry and pharmacology of arachidonylethanolamide, a putative endogenous cannabinoid. J. Lipid Res. 38, 2383–2398.

Hillard, C.J., Jarrahian, A., 2000. The movement of N-arachidonoylethanolamine (anandamide) across cellular membranes. Chem. Phys. Lipids 108, 123–134.

Hillard, C.J., Manna, S., Greenberg, M.J., DiCamelli, R., Ross, R.A., Stevenson, L.A., Murphy, V., Pertwee, R.G., Campbell, W.B., 1999. Synthesis and Characterization of Potent and Selective Agonists of the Neuronal Cannabinoid Receptor (CB1). J. Pharmacol. Exp. Ther. 289, 1427–1433.

Hirano, A., Nakano, I., Kurland, L.T., Mulder, D.W., Holley, P.W., Saccomanno, G., 1984. Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 43, 471–480.

Hoesch, R.E., Geocadin, R.G., 2007. Therapeutic hypothermia for global and focal ischemic brain injury--a cool way to improve neurologic outcomes. The Neurologist 13, 331–342.

Hoffman, A.F., Lupica, C.R., 2000. Mechanisms of Cannabinoid Inhibition of GABAASynaptic Transmission in the Hippocampus. J. Neurosci. 20, 2470–2479.

Hollmann, M., Hartley, M., Heinemann, S., 1991. Ca2+ permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition. Science 252, 851–853.

Horner, R.D., Kamins, K.G., Feussner, J.R., Grambow, S.C., Hoff-Lindquist, J., Harati, Y., Mitsumoto, H., Pascuzzi, R., Spencer, P.S., Tim, R., Howard, D., Smith, T.C., Ryan, M. a. K., Coffman, C.J., Kasarskis, E.J., 2003. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. Neurology 61, 742–749.

Horner, R.L., 2008. Neuromodulation of hypoglossal motoneurons during sleep. Respir. Physiol. Neurobiol. 164, 179–196.

Horner, R.L., 2009. Emerging principles and neural substrates underlying tonic sleep-state-dependent influences on respiratory motor activity. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 2553–2564.

Howland, D.S., Liu, J., She, Y., Goad, B., Maragakis, N.J., Kim, B., Erickson, J., Kulik, J., DeVito, L., Psaltis, G., DeGennaro, L.J., Cleveland, D.W., Rothstein, J.D., 2002. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc. Natl. Acad. Sci. U. S. A. 99, 1604–1609.

Howlett, A.C., Blume, L.C., Dalton, G.D., 2010. CB1 Cannabinoid Receptors and their Associated Proteins. Curr. Med. Chem. 17, 1382–1393.

Howlett, A.C., Fleming, R.M., 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol. Pharmacol. 26, 532–538.

Howlett, A.C., Johnson, M.R., Melvin, L.S., Milne, G.M., 1988. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. Mol. Pharmacol. 33, 297–302.

Howlett, A.C., Mukhopadhyay, S., 2000. Cellular signal transduction by anandamide and 2-arachidonoylglycerol. Chem. Phys. Lipids 108, 53–70.

Howlett, A.C., Qualy, J.M., Khachatrian, L.L., 1986. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol. Pharmacol. 29, 307–313.

Huang, H., Bordey, A., 2004. Glial Glutamate Transporters Limit Spillover Activation of Presynaptic NMDA Receptors and Influence Synaptic Inhibition of Purkinje Neurons. J. Neurosci. 24, 5659–5669.

Huang, S.M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F., Tognetto, M., Petros, T.J., Krey, J.F., Chu, C.J., Miller, J.D., Davies, S.N., Geppetti, P., Walker, J.M., Di Marzo, V., 2002. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc. Natl. Acad. Sci. U. S. A. 99, 8400–8405.

Huang, Y.H., Sinha, S.R., Tanaka, K., Rothstein, J.D., Bergles, D.E., 2004. Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons. J. Neurosci. 24, 4551–4559.

Hughes, S.W., Cope, D.W., Blethyn, K.L., Crunelli, V., 2002. Cellular mechanisms of the slow (<1 Hz) oscillation in thalamocortical neurons in vitro. Neuron 33, 947–958.

Hwang, J.C., Bartlett, D., St John, W.M., 1983. Characterization of respiratory-modulated activities of hypoglossal motoneurons. J. Appl. Physiol. 55, 793–798.

hypoglossal nerve [WWW Document], n.d. . TheFreeDictionary.com. URL http://medical-dictionary.thefreedictionary.com/hypoglossal+nerve (accessed 5.27.15).

Ikeda, S.R., 1996. Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. Nature 380, 255–258.

Ilieva, E.V., Ayala, V., Jové, M., Dalfó, E., Cacabelos, D., Povedano, M., Bellmunt, M.J., Ferrer, I., Pamplona, R., Portero-Otín, M., 2007. Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain J. Neurol. 130, 3111–3123.

Ishac, E.J., Jiang, L., Lake, K.D., Varga, K., Abood, M.E., Kunos, G., 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. Br. J. Pharmacol. 118, 2023–2028.

Jaarsma, D., Haasdijk, E.D., Grashorn, J.A., Hawkins, R., van Duijn, W., Verspaget, H.W., London, J., Holstege, J.C., 2000. Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. Neurobiol. Dis. 7, 623–643.

Janzen, V.D., Rae, R.E., Hudson, A.J., 1988. Otolaryngologic manifestations of amyotrophic lateral sclerosis. J. Otolaryngol. 17, 41–42.

Jarrahian, A., Watts, V.J., Barker, E.L., 2004. D2 dopamine receptors modulate Galpha-subunit coupling of the CB1 cannabinoid receptor. J. Pharmacol. Exp. Ther. 308, 880–886.

Jean-Gilles, L., Feng, S., Tench, C.R., Chapman, V., Kendall, D.A., Barrett, D.A., Constantinescu, C.S., 2009. Plasma endocannabinoid levels in multiple sclerosis. J. Neurol. Sci. 287, 212–215.

Jean, A., 2001. Brain stem control of swallowing: neuronal network and cellular mechanisms. Physiol. Rev. 81, 929–969.

Jennings, E.A., Vaughan, C.W., Christie, M.J., 2001. Cannabinoid actions on rat superficial medullary dorsal horn neurons in vitro. J. Physiol. 534, 805–812.

Jia, Z., Agopyan, N., Miu, P., Xiong, Z., Henderson, J., Gerlai, R., Taverna, F.A., Velumian, A., MacDonald, J., Carlen, P., Abramow-Newerly, W., Roder, J., 1996. Enhanced LTP in mice deficient in the AMPA receptor GluR2. Neuron 17, 945–956.

Jin, K.L., Mao, X.O., Goldsmith, P.C., Greenberg, D.A., 2000. CB1 cannabinoid receptor induction in experimental stroke. Ann. Neurol. 48, 257–261.

Jin, L., Pan, L., Guo, Y., Zheng, Y., Nie, Z., Zhu, R., 2014. Expression and localization of cannabinoid receptor 1 in rats' brain treated with acute and repeated morphine. Acta Neurobiol. Exp. (Warsz.) 74, 288–297.

Joo, I.-S., Hwang, D.-H., Seok, J.-I., Shin, S.-K., Kim, S.-U., 2007. Oral administration of memantine prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. J. Clin. Neurol. Seoul Korea 3, 181–186.

Julien, J.P., 1997. Neurofilaments and motor neuron disease. Trends Cell Biol. 7, 243–249.

Jung, C., Higgins, C.M.J., Xu, Z., 2002. A quantitative histochemical assay for activities of mitochondrial electron transport chain complexes in mouse spinal cord sections. J. Neurosci. Methods 114, 165–172.

Jung, C., Lee, S., Ortiz, D., Zhu, Q., Julien, J.-P., Shea, T.B., 2005. The high and middle molecular weight neurofilament subunits regulate the association of neurofilaments with kinesin: inhibition by phosphorylation of the high molecular weight subunit. Brain Res. Mol. Brain Res. 141, 151–155.

Jung, K.-M., Astarita, G., Zhu, C., Wallace, M., Mackie, K., Piomelli, D., 2007. A key role for diacylglycerol lipase-alpha in metabotropic glutamate receptor-dependent endocannabinoid mobilization. Mol. Pharmacol. 72, 612–621.

Jung, K.-M., Mangieri, R., Stapleton, C., Kim, J., Fegley, D., Wallace, M., Mackie, K., Piomelli, D., 2005b. Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. Mol. Pharmacol. 68, 1196–1202.

Kabashi, E., Agar, J.N., Hong, Y., Taylor, D.M., Minotti, S., Figlewicz, D.A., Durham, H.D., 2008. Proteasomes remain intact, but show early focal alteration in their composition in a mouse model of amyotrophic lateral sclerosis. J. Neurochem. 105, 2353–2366.

Kalb, R.G., 1994. Regulation of motor neuron dendrite growth by NMDA receptor activation. Dev. Camb. Engl. 120, 3063–3071.

Kamel, F., Umbach, D.M., Stallone, L., Richards, M., Hu, H., Sandler, D.P., 2008. Association of lead exposure with survival in amyotrophic lateral sclerosis. Environ. Health Perspect. 116, 943–947.

Kano, M., 2014. Control of synaptic function by endocannabinoid-mediated retrograde signaling. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 90, 235–250.

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., Watanabe, M., 2009. Endocannabinoid-mediated control of synaptic transmission. Physiol. Rev. 89, 309–380.

Katona, I., Sperlágh, B., Maglóczky, Z., Sántha, E., Köfalvi, A., Czirják, S., Mackie, K., Vizi, E.S., Freund, T.F., 2000. GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience 100, 797–804.

