

A new method for modulating the activity of parvalbumin-positive neurons and cerebellar Purkinje cells *in vivo*

Elli Leppä

Institute of Biomedicine

Pharmacology

University of Helsinki

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki,
for public examination in Lecture Hall 2, Biomedicum Helsinki 1, Haartmaninkatu 8,
on September 23rd 2011 at 12 noon.

HELSINKI 2011

Supervisors

Professor Esa Korpi, MD, PhD

Institute of Biomedicine

Pharmacology

University of Helsinki

Docent Anni-Maija Linden, PhD

Institute of Biomedicine

Pharmacology

University of Helsinki

Reviewers

Professor Garry Wong, PhD

Department of Biosciences

Department of Neurobiology, A.I. Virtanen Institute

University of Eastern Finland

Docent Eriika Savontaus, MD, PhD

Department of Pharmacology, Drug Development and Therapeutics

University of Turku

Dissertation opponent

Professor Raimo Tuominen, MD, PhD

Faculty of Pharmacy

Division of Pharmacology and Toxicology

University of Helsinki

ISBN 978-952-10-7131-7

ISBN 978-952-10-7132-4 (PDF)

<http://ethesis.helsinki.fi>

Unigrafia OY

Helsinki 2011

To Juha and Otso

Table of Contents

ABSTRACT	6
LIST OF ORIGINAL PUBLICATIONS	8
ABBREVIATIONS	9
1 INTRODUCTION	10
2 REVIEW OF THE LITERATURE	12
2.1 Neuronal networks.....	12
2.1.1 Feedforward and feedback inhibition	13
2.2 Interneurons	14
2.2.1 Classification of GABAergic interneurons	15
2.2.2 Parvalbumin-positive interneurons	17
2.2.3 Coordinating function and Pv-interneurons: network oscillations	23
2.2.4 Other parvalbumin-positive neuronal types	25
2.2.5 Significance of Pv-positive neurons in pathophysiology.....	26
2.3 Cerebellar circuits and Purkinje cells.....	29
2.3.1 Cerebellar mutant mice	31
2.4 The GABA _A receptor	32
2.5 GABA _A receptor subunits	33
2.5.1 The α subunits.....	34
2.5.2 The β , δ , θ , ϵ and π subunits	35
2.5.3 The γ subunits	35
2.6 GABA _A receptor assembly.....	37
2.7 Synaptic and extrasynaptic inhibition	37
2.8 Ligand binding sites of the GABA _A receptor.....	39
2.8.1 The benzodiazepine site	41
2.8.2 The loreclezole site	42
2.8.3 Other binding sites	43
3 AIMS OF THE STUDY.....	47
4 MATERIALS AND METHODS.....	48
4.1 Experimental animals.....	48
4.1.1 Generation of mouse lines.....	48
4.2 Culturing and transfection of HEK-293 cells	49
4.3 Ligand autoradiography and binding assays	49
4.3.1 [³⁵ S]TBPS autoradiography (I, II, IV)	50
4.3.2 [³ H]Ro 15-4513 autoradiography (II, IV)	50
4.3.3 [3H]EBOB binding assay (II).....	50
4.3.4 Quantification from films (I, II, IV).....	50
4.4 In situ hybridization (III, IV).....	51
4.5 Immunohistochemistry (III, IV)	51
4.5.1 Enhanced green fluorescent protein (eGFP) imaging and β -galactosidase staining (IV).....	51
4.5.2 Triple labeling (III)	52
4.5.3 c-Fos expression (I, II).....	52
4.6 Electrophysiology	52
4.6.1 Recordings from HEK 293 cells (II).....	53
4.6.2 Recordings from brain slices (III).....	53
4.7 Behavioral studies.....	53
4.7.1 SHIRPA screen (IV)	54

4.7.2	Elevated plus-maze (I, IV)	54
4.7.3	Open field exploration (II)	54
4.7.4	Startle reflex and prepulse inhibition (IV)	55
4.7.5	Morris water maze (IV).....	56
4.7.6	Fear conditioning (II).....	56
4.7