Katona, I., Sperlágh, B., Sík, A., Käfalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J. Neurosci. Off. J. Soc. Neurosci. 19, 4544–4558.

Kawamura, Y., Dyck, P.J., Shimono, M., Okazaki, H., Tateishi, J., Doi, H., 1981. Morphometric comparison of the vulnerability of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 40, 667–675.

Kearn, C.S., Blake-Palmer, K., Daniel, E., Mackie, K., Glass, M., 2005. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Mol. Pharmacol. 67, 1697–1704.

Kearn, C.S., Greenberg, M.J., DiCamelli, R., Kurzawa, K., Hillard, C.J., 1999. Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. J. Neurochem. 72, 2379–2387.

Khaspekov, L.G., Brenz Verca, M.S., Frumkina, L.E., Hermann, H., Marsicano, G., Lutz, B., 2004. Involvement of brain-derived neurotrophic factor in cannabinoid receptor-dependent protection against excitotoxicity. Eur. J. Neurosci. 19, 1691–1698.

Khrapko, K., Vijg, J., 2007. Mitochondrial DNA mutations and aging: a case closed? Nat. Genet. 39, 445–446.

Kikuchi, H., Almer, G., Yamashita, S., Guégan, C., Nagai, M., Xu, Z., Sosunov, A.A., McKhann, G.M., Przedborski, S., 2006. Spinal cord endoplasmic reticulum stress associated with a microsomal accumulation of mutant superoxide dismutase-1 in an ALS model. Proc. Natl. Acad. Sci. U. S. A. 103, 6025–6030.

Kim, J., Isokawa, M., Ledent, C., Alger, B.E., 2002. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. J. Neurosci. Off. J. Soc. Neurosci. 22, 10182–10191.

Kim, P.M., Aizawa, H., Kim, P.S., Huang, A.S., Wickramasinghe, S.R., Kashani, A.H., Barrow, R.K., Huganir, R.L., Ghosh, A., Snyder, S.H., 2005. Serine racemase: activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. Proc. Natl. Acad. Sci. U. S. A. 102, 2105–2110.

Kirkinezos, I.G., Bacman, S.R., Hernandez, D., Oca-Cossio, J., Arias, L.J., Perez-Pinzon, M.A., Bradley, W.G., Moraes, C.T., 2005. Cytochrome c association with the inner mitochondrial membrane is impaired in the CNS of G93A-SOD1 mice. J. Neurosci. Off. J. Soc. Neurosci. 25, 164–172.

Kobayashi, Y., Arai, S., Waku, K., Sugiura, T., 2001. Activation by 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, of p42/44 mitogen-activated protein kinase in HL-60 cells. J. Biochem. (Tokyo) 129, 665–669.

Köfalvi, A., Rodrigues, R.J., Ledent, C., Mackie, K., Vizi, E.S., Cunha, R.A., Sperlágh, B., 2005. Involvement of Cannabinoid Receptors in the Regulation of Neurotransmitter Release in the Rodent Striatum: A Combined Immunochemical and Pharmacological Analysis. J. Neurosci. 25, 2874–2884.

Kondo, S., Kondo, H., Nakane, S., Kodaka, T., Tokumura, A., Waku, K., Sugiura, T., 1998. 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through CA2+-dependent and -independent mechanisms. FEBS Lett. 429, 152–156.

Kozutsumi, Y., Segal, M., Normington, K., Gething, M.J., Sambrook, J., 1988. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature 332, 462–464.

Krammer, E.B., Rath, T., Lischka, M.F., 1979. Somatotopic organization of the hypoglossal nucleus: a HRP study in the rat. Brain Res. 170, 533–537.

Kreitzer, A.C., Carter, A.G., Regehr, W.G., 2002. Inhibition of Interneuron Firing Extends the Spread of Endocannabinoid Signaling in the Cerebellum. Neuron 34, 787–796.

Kreitzer, A.C., Regehr, W.G., 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29, 717–727.

Krieger, C., Jones, K., Kim, S.U., Eisen, A.A., 1994. The role of intracellular free calcium in motor neuron disease. J. Neurol. Sci. 124 Suppl, 27–32.

Krieger, D.W., Yenari, M.A., 2004. Therapeutic hypothermia for acute ischemic stroke: what do laboratory studies teach us? Stroke J. Cereb. Circ. 35, 1482–1489.

Kuhse, J., Betz, H., Kirsch, J., 1995. The inhibitory glycine receptor: architecture, synaptic localization and molecular pathology of a postsynaptic ion-channel complex. Curr. Opin. Neurobiol. 5, 318–323.

Kujoth, G.C., Hiona, A., Pugh, T.D., Someya, S., Panzer, K., Wohlgemuth, S.E., Hofer, T., Seo, A.Y., Sullivan, R., Jobling, W.A., Morrow, J.D., Van Remmen, H., Sedivy, J.M., Yamasoba, T., Tanokura, M., Weindruch, R., Leeuwenburgh, C., Prolla, T.A., 2005. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 309, 481–484.

Kumar, D.R., Aslinia, F., Yale, S.H., Mazza, J.J., 2011. Jean-Martin Charcot: The Father of Neurology. Clin. Med. Res. 9, 46–49.

Lacomblez, L., Bensimon, G., Leigh, P.N., Guillet, P., Meininger, V., 1996. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Lancet 347, 1425–1431.

Lamanauskas, N., Nistri, A., 2008. Riluzole blocks persistent Na+ and Ca2+ currents and modulates release of glutamate via presynaptic NMDA receptors on neonatal rat hypoglossal motoneurons in vitro. Eur. J. Neurosci. 27, 2501–2514.

Langmore, S.E., Lehman, M.E., 1994. Physiologic deficits in the orofacial system underlying dysarthria in amyotrophic lateral sclerosis. J. Speech Hear. Res. 37, 28–37.

Lape, R., Nistri, A., 1999. Voltage-activated K+ currents of hypoglossal motoneurons in a brain stem slice preparation from the neonatal rat. J. Neurophysiol. 81, 140–148.

Lape, R., Nistri, A., 2000. Current and voltage clamp studies of the spike medium afterhyperpolarization of hypoglossal motoneurons in a rat brain stem slice preparation. J. Neurophysiol. 83, 2987–2995.

Lape, R., Nistri, A., 2001. Characteristics of fast Na(+) current of hypoglossal motoneurons in a rat brainstem slice preparation. Eur. J. Neurosci. 13, 763–772.

Laslo, P., Lipski, J., Nicholson, L.F., Miles, G.B., Funk, G.D., 2001. GluR2 AMPA receptor subunit expression in motoneurons at low and high risk for degeneration in amyotrophic lateral sclerosis. Exp. Neurol. 169, 461–471.

Lastres-Becker, I., Bizat, N., Boyer, F., Hantraye, P., Fernández-Ruiz, J., Brouillet, E., 2004. Potential involvement of cannabinoid receptors in 3-nitropropionic acid toxicity in vivo. Neuroreport 15, 2375–2379.

Lastres-Becker, I., Hansen, H.H., Berrendero, F., De Miguel, R., Pérez-Rosado, A., Manzanares, J., Ramos, J.A., Fernández-Ruiz, J., 2002. Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. Synap. N. Y. N 44, 23–35.

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.F., Beslot, F., Böhme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283, 401–404.

Lee, M.K., Marszalek, J.R., Cleveland, D.W., 1994. A mutant neurofilament subunit causes massive, selective motor neuron death: implications for the pathogenesis of human motor neuron disease. Neuron 13, 975–988.

Leigh, P.N., Whitwell, H., Garofalo, O., Buller, J., Swash, M., Martin, J.E., Gallo, J.M., Weller, R.O., Anderton, B.H., 1991. Ubiquitin-immunoreactive intraneuronal inclusions in amyotrophic lateral sclerosis. Morphology, distribution, and specificity. Brain J. Neurol. 114 (Pt 2), 775–788.

Leker, R.R., Gai, N., Mechoulam, R., Ovadia, H., 2003. Drug-induced hypothermia reduces ischemic damage: effects of the cannabinoid HU-210. Stroke J. Cereb. Circ. 34, 2000–2006.

Leker, R.R., Shohami, E., 2002. Cerebral ischemia and trauma-different etiologies yet similar mechanisms: neuroprotective opportunities. Brain Res. Brain Res. Rev. 39, 55–73.

Leung, D., Saghatelian, A., Simon, G.M., Cravatt, B.F., 2006. Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. Biochemistry (Mosc.) 45, 4720–4726.

Lévénés, C., Daniel, H., Soubrié, P., Crépel, F., 1998. Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. J. Physiol. 510 (Pt 3), 867–879.

Li, Q., Ma, H.-J., Zhang, H., Qi, Z., Guan, Y., Zhang, Y., 2009. Electrophysiological effects of anandamide on rat myocardium. Br. J. Pharmacol. 158, 2022–2029.

Li, Y.Q., Takada, M., Kaneko, T., Mizuno, N., 1997. Distribution of GABAergic and glycinergic premotor neurons projecting to the facial and hypoglossal nuclei in the rat. J. Comp. Neurol. 378, 283–294.

Ligresti, A., Morera, E., Van Der Stelt, M., Monory, K., Lutz, B., Ortar, G., Di Marzo, V., 2004. Further evidence for the existence of a specific process for the membrane transport of anandamide. Biochem. J. 380, 265–272.

Lim, R., Hoang, P., Berger, A.J., 2004. Blockade of glycine transporter-1 (GLYT-1) potentiates NMDA receptor-mediated synaptic transmission in hypoglossal motorneurons. J. Neurophysiol. 92, 2530–2537.

Lips, M.B., Keller, B.U., 1998. Endogenous calcium buffering in motoneurones of the nucleus hypoglossus from mouse. J. Physiol. 511 (Pt 1), 105–117.

Lipton, P., 1999. Ischemic cell death in brain neurons. Physiol. Rev. 79, 1431–1568.

Lipton, S.A., Rosenberg, P.A., 1994. Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med. 330, 613–622.

Liu, D., Wen, J., Liu, J., Li, L., 1999. The roles of free radicals in amyotrophic lateral sclerosis: reactive oxygen species and elevated oxidation of protein, DNA, and membrane phospholipids. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 13, 2318–2328.

Liu, J., Lillo, C., Jonsson, P.A., Vande Velde, C., Ward, C.M., Miller, T.M., Subramaniam, J.R., Rothstein, J.D., Marklund, S., Andersen, P.M., Brännström, T., Gredal, O., Wong, P.C., Williams,

D.S., Cleveland, D.W., 2004. Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. Neuron 43, 5–17.

Lloyd, C.M., Richardson, M.P., Brooks, D.J., Al-Chalabi, A., Leigh, P.N., 2000. Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. Brain J. Neurol. 123 (Pt 11), 2289–2296.

Lowe, A.A., 1980. The neural regulation of tongue movements. Prog. Neurobiol. 15, 295–344.

Lozovaya, N., Mukhtarov, M., Tsintsadze, T., Ledent, C., Burnashev, N., Bregestovski, P., 2011. Frequency-Dependent Cannabinoid Receptor-Independent Modulation of Glycine Receptors by Endocannabinoid 2-AG. Front. Mol. Neurosci. 4, 13.

Mackie, K., 2005. Cannabinoid receptor homo- and heterodimerization. Life Sci. 77, 1667–1673.

Mackie, K., 2006. Mechanisms of CB1 receptor signaling: endocannabinoid modulation of synaptic strength. Int. J. Obes. 2005 30 Suppl 1, S19–23.

Mackie, K., Devane, W.A., Hille, B., 1993. Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. Mol. Pharmacol. 44, 498–503.

Mackie, K., Lai, Y., Westenbroek, R., Mitchell, R., 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. J. Neurosci. Off. J. Soc. Neurosci. 15, 6552–6561.

Maejima, T., Hashimoto, K., Yoshida, T., Aiba, A., Kano, M., 2001. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. Neuron 31, 463–475.

Magrané, J., Manfredi, G., 2009. Mitochondrial function, morphology, and axonal transport in amyotrophic lateral sclerosis. Antioxid. Redox Signal. 11, 1615–1626.

Mailleux, P., Parmentier, M., Vanderhaeghen, J.J., 1992. Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. Neurosci. Lett. 143, 200–204.

Mailleux, P., Vanderhaeghen, J.J., 1992. Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience 48, 655–668.

Maingret, F., Patel, A.J., Lazdunski, M., Honoré, E., 2001. The endocannabinoid anandamide is a direct and selective blocker of the background K(+) channel TASK-1. EMBO J. 20, 47–54.

Malessa, S., Leigh, P.N., Bertel, O., Sluga, E., Hornykiewicz, O., 1991. Amyotrophic lateral sclerosis: glutamate dehydrogenase and transmitter amino acids in the spinal cord. J. Neurol. Neurosurg. Psychiatry 54, 984–988.

Mali, Y., Zisapels, N., 2008. Gain of interaction of ALS-linked G93A superoxide dismutase with cytosolic malate dehydrogenase. Neurobiol. Dis. 32, 133–141.

Mancuso, R., Osta, R., Navarro, X., 2014. Presymptomatic electrophysiological tests predict clinical onset and survival in SOD1(G93A) ALS mice. Muscle Nerve 50, 943–949.

Mancuso, R., Santos-Nogueira, E., Osta, R., Navarro, X., 2011. Electrophysiological analysis of a murine model of motoneuron disease. Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol. 122, 1660–1670.

Maneuf, Y.P., Brotchie, J.M., 1997. Paradoxical action of the cannabinoid WIN 55,212-2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices. Br. J. Pharmacol. 120, 1397–1398.

Marchetti, C., Pagnotta, S., Donato, R., Nistri, A., 2002. Inhibition of spinal or hypoglossal motoneurons of the newborn rat by glycine or GABA. Eur. J. Neurosci. 15, 975–983.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., López-Rodriguez, M.L., Casanova, E., Schütz, G., Zieglgänsberger, W., Di Marzo, V., Behl, C., Lutz, B., 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302, 84–88.

Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur. J. Neurosci. 11, 4213–4225.

Martin, L.J., 2007. Transgenic mice with human mutant genes causing Parkinson's disease and amyotrophic lateral sclerosis provide common insight into mechanisms of motor neuron selective vulnerability to degeneration. Rev. Neurosci. 18, 115–136.

Martin, L.J., Liu, Z., Chen, K., Price, A.C., Pan, Y., Swaby, J.A., Golden, W.C., 2007. Motor neuron degeneration in amyotrophic lateral sclerosis mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell death. J. Comp. Neurol. 500, 20–46.

Martins, D., Tavares, I., Morgado, C., 2014. "Hotheaded": the role OF TRPV1 in brain functions. Neuropharmacology 85, 151–157.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., Wada, K., 1995. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J. Neurochem. 65, 454–458.

Mattiazzi, M., D'Aurelio, M., Gajewski, C.D., Martushova, K., Kiaei, M., Beal, M.F., Manfredi, G., 2002. Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J. Biol. Chem. 277, 29626–29633.

Mattsson, P., Lönnstedt, I., Nygren, I., Askmark, H., 2012. Physical fitness, but not muscle strength, is a risk factor for death in amyotrophic lateral sclerosis at an early age. J. Neurol. Neurosurg. Psychiatry 83, 390–394.

Mazza, E., Núñez-Abades, P.A., Spielmann, J.M., Cameron, W.E., 1992. Anatomical and electrotonic coupling in developing genioglossal motoneurons of the rat. Brain Res. 598, 127–137.

McAllister, S.D., Griffin, G., Satin, L.S., Abood, M.E., 1999. Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a xenopus oocyte expression system. J. Pharmacol. Exp. Ther. 291, 618–626.

McFarland, M.J., Barker, E.L., 2004. Anandamide transport. Pharmacol. Ther. 104, 117–135.

McFarland, M.J., Porter, A.C., Rakhshan, F.R., Rawat, D.S., Gibbs, R.A., Barker, E.L., 2004. A role for caveolae/lipid rafts in the uptake and recycling of the endogenous cannabinoid anandamide. J. Biol. Chem. 279, 41991–41997.

McGuire, V., Longstreth, W.T., Nelson, L.M., Koepsell, T.D., Checkoway, H., Morgan, M.S., van Belle, G., 1997. Occupational exposures and amyotrophic lateral sclerosis. A population-based case-control study. Am. J. Epidemiol. 145, 1076–1088.

McIntosh, H.H., Song, C., Howlett, A.C., 1998. CB1 cannabinoid receptor: cellular regulation and distribution in N18TG2 neuroblastoma cells. Mol. Brain Res. 53, 163–173.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83–90.

Mechoulam, R., Fride, E., Di Marzo, V., 1998. Endocannabinoids. Eur. J. Pharmacol. 359, 1–18.

Mechoulam, R., Panikashvili, D., Shohami, E., 2002a. Cannabinoids and brain injury: therapeutic implications. Trends Mol. Med. 8, 58–61.

Mechoulam, R., Spatz, M., Shohami, E., 2002b. Endocannabinoids and Neuroprotection. Sci. Signal. 2002, re5–re5.

Meininger, V., Lacomblez, L., Salachas, F., 2000. What has changed with riluzole? J. Neurol. 247 Suppl 6, VI/19–22.

Mendonça, D.M.F., Chimelli, L., Martinez, A.M.B., 2006. Expression of ubiquitin and proteasome in motorneurons and astrocytes of spinal cords from patients with amyotrophic lateral sclerosis. Neurosci. Lett. 404, 315–319.

Millecamps, S., Robertson, J., Lariviere, R., Mallet, J., Julien, J.-P., 2006. Defective axonal transport of neurofilament proteins in neurons overexpressing peripherin. J. Neurochem. 98, 926–938.

Miller, R.G., Mitchell, J.D., Lyon, M., Moore, D.H., 1996. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND), in: Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd.

Mitsumoto, H., Hanson, M.R., Chad, D.A., 1988. Amyotrophic lateral sclerosis. Recent advances in pathogenesis and therapeutic trials. Arch. Neurol. 45, 189–202.

Mombouli, J.V., Schaeffer, G., Holzmann, S., Kostner, G.M., Graier, W.F., 1999. Anandamide-induced mobilization of cytosolic Ca2+ in endothelial cells. Br. J. Pharmacol. 126, 1593–1600.

Monory, K., Massa, F., Egertová, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W., Jacob, W., Marsch, R., Ekker, M., Long, J., Rubenstein, J.L., Goebbels, S., Nave, K.-A., During, M., Klugmann, M., Wölfel, B., Dodt, H.-U., Zieglgänsberger, W., Wotjak, C.T., Mackie, K., Elphick, M.R., Marsicano, G., Lutz, B., 2006. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 51, 455–466.

Moritz, A.T., Newkirk, G., Powers, R.K., Binder, M.D., 2007. Facilitation of somatic calcium channels can evoke prolonged tail currents in rat hypoglossal motoneurons. J. Neurophysiol. 98, 1042–1047.

Morozova, N., Weisskopf, M.G., McCullough, M.L., Munger, K.L., Calle, E.E., Thun, M.J., Ascherio, A., 2008. Diet and amyotrophic lateral sclerosis. Epidemiol. Camb. Mass 19, 324–337.

Mosfeldt Laursen, A., Rekling, J.C., 1989. Electrophysiological properties of hypoglossal motoneurons of guinea-pigs studied in vitro. Neuroscience 30, 619–637.

Mothet, J.P., Parent, A.T., Wolosker, H., Brady, R.O., Linden, D.J., Ferris, C.D., Rogawski, M.A., Snyder, S.H., 2000. D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proc. Natl. Acad. Sci. U. S. A. 97, 4926–4931.

Movsesyan, V.A., Stoica, B.A., Yakovlev, A.G., Knoblach, S.M., Lea, P.M., Cernak, I., Vink, R., Faden, A.I., 2004. Anandamide-induced cell death in primary neuronal cultures: role of calpain and caspase pathways. Cell Death Differ. 11, 1121–1132.

Mu, J., Zhuang, S.Y., Hampson, R.E., Deadwyler, S.A., 2000. Protein kinase-dependent phosphorylation and cannabinoid receptor modulation of potassium A current (IA) in cultured rat hippocampal neurons. Pflüg. Arch. Eur. J. Physiol. 439, 541–546.

Mu, J., Zhuang, S.Y., Kirby, M.T., Hampson, R.E., Deadwyler, S.A., 1999. Cannabinoid receptors differentially modulate potassium A and D currents in hippocampal neurons in culture. J. Pharmacol. Exp. Ther. 291, 893–902.

Mukhopadhyay, S., McIntosh, H.H., Houston, D.B., Howlett, A.C., 2000. The CB(1) cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. Mol. Pharmacol. 57, 162–170.

Mukhtarov, M., Ragozzino, D., Bregestovski, P., 2005. Dual Ca2+ modulation of glycinergic synaptic currents in rodent hypoglossal motoneurones. J. Physiol. 569, 817–831.

Muller, E., Triller, A., Legendre, P., 2004. Glycine receptors and GABA receptor alpha 1 and gamma 2 subunits during the development of mouse hypoglossal nucleus. Eur. J. Neurosci. 20, 3286–3300.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Muthian, S., Rademacher, D.J., Roelke, C.T., Gross, G.J., Hillard, C.J., 2004. Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. Neuroscience 129, 743–750.

Nagai, M., Re, D.B., Nagata, T., Chalazonitis, A., Jessell, T.M., Wichterle, H., Przedborski, S., 2007. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat. Neurosci. 10, 615–622.

Nagayama, T., Sinor, A.D., Simon, R.P., Chen, J., Graham, S.H., Jin, K., Greenberg, D.A., 1999. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. J. Neurosci. Off. J. Soc. Neurosci. 19, 2987–2995.

Nakamura, Y., Katakura, N., 1995. Generation of masticatory rhythm in the brainstem. Neurosci. Res. 23, 1–19.

Nakane, S., Oka, S., Arai, S., Waku, K., Ishima, Y., Tokumura, A., Sugiura, T., 2002. 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. Arch. Biochem. Biophys. 402, 51–58.

Nakazi, M., Bauer, U., Nickel, T., Kathmann, M., Schlicker, E., 2000. Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors. Naunyn. Schmiedebergs Arch. Pharmacol. 361, 19–24.

Netzeband, J.G., Conroy, S.M., Parsons, K.L., Gruol, D.L., 1999. Cannabinoids enhance NMDA-elicited Ca2+ signals in cerebellar granule neurons in culture. J. Neurosci. Off. J. Soc. Neurosci. 19, 8765–8777.

Nicholson, R.A., Liao, C., Zheng, J., David, L.S., Coyne, L., Errington, A.C., Singh, G., Lees, G., 2003. Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. Brain Res. 978, 194–204.

Niebroj-Dobosz, I., Jamrozik, Z., Janik, P., Hausmanowa-Petrusewicz, I., Kwieciński, H., 1999. Anti-neural antibodies in serum and cerebrospinal fluid of amyotrophic lateral sclerosis (ALS) patients. Acta Neurol. Scand. 100, 238–243.

Nishimura, A.L., Mitne-Neto, M., Silva, H.C.A., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J.R.M., Gillingwater, T., Webb, J., Skehel, P., Zatz, M., 2004. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am. J. Hum. Genet. 75, 822–831.

Nishitoh, H., Kadowaki, H., Nagai, A., Maruyama, T., Yokota, T., Fukutomi, H., Noguchi, T., Matsuzawa, A., Takeda, K., Ichijo, H., 2008. ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. Genes Dev. 22, 1451–1464.

Núñez-Abades, P.A., Cameron, W.E., 1995. Morphology of developing rat genioglossal motoneurons studied in vitro: relative changes in diameter and surface area of somata and dendrites. J. Comp. Neurol. 353, 129–142.

Nüñez-Abades, P.A., He, F., Barrionuevo, G., Cameron, W.E., 1994. Morphology of developing rat genioglossal motoneurons studied in vitro: Changes in length, branching pattern, and spatial distribution of dendrites. J. Comp. Neurol. 339, 401–420.

O'Brien, J.A., Berger, A.J., 1999. Cotransmission of GABA and glycine to brain stem motoneurons. J. Neurophysiol. 82, 1638–1641.

O'Brien, J.A., Isaacson, J.S., Berger, A.J., 1997. NMDA and non-NMDA receptors are co-localized at excitatory synapses of rat hypoglossal motoneurons. Neurosci. Lett. 227, 5–8.

Ohno-Shosaku, T., Maejima, T., Kano, M., 2001. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29, 729–738.

Ohno-Shosaku, T., Shosaku, J., Tsubokawa, H., Kano, M., 2002. Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. Eur. J. Neurosci. 15, 953–961.

Okamoto, K., Kihira, T., Kondo, T., Kobashi, G., Washio, M., Sasaki, S., Yokoyama, T., Miyake, Y., Sakamoto, N., Inaba, Y., Nagai, M., 2007b. Nutritional status and risk of amyotrophic lateral sclerosis in Japan. Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 8, 300–304.

Okamoto, Y., Wang, J., Morishita, J., Ueda, N., 2007a. Biosynthetic pathways of the endocannabinoid anandamide. Chem. Biodivers. 4, 1842–1857.

Ono, T., Ishiwata, Y., Inaba, N., Kuroda, T., Nakamura, Y., 1998. Modulation of the inspiratory-related activity of hypoglossal premotor neurons during ingestion and rejection in the decerebrate cat. J. Neurophysiol. 80, 48–58.

Orrell, R.W., Lane, R.J.M., Ross, M., 2008. A systematic review of antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Amyotroph. Lateral Scler. 9, 195–211.

Oz, M., Ravindran, A., Diaz-Ruiz, O., Zhang, L., Morales, M., 2003. The endogenous cannabinoid anandamide inhibits alpha7 nicotinic acetylcholine receptor-mediated responses in Xenopus oocytes. J. Pharmacol. Exp. Ther. 306, 1003–1010.

Oz, M., Yang, K.-H., Dinc, M., Shippenberg, T.S., 2007. The endogenous cannabinoid anandamide inhibits cromakalim-activated K+ currents in follicle-enclosed Xenopus oocytes. J. Pharmacol. Exp. Ther. 323, 547–554.

Oz, M., Zhang, L., Morales, M., 2002. Endogenous cannabinoid, anandamide, acts as a noncompetitive inhibitor on 5-HT3 receptor-mediated responses in Xenopus oocytes. Synap. N. Y. N 46, 150–156.

Oz, M., Zhang, L., Ravindran, A., Morales, M., Lupica, C.R., 2004. Differential effects of endogenous and synthetic cannabinoids on alpha7-nicotinic acetylcholine receptor-mediated responses in Xenopus Oocytes. J. Pharmacol. Exp. Ther. 310, 1152–1160.

Pacher , P., Bátkai, S., Kunos, G., 2006. The Endocannabinoid System as an Emerging Target of Pharmacotherapy. Pharmacol. Rev. 58, 389–462.

Palecek, J., Lips, M.B., Keller, B.U., 1999. Calcium dynamics and buffering in motoneurones of the mouse spinal cord. J. Physiol. 520, 485–502.

Pan, X., Ikeda, S.R., Lewis, D.L., 1996. Rat brain cannabinoid receptor modulates N-type Ca2+ channels in a neuronal expression system. Mol. Pharmacol. 49, 707–714.

Panatier, A., Theodosis, D.T., Mothet, J.-P., Touquet, B., Pollegioni, L., Poulain, D.A., Oliet, S.H.R., 2006. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell 125, 775–784.

Panikashvili, D., Mechoulam, R., Beni, S.M., Alexandrovich, A., Shohami, E., 2005. CB1 cannabinoid receptors are involved in neuroprotection via NF-kappa B inhibition. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 25, 477–484.

Panikashvili, D., Simeonidou, C., Ben-Shabat, S., Hanus, L., Breuer, A., Mechoulam, R., Shohami, E., 2001. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 413, 527–531.

Papadimitriou, D., Le Verche, V., Jacquier, A., Ikiz, B., Przedborski, S., Re, D.B., 2010. Inflammation in ALS and SMA: sorting out the good from the evil. Neurobiol. Dis. 37, 493–502.

Park, R.M., Schulte, P.A., Bowman, J.D., Walker, J.T., Bondy, S.C., Yost, M.G., Touchstone, J.A., Dosemeci, M., 2005. Potential occupational risks for neurodegenerative diseases. Am. J. Ind. Med. 48, 63–77.

Parmentier-Batteur, S., Jin, K., Mao, X.O., Xie, L., Greenberg, D.A., 2002. Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. J. Neurosci. Off. J. Soc. Neurosci. 22, 9771–9775.

Pasinelli, P., Brown, R.H., 2006. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Nat. Rev. Neurosci. 7, 710–723.

Paton, J.F., Richter, D.W., 1995. Role of fast inhibitory synaptic mechanisms in respiratory rhythm generation in the maturing mouse. J. Physiol. 484 (Pt 2), 505–521.

Peever, J.H., Shen, L., Duffin, J., 2002. Respiratory pre-motor control of hypoglossal motoneurons in the rat. Neuroscience 110, 711–722.

Perlson, E., Jeong, G.-B., Ross, J.L., Dixit, R., Wallace, K.E., Kalb, R.G., Holzbaur, E.L.F., 2009. A switch in retrograde signaling from survival to stress in rapid-onset neurodegeneration. J. Neurosci. Off. J. Soc. Neurosci. 29, 9903–9917.

Perry, T.L., Krieger, C., Hansen, S., Eisen, A., 1990. Amyotrophic lateral sclerosis: amino acid levels in plasma and cerebrospinal fluid. Ann. Neurol. 28, 12–17.

Pertwee, R.G., 1997. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol. Ther. 74, 129–180.

Pertwee, R.G., 2005. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J. 7, E625–E654.

Pertwee, R.G., 2006. The pharmacology of cannabinoid receptors and their ligands: an overview. Int. J. Obes. 2005 30 Suppl 1, S13–18.

Pertwee, R.G., Ross, R.A., 2002. Cannabinoid receptors and their ligands. Prostaglandins Leukot. Essent. Fat. Acids PLEFA 66, 101–121.

Petri, S., Kollewe, K., Grothe, C., Hori, A., Dengler, R., Bufler, J., Krampfl, K., 2006. GABA(A)-receptor mRNA expression in the prefrontal and temporal cortex of ALS patients. J. Neurol. Sci. 250, 124–132.

Petri, S., Krampfl, K., Hashemi, F., Grothe, C., Hori, A., Dengler, R., Bufler, J., 2003. Distribution of GABAA receptor mRNA in the motor cortex of ALS patients. J. Neuropathol. Exp. Neurol. 62, 1041–1051.

Petrocellis, L.D., Cascio, M.G., Marzo, V.D., 2004. The endocannabinoid system: a general view and latest additions. Br. J. Pharmacol. 141, 765–774.

Pettit, D.A., Harrison, M.P., Olson, J.M., Spencer, R.F., Cabral, G.A., 1998. Immunohistochemical localization of the neural cannabinoid receptor in rat brain. J. Neurosci. Res. 51, 391–402.

Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. Nat. Rev. Neurosci. 4, 873–884.

Piomelli, D., Beltramo, M., Giuffrida, A., Stella, N., 1998. Endogenous cannabinoid signaling. Neurobiol. Dis. 5, 462–473.

Piomelli, D., Beltramo, M., Glasnapp, S., Lin, S.Y., Goutopoulos, A., Xie, X.Q., Makriyannis, A., 1999. Structural determinants for recognition and translocation by the anandamide transporter. Proc. Natl. Acad. Sci. U. S. A. 96, 5802–5807.

Poon, H.F., Hensley, K., Thongboonkerd, V., Merchant, M.L., Lynn, B.C., Pierce, W.M., Klein, J.B., Calabrese, V., Butterfield, D.A., 2005. Redox proteomics analysis of oxidatively modified proteins in G93A-SOD1 transgenic mice--a model of familial amyotrophic lateral sclerosis. Free Radic. Biol. Med. 39, 453–462.

Popratiloff, A.S., Streppel, M., Gruart, A., Guntinas-Lichius, O., Angelov, D.N., Stennert, E., Delgado-García, J.M., Neiss, W.F., 2001. Hypoglossal and reticular interneurons involved in oro-facial coordination in the rat. J. Comp. Neurol. 433, 364–379.

Porter, A.C., Sauer, J.-M., Knierman, M.D., Becker, G.W., Berna, M.J., Bao, J., Nomikos, G.G., Carter, P., Bymaster, F.P., Leese, A.B., Felder, C.C., 2002. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. J. Pharmacol. Exp. Ther. 301, 1020–1024.

Porter, R., 1967. The synaptic basis of a bilateral lingual-hypoglossal reflex in cats. J. Physiol. 190, 611–627.

Powers, R.K., Binder, M.D., 2003. Persistent sodium and calcium currents in rat hypoglossal motoneurons. J. Neurophysiol. 89, 615–624.

Puttaparthi, K., Elliott, J.L., 2005. Non-neuronal induction of immunoproteasome subunits in an ALS model: possible mediation by cytokines. Exp. Neurol. 196, 441–451.

Raman, C., McAllister, S.D., Rizvi, G., Patel, S.G., Moore, D.H., Abood, M.E., 2004. Amyotrophic lateral sclerosis: delayed disease progression in mice by treatment with a cannabinoid. Amyotroph.

Lateral Scler. Mot. Neuron Disord. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 5, 33–39.

Ramírez, B.G., Blázquez, C., Gómez del Pulgar, T., Guzmán, M., de Ceballos, M.L., 2005. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. J. Neurosci. Off. J. Soc. Neurosci. 25, 1904–1913.

Ramirez, J.-M., Garcia, A.J., Anderson, T.M., Koschnitzky, J.E., Peng, Y.-J., Kumar, G., Prabhakar, N., 2013. Central and Peripheral factors contributing to Obstructive Sleep Apneas. Respir. Physiol. Neurobiol. 189, 344–353.

Ramirez, J.M., Quellmalz, U.J., Wilken, B., 1997. Developmental changes in the hypoxic response of the hypoglossus respiratory motor output in vitro. J. Neurophysiol. 78, 383–392.

Raoul, C., Estévez, A.G., Nishimune, H., Cleveland, D.W., deLapeyrière, O., Henderson, C.E., Haase, G., Pettmann, B., 2002. Motoneuron death triggered by a specific pathway downstream of Fas. potentiation by ALS-linked SOD1 mutations. Neuron 35, 1067–1083.

Redler, R.L., Dokholyan, N.V., 2012. The Complex Molecular Biology of Amyotrophic Lateral Sclerosis (ALS). Prog. Mol. Biol. Transl. Sci. 107, 215–262.

Rekling, J.C., 1992. Interaction between thyrotropin-releasing hormone (TRH) and NMDA-receptor-mediated responses in hypoglossal motoneurones. Brain Res. 578, 289–296.

Rekling, J.C., Feldman, J.L., 1998. PreBötzinger complex and pacemaker neurons: hypothesized site and kernel for respiratory rhythm generation. Annu. Rev. Physiol. 60, 385–405.

Rekling, J.C., Funk, G.D., Bayliss, D.A., Dong, X.W., Feldman, J.L., 2000. Synaptic control of motoneuronal excitability. Physiol. Rev. 80, 767–852.

Rekling, J.C., Laursen, A.M., 1989. Evidence for a persistent sodium conductance in neurons from the nucleus prepositus hypoglossi. Brain Res. 500, 276–286.

Remmers, J.E., deGroot, W.J., Sauerland, E.K., Anch, A.M., 1978. Pathogenesis of upper airway occlusion during sleep. J. Appl. Physiol. 44, 931–938.

Robbe, D., Alonso, G., Duchamp, F., Bockaert, J., Manzoni, O.J., 2001. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J. Neurosci. Off. J. Soc. Neurosci. 21, 109–116.

Roda, F., Gestreau, C., Bianchi, A.L., 2002. Discharge patterns of hypoglossal motoneurons during fictive breathing, coughing, and swallowing. J. Neurophysiol. 87, 1703–1711.

Rog, D.J., Nurmikko, T.J., Friede, T., Young, C.A., 2005. Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. Neurology 65, 812–819.

Rog, D.J., Nurmikko, T.J., Young, C.A., 2007. Oromucosal delta9-tetrahydrocannabinol/cannabidiol for neuropathic pain associated with multiple sclerosis: an uncontrolled, open-label, 2-year extension trial. Clin. Ther. 29, 2068–2079.

Rosen, A.D., 1978. Amyotrophic lateral sclerosis. Clinical features and prognosis. Arch. Neurol. 35, 638–642.

Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J.P., Deng, H.X., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362, 59–62.

Ross, R.A., 2003. Anandamide and vanilloid TRPV1 receptors. Br. J. Pharmacol. 140, 790–801.

Rossi, S., De Chiara, V., Musella, A., Cozzolino, M., Bernardi, G., Maccarrone, M., Mercuri, N.B., Carrì, M.T., Centonze, D., 2010. Abnormal sensitivity of cannabinoid CB1 receptors in the striatum of mice with experimental amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 11, 83–90.

Rothstein, J.D., Dykes-Hoberg, M., Pardo, C.A., Bristol, L.A., Jin, L., Kuncl, R.W., Kanai, Y., Hediger, M.A., Wang, Y., Schielke, J.P., Welty, D.F., 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–686.

Rothstein, J.D., Kuncl, R.W., 1995. Neuroprotective Strategies in a Model of Chronic Glutamate-Mediated Motor Neuron Toxicity. J. Neurochem. 65, 643–651.

Rothstein, J.D., Martin, L.J., Kuncl, R.W., 1992. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. N. Engl. J. Med. 326, 1464–1468.

Rothstein, J.D., Van Kammen, M., Levey, A.I., Martin, L.J., Kuncl, R.W., 1995. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann. Neurol. 38, 73–84.

Rowland, K.C., Irby, N.K., Spirou, G.A., 2000. Specialized synapse-associated structures within the calyx of Held. J. Neurosci. Off. J. Soc. Neurosci. 20, 9135–9144.

Rozzo, A., Ballerini, L., Abbate, G., Nistri, A., 2002. Experimental and modeling studies of novel bursts induced by blocking na(+) pump and synaptic inhibition in the rat spinal cord. J. Neurophysiol. 88, 676–691.

Saboisky, J.P., Chamberlin, N.L., Malhotra, A., 2009. Potential therapeutic targets in obstructive sleep apnoea. Expert Opin. Ther. Targets 13, 795–809.

Sánchez, C., Galve-Roperh, I., Rueda, D., Guzmán, M., 1998. Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Delta9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. Mol. Pharmacol. 54, 834–843.

Sargsyan, S.A., Monk, P.N., Shaw, P.J., 2005. Microglia as potential contributors to motor neuron injury in amyotrophic lateral sclerosis. Glia 51, 241–253.

Sasabe, J., Chiba, T., Yamada, M., Okamoto, K., Nishimoto, I., Matsuoka, M., Aiso, S., 2007. D-serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. EMBO J. 26, 4149–4159.

Sasaki, S., 2010. Endoplasmic reticulum stress in motor neurons of the spinal cord in sporadic amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 69, 346–355.

Sasaki, S., Iwata, M., 1996. Impairment of fast axonal transport in the proximal axons of anterior horn neurons in amyotrophic lateral sclerosis. Neurology 47, 535–540.

Sasaki, S., Komori, T., Iwata, M., 2000. Excitatory amino acid transporter 1 and 2 immunoreactivity in the spinal cord in amyotrophic lateral sclerosis. Acta Neuropathol. (Berl.) 100, 138–144.

Satoh, I., Shiba, K., Kobayashi, N., Nakajima, Y., Konno, A., 1998. Upper airway motor outputs during sneezing and coughing in decerebrate cats. Neurosci. Res. 32, 131–135.

Sau, D., De Biasi, S., Vitellaro-Zuccarello, L., Riso, P., Guarnieri, S., Porrini, M., Simeoni, S., Crippa, V., Onesto, E., Palazzolo, I., Rusmini, P., Bolzoni, E., Bendotti, C., Poletti, A., 2007. Mutation of SOD1 in ALS: a gain of a loss of function. Hum. Mol. Genet. 16, 1604–1618.

Sawczuk, A., Mosier, K.M., 2001. Neural control of tongue movement with respect to respiration and swallowing. Crit. Rev. Oral Biol. Med. Off. Publ. Am. Assoc. Oral Biol. 12, 18–37.

Sawczuk, A., Powers, R.K., Binder, M.D., 1995. Spike frequency adaptation studied in hypoglossal motoneurons of the rat. J. Neurophysiol. 73, 1799–1810.

Saxena, S., Cabuy, E., Caroni, P., 2009. A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. Nat. Neurosci. 12, 627–636.

Schell, M.J., Molliver, M.E., Snyder, S.H., 1995. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc. Natl. Acad. Sci. U. S. A. 92, 3948–3952.

Schlicker, E., Kathmann, M., 2001. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol. Sci. 22, 565–572.

Schmid, H.H.O., Schmid, P.C., Berdyshev, E.V., 2002. Cell signaling by endocannabinoids and their congeners: questions of selectivity and other challenges. Chem. Phys. Lipids 121, 111–134.

Schmidt, S., Kwee, L.C., Allen, K.D., Oddone, E.Z., 2010. Association of ALS with head injury, cigarette smoking and APOE genotypes. J. Neurol. Sci. 291, 22–29.

Schoepp, D.D., Jane, D.E., Monn, J.A., 1999. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 38, 1431–1476.

Schröder, M., Kaufman, R.J., 2005. ER stress and the unfolded protein response. Mutat. Res. 569, 29–63.

Schweitzer, P., 2000. Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. J. Neurosci. Off. J. Soc. Neurosci. 20, 51–58.

Schweitzer, P., 2000. Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. J. Neurosci. Off. J. Soc. Neurosci. 20, 51–58.

Scotter, E.L., Abood, M.E., Glass, M., 2010. The endocannabinoid system as a target for the treatment of neurodegenerative disease. Br. J. Pharmacol. 160, 480–498.

Selley, D.E., Stark, S., Sim, L.J., Childers, S.R., 1996. Cannabinoid receptor stimulation of guanosine-5'-O-(3-[35S]thio)triphosphate binding in rat brain membranes. Life Sci. 59, 659–668.

Semyanov, A., Walker, M.C., Kullmann, D.M., 2003. GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat. Neurosci. 6, 484–490.

Shao, X.M., Feldman, J.L., 2005. Cholinergic neurotransmission in the preBötzinger Complex modulates excitability of inspiratory neurons and regulates respiratory rhythm. Neuroscience 130, 1069–1081.

Sharifullina, E., 2006. Oscillatory motor discharges by brainstem neurons: functional mechanisms and consequences for cell survival.

Sharifullina, E., Nistri, A., 2006. Glutamate uptake block triggers deadly rhythmic bursting of neonatal rat hypoglossal motoneurons. J. Physiol. 572, 407–423.

Sharifullina, E., Ostroumov, K., Grandolfo, M., Nistri, A., 2008. N-methyl-D-aspartate triggers neonatal rat hypoglossal motoneurons in vitro to express rhythmic bursting with unusual Mg2+ sensitivity. Neuroscience 154, 804–820.

Sharifullina, E., Ostroumov, K., Nistri, A., 2005. Metabotropic glutamate receptor activity induces a novel oscillatory pattern in neonatal rat hypoglossal motoneurones. J. Physiol. 563, 139–159.

Shaw, P.J., Eggett, C.J., 2000. Molecular factors underlying selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. J. Neurol. 247 Suppl 1, I17–27.

Shaw, P.J., Ince, P.G., Falkous, G., Mantle, D., 1995. Oxidative damage to protein in sporadic motor neuron disease spinal cord. Ann. Neurol. 38, 691–695.

Shaw, P.J., Williams, T.L., Slade, J.Y., Eggett, C.J., Ince, P.G., 1999. Low expression of GluR2 AMPA receptor subunit protein by human motor neurons. Neuroreport 10, 261–265.

Shea, T.B., Zheng, Y.-L., Ortiz, D., Pant, H.C., 2004. Cyclin-dependent kinase 5 increases perikaryal neurofilament phosphorylation and inhibits neurofilament axonal transport in response to oxidative stress. J. Neurosci. Res. 76, 795–800.

Shen, M., Thayer, S.A., 1998. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Mol. Pharmacol. 54, 459–462.

Shintani, T., Anker, A.R., Billig, I., Card, J.P., Yates, B.J., 2003. Transneuronal tracing of neural pathways influencing both diaphragm and genioglossal muscle activity in the ferret. J. Appl. Physiol. 95, 1453–1459.

Shivachar, A.C., Martin, B.R., Ellis, E.F., 1996. Anandamide- and delta9-tetrahydrocannabinol-evoked arachidonic acid mobilization and blockade by SR141716A [N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4 -methyl-1H-pyrazole-3-carboximide hydrochloride]. Biochem. Pharmacol. 51, 669–676.

Shleper, M., Kartvelishvily, E., Wolosker, H., 2005. D-serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. J. Neurosci. Off. J. Soc. Neurosci. 25, 9413–9417.

Shouman, B., Fontaine, R.H., Baud, O., Schwendimann, L., Keller, M., Spedding, M., Lelièvre, V., Gressens, P., 2006. Endocannabinoids potently protect the newborn brain against AMPA-kainate receptor-mediated excitotoxic damage. Br. J. Pharmacol. 148, 442–451.

Siklós, L., Engelhardt, J., Harati, Y., Smith, R.G., Joó, F., Appel, S.H., 1996. Ultrastructural evidence for altered calcium in motor nerve terminals in amyotropic lateral sclerosis. Ann. Neurol. 39, 203–216.

Singer, J.H., Berger, A.J., 2000. Development of inhibitory synaptic transmission to motoneurons. Brain Res. Bull. 53, 553–560.

Sink, K.S., Segovia, K.N., Sink, J., Randall, P.A., Collins, L.E., Correa, M., Markus, E.J., Vemuri, V.K., Makriyannis, A., Salamone, J.D., 2010. Potential anxiogenic effects of cannabinoid CB1 receptor antagonists/inverse agonists in rats: comparisons between AM4113, AM251, and the benzodiazepine inverse agonist FG-7142. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 20, 112–122.

Slipetz, D.M., O'Neill, G.P., Favreau, L., Dufresne, C., Gallant, M., Gareau, Y., Guay, D., Labelle, M., Metters, K.M., 1995. Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. Mol. Pharmacol. 48, 352–361.

Smart, D., Gunthorpe, M.J., Jerman, J.C., Nasir, S., Gray, J., Muir, A.I., Chambers, J.K., Randall, A.D., Davis, J.B., 2000. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). Br. J. Pharmacol. 129, 227–230.

Smith, J.C., Abdala, A.P.L., Koizumi, H., Rybak, I.A., Paton, J.F.R., 2007. Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. J. Neurophysiol. 98, 3370–3387.

Smith, J.C., Ellenberger, H.H., Ballanyi, K., Richter, D.W., Feldman, J.L., 1991. Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254, 726–729.

Sokoloff, A.J., Deacon, T.W., 1992. Musculotopic organization of the hypoglossal nucleus in the cynomolgus monkey, Macaca fascicularis. J. Comp. Neurol. 324, 81–93.

Sommer, C., Schomacher, M., Berger, C., Kuhnert, K., Müller, H.D., Schwab, S., Schäbitz, W.-R., 2006. Neuroprotective cannabinoid receptor antagonist SR141716A prevents downregulation of excitotoxic NMDA receptors in the ischemic penumbra. Acta Neuropathol. (Berl.) 112, 277–286.

Spivak, C.E., Lupica, C.R., Oz, M., 2007. The endocannabinoid anandamide inhibits the function of alpha4beta2 nicotinic acetylcholine receptors. Mol. Pharmacol. 72, 1024–1032.

Spreux-Varoquaux, O., Bensimon, G., Lacomblez, L., Salachas, F., Pradat, P.F., Le Forestier, N., Marouan, A., Dib, M., Meininger, V., 2002. Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. J. Neurol. Sci. 193, 73–78.

Starowicz, K., Nigam, S., Di Marzo, V., 2007. Biochemistry and pharmacology of endovanilloids. Pharmacol. Ther. 114, 13–33.

Steenland, H.W., Liu, H., Horner, R.L., 2008. Endogenous glutamatergic control of rhythmically active mammalian respiratory motoneurons in vivo. J. Neurosci. Off. J. Soc. Neurosci. 28, 6826–6835.

Steenland, H.W., Liu, H., Sood, S., Liu, X., Horner, R.L., 2006. Respiratory activation of the genioglossus muscle involves both non-NMDA and NMDA glutamate receptors at the hypoglossal motor nucleus in vivo. Neuroscience 138, 1407–1424.

Stell, B.M., Brickley, S.G., Tang, C.Y., 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. U. S. A. 100, 14439–14444.

Stella, N., Piomelli, D., 2001. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. Eur. J. Pharmacol. 425, 189–196.

Stella, N., Schweitzer, P., Piomelli, D., 1997. A second endogenous cannabinoid that modulates long-term potentiation. Nature 388, 773–778.

Straiker, A., Stella, N., Piomelli, D., Mackie, K., Karten, H.J., Maguire, G., 1999. Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc. Natl. Acad. Sci. U. S. A. 96, 14565–14570.

Straiker, A., Sullivan, J.M., 2003. Cannabinoid receptor activation differentially modulates ion channels in photoreceptors of the tiger salamander. J. Neurophysiol. 89, 2647–2654.

Sugiura, T., Kishimoto, S., Oka, S., Gokoh, M., 2006. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog. Lipid Res. 45, 405–446.

Sugiura, T., Kobayashi, Y., Oka, S., Waku, K., 2002. Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. Prostaglandins Leukot. Essent. Fatty Acids 66, 173–192.

Sugiura, T., Kodaka, T., Kondo, S., Tonegawa, T., Nakane, S., Kishimoto, S., Yamashita, A., Waku, K., 1997. Inhibition by 2-arachidonoylglycerol, a novel type of possible neuromodulator, of

the depolarization-induced increase in intracellular free calcium in neuroblastoma x glioma hybrid NG108-15 cells. Biochem. Biophys. Res. Commun. 233, 207–210.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Sumino, R., Nakamura, Y., 1974. Synaptic potentials of hypoglossal motoneurons and a common inhibitory interneuron in the trigemino-hypoglossal reflex. Brain Res. 73, 439–454.

Sutedja, N.A., Veldink, J.H., Fischer, K., Kromhout, H., Wokke, J.H.J., Huisman, M.H.B., Heederik, D.J.J., Van den Berg, L.H., 2007. Lifetime occupation, education, smoking, and risk of ALS. Neurology 69, 1508–1514.

Suzue, T., 1984. Respiratory rhythm generation in the in vitro brain stem-spinal cord preparation of the neonatal rat. J. Physiol. 354, 173–183.

Swash, M., Ingram, D., 1988. Preclinical and subclinical events in motor neuron disease. J. Neurol. Neurosurg. Psychiatry 51, 165–168.

Swerdlow, R.H., Parks, J.K., Cassarino, D.S., Trimmer, P.A., Miller, S.W., Maguire, D.J., Sheehan, J.P., Maguire, R.S., Pattee, G., Juel, V.C., Phillips, L.H., Tuttle, J.B., Bennett, J.P., Davis, R.E., Parker, W.D., 1998. Mitochondria in sporadic amyotrophic lateral sclerosis. Exp. Neurol. 153, 135–142.

Szabo, B., Dörner, L., Pfreundtner, C., Nörenberg, W., Starke, K., 1998. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 85, 395–403.

Takasu, N., Hashimoto, P.H., 1988. Morphological identification of an interneuron in the hypoglossal nucleus of the rat: a combined Golgi-electron microscopic study. J. Comp. Neurol. 271, 461–471.

Telgkamp, P., Ramirez, J.M., 1999. Differential responses of respiratory nuclei to anoxia in rhythmic brain stem slices of mice. J. Neurophysiol. 82, 2163–2170.

Tohgi, H., Abe, T., Yamazaki, K., Murata, T., Ishizaki, E., Isobe, C., 1999. Increase in oxidized NO products and reduction in oxidized glutathione in cerebrospinal fluid from patients with sporadic form of amyotrophic lateral sclerosis. Neurosci. Lett. 260, 204–206.

Tomomune, N., Takata, M., 1988. Excitatory and inhibitory postsynaptic potentials in cat hypoglossal motoneurons during swallowing. Exp. Brain Res. 71, 262–272.

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. 62, 405–496.

Tree, K., Caravagna, C., Hilaire, G., Peyronnet, J., Cayetanot, F., 2010. Anandamide centrally depresses the respiratory rhythm generator of neonatal mice. Neuroscience 170, 1098–1109.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E., Bohlooly-Y, M., Gidlöf, S., Oldfors, A., Wibom, R., Törnell, J., Jacobs, H.T., Larsson, N.-G., 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429, 417–423.

Trotti, D., Danbolt, N.C., Volterra, A., 1998. Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? Trends Pharmacol. Sci. 19, 328–334.

Trotti, D., Nussberger, S., Volterra, A., Hediger, M.A., 1997. Differential modulation of the uptake currents by redox interconversion of cysteine residues in the human neuronal glutamate transporter EAAC1. Eur. J. Neurosci. 9, 2207–2212.

Tsou, K., Brown, S., Sañudo-Peña, M.C., Mackie, K., Walker, J.M., 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83, 393–411.

Tsou, K., Mackie, K., Sañudo-Peña, M.C., Walker, J.M., 1999. Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience 93, 969–975.

Tsutsumi, T., Kobayashi, T., Ueda, H., Yamauchi, E., Watanabe, S., Okuyama, H., 1994. Lysophosphoinositide-specific phospholipase C in rat brain synaptic plasma membranes. Neurochem. Res. 19, 399–406.

Tu, P.H., Gurney, M.E., Julien, J.P., Lee, V.M., Trojanowski, J.Q., 1997. Oxidative stress, mutant SOD1, and neurofilament pathology in transgenic mouse models of human motor neuron disease. Lab. Investig. J. Tech. Methods Pathol. 76, 441–456.

Twitchell, W., Brown, S., Mackie, K., 1997. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J. Neurophysiol. 78, 43–50.

Ueda, H., Kobayashi, T., Kishimoto, M., Tsutsumi, T., Okuyama, H., 1993. A possible pathway of phosphoinositide metabolism through EDTA-insensitive phospholipase A1 followed by lysophosphoinositide-specific phospholipase C in rat brain. J. Neurochem. 61, 1874–1881.

Umemiya, M., Berger, A.J., 1994. Properties and function of low- and high-voltage-activated Ca2+ channels in hypoglossal motoneurons. J. Neurosci. Off. J. Soc. Neurosci. 14, 5652–5660.

Umemiya, M., Berger, A.J., 1995. Presynaptic inhibition by serotonin of glycinergic inhibitory synaptic currents in the rat brain stem. J. Neurophysiol. 73, 1192–1201.

Umezaki, T., Nakazawa, K., Miller, A.D., 1998. Behaviors of hypoglossal hyoid motoneurons in laryngeal and vestibular reflexes and in deglutition and emesis. Am. J. Physiol. 274, R950–955.

Van Brederode, J.F.M., Yanagawa, Y., Berger, A.J., 2011. GAD67-GFP+ neurons in the Nucleus of Roller: a possible source of inhibitory input to hypoglossal motoneurons. I. Morphology and firing properties. J. Neurophysiol. 105, 235–248.

Van Damme, P., Braeken, D., Callewaert, G., Robberecht, W., Van Den Bosch, L., 2005. GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 64, 605–612.

Van Damme, P., Leyssen, M., Callewaert, G., Robberecht, W., Van Den Bosch, L., 2003. The AMPA receptor antagonist NBQX prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. Neurosci. Lett. 343, 81–84.

Van Damme, P., Van Den Bosch, L., Van Houtte, E., Callewaert, G., Robberecht, W., 2002. GluR2-dependent properties of AMPA receptors determine the selective vulnerability of motor neurons to excitotoxicity. J. Neurophysiol. 88, 1279–1287.

Van Den Bosch, L., Van Damme, P., Bogaert, E., Robberecht, W., 2006. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. Biochim. Biophys. Acta 1762, 1068–1082.

Van der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., Micale, V., Steardo, L., Drago, F., Iuvone, T., Di Marzo, V., 2006. Endocannabinoids and beta-amyloid-

induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. Cell. Mol. Life Sci. CMLS 63, 1410–1424.

Van der Stelt, M., Veldhuis, W.B., Maccarrone, M., Bär, P.R., Nicolay, K., Veldink, G.A., Di Marzo, V., Vliegenthart, J.F.G., 2002. Acute neuronal injury, excitotoxicity, and the endocannabinoid system. Mol. Neurobiol. 26, 317–346.

Vande Velde, C., Miller, T.M., Cashman, N.R., Cleveland, D.W., 2008. Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria. Proc. Natl. Acad. Sci. U. S. A. 105, 4022–4027.

Vandevoorde, S., Lambert, D.M., 2007. The multiple pathways of endocannabinoid metabolism: a zoom out. Chem. Biodivers. 4, 1858–1881.

Varma, N., Carlson, G.C., Ledent, C., Alger, B.E., 2001. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J. Neurosci. Off. J. Soc. Neurosci. 21, RC188.

Veldhuis, W.B., van der Stelt, M., Wadman, M.W., van Zadelhoff, G., Maccarrone, M., Fezza, F., Veldink, G.A., Vliegenthart, J.F.G., Bär, P.R., Nicolay, K., Di Marzo, V., 2003. Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. J. Neurosci. Off. J. Soc. Neurosci. 23, 4127–4133.

Vermulst, M., Bielas, J.H., Kujoth, G.C., Ladiges, W.C., Rabinovitch, P.S., Prolla, T.A., Loeb, L.A., 2007. Mitochondrial point mutations do not limit the natural lifespan of mice. Nat. Genet. 39, 540–543.

Viana, F., Bayliss, D.A., Berger, A.J., 1993a. Calcium conductances and their role in the firing behavior of neonatal rat hypoglossal motoneurons. J. Neurophysiol. 69, 2137–2149.

Viana, F., Bayliss, D.A., Berger, A.J., 1993b. Multiple potassium conductances and their role in action potential repolarization and repetitive firing behavior of neonatal rat hypoglossal motoneurons. J. Neurophysiol. 69, 2150–2163.

Viana, F., Bayliss, D.A., Berger, A.J., 1994. Postnatal changes in rat hypoglossal motoneuron membrane properties. Neuroscience 59, 131–148.

Viana, F., Bayliss, D.A., Berger, A.J., 1995. Repetitive firing properties of developing rat brainstem motoneurones. J. Physiol. 486 (Pt 3), 745–761.

Vielhaber, S., Kunz, D., Winkler, K., Wiedemann, F.R., Kirches, E., Feistner, H., Heinze, H.J., Elger, C.E., Schubert, W., Kunz, W.S., 2000. Mitochondrial DNA abnormalities in skeletal muscle of patients with sporadic amyotrophic lateral sclerosis. Brain J. Neurol. 123 (Pt 7), 1339–1348.

Vinceti, M., Nacci, G., Rocchi, E., Cassinadri, T., Vivoli, R., Marchesi, C., Bergomi, M., 2000. Mortality in a population with long-term exposure to inorganic selenium via drinking water. J. Clin. Epidemiol. 53, 1062–1068.

Vogel, Z., Barg, J., Levy, R., Saya, D., Heldman, E., Mechoulam, R., 1993. Anandamide, a Brain Endogenous Compound, Interacts Specifically with Cannabinoid Receptors and Inhibits Adenylate Cyclase. J. Neurochem. 61, 352–355.

Volterra, A., Trotti, D., Tromba, C., Floridi, S., Racagni, G., 1994. Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. J. Neurosci. Off. J. Soc. Neurosci. 14, 2924–2932.

Wacnik, P.W., Luhr, K.M., Hill, R.H., Ljunggren, H.-G., Kristensson, K., Svensson, M., 2008. Cannabinoids Affect Dendritic Cell (DC) Potassium Channel Function and Modulate DC T Cell Stimulatory Capacity. J. Immunol. 181, 3057–3066.

Wade, D.T., Makela, P.M., House, H., Bateman, C., Robson, P., 2006. Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. Mult. Scler. Houndmills Basingstoke Engl. 12, 639–645.

Wade, M.R., Tzavara, E.T., Nomikos, G.G., 2004. Cannabinoids reduce cAMP levels in the striatum of freely moving rats: an in vivo microdialysis study. Brain Res. 1005, 117–123.

Wager-Miller, J., Westenbroek, R., Mackie, K., 2002. Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. Chem. Phys. Lipids 121, 83–89.

Wagner, O.I., Ascaño, J., Tokito, M., Leterrier, J.-F., Janmey, P.A., Holzbaur, E.L.F., 2004. The interaction of neurofilaments with the microtubule motor cytoplasmic dynein. Mol. Biol. Cell 15, 5092–5100.

Wang, H., O'Reilly, É.J., Weisskopf, M.G., Logroscino, G., McCullough, M.L., Thun, M.J., Schatzkin, A., Kolonel, L.N., Ascherio, A., 2011. Smoking and risk of amyotrophic lateral sclerosis: a pooled analysis of 5 prospective cohorts. Arch. Neurol. 68, 207–213.

Wang, R., Zhang, D., 2005. Memantine prolongs survival in an amyotrophic lateral sclerosis mouse model. Eur. J. Neurosci. 22, 2376–2380.

Warita, H., Itoyama, Y., Abe, K., 1999. Selective impairment of fast anterograde axonal transport in the peripheral nerves of asymptomatic transgenic mice with a G93A mutant SOD1 gene. Brain Res. 819, 120–131.

Watanabe, M., Dykes-Hoberg, M., Culotta, V.C., Price, D.L., Wong, P.C., Rothstein, J.D., 2001. Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. Neurobiol. Dis. 8, 933–941.

Werner, C., Engelhard, K., 2007. Pathophysiology of traumatic brain injury. Br. J. Anaesth. 99, 4–9.

Westlake, T.M., Howlett, A.C., Bonner, T.I., Matsuda, L.A., Herkenham, M., 1994. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63, 637–652.

Weydt, P., Hong, S., Witting, A., Möller, T., Stella, N., Kliot, M., 2005. Cannabinol delays symptom onset in SOD1 (G93A) transgenic mice without affecting survival. Amyotroph. Lateral Scler. Mot. Neuron Disord. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 6, 182–184.

Whitehouse, P.J., Wamsley, J.K., Zarbin, M.A., Price, D.L., Tourtellotte, W.W., Kuhar, M.J., 1983. Amyotrophic lateral sclerosis: alterations in neurotransmitter receptors. Ann. Neurol. 14, 8–16.

Whittington, M.A., Traub, R.D., Jefferys, J.G., 1995. Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. Nature 373, 612–615.

Wijesekera, L.C., Leigh, P.N., 2009. Amyotrophic lateral sclerosis. Orphanet J. Rare Dis. 4, 3. doi:10.1186/1750-1172-4-3

Williams, T.L., Ince, P.G., Oakley, A.E., Shaw, P.J., 1996. An immunocytochemical study of the distribution of AMPA selective glutamate receptor subunits in the normal human motor system. Neuroscience 74, 185–198.

Williamson, T.L., Cleveland, D.W., 1999. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat. Neurosci. 2, 50–56. doi:10.1038/4553

Wilson, R.I., Kunos, G., Nicoll, R.A., 2001. Presynaptic specificity of endocannabinoid signaling in the hippocampus. Neuron 31, 453–462.

Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410, 588–592. doi:10.1038/35069076

Withington-Wray, D.J., Mifflin, S.W., Spyer, K.M., 1988. Intracellular analysis of respiratory-modulated hypoglossal motoneurons in the cat. Neuroscience 25, 1041–1051.

Witting, A., Weydt, P., Hong, S., Kliot, M., Moller, T., Stella, N., 2004. Endocannabinoids accumulate in spinal cord of SOD1 G93A transgenic mice. J. Neurochem. 89, 1555–1557.

Wong, P.C., Pardo, C.A., Borchelt, D.R., Lee, M.K., Copeland, N.G., Jenkins, N.A., Sisodia, S.S., Cleveland, D.W., Price, D.L., 1995. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron 14, 1105–1116.

Wu, S., Barger, S.W., 2004. Induction of serine racemase by inflammatory stimuli is dependent on AP-1. Ann. N. Y. Acad. Sci. 1035, 133–146.

Xing, G., Carlton, J., Zhang, L., Jiang, X., Fullerton, C., Li, H., Ursano, R., 2011. Cannabinoid receptor expression and phosphorylation are differentially regulated between male and female cerebellum and brain stem after repeated stress: implication for PTSD and drug abuse. Neurosci. Lett. 502, 5–9.

Yamauchi, R., Imai, T., Tsuda, E., Hozuki, T., Yamamoto, D., Shimohama, S., 2014. Respiratory insufficiency with preserved diaphragmatic function in amyotrophic lateral sclerosis. Intern. Med. Tokyo Jpn. 53, 1325–1331.

Yang, Y., Gozen, O., Watkins, A., Lorenzini, I., Lepore, A., Gao, Y., Vidensky, S., Brennan, J., Poulsen, D., Won Park, J., Li Jeon, N., Robinson, M.B., Rothstein, J.D., 2009. Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. Neuron 61, 880–894.

Yuan, A., Rao, M.V., Kumar, A., Julien, J.-P., Nixon, R.A., 2003. Neurofilament transport in vivo minimally requires hetero-oligomer formation. J. Neurosci. Off. J. Soc. Neurosci. 23, 9452–9458.

Zeng, J., Powers, R.K., Newkirk, G., Yonkers, M., Binder, M.D., 2005. Contribution of persistent sodium currents to spike-frequency adaptation in rat hypoglossal motoneurons. J. Neurophysiol. 93, 1035–1041.

Zhao, P., Ignacio, S., Beattie, E.C., Abood, M.E., 2008. Altered presymptomatic AMPA and cannabinoid receptor trafficking in motor neurons of ALS model mice: implications for excitotoxicity. Eur. J. Neurosci. 27, 572–579.

Zogopoulos, P., Vasileiou, I., Patsouris, E., Theocharis, S., 2013. The neuroprotective role of endocannabinoids against chemical-induced injury and other adverse effects. J. Appl. Toxicol. JAT 33, 246–264.

Zoratti, C., Kipmen-Korgun, D., Osibow, K., Malli, R., Graier, W.F., 2003. Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. Br. J. Pharmacol. 140, 1351–1362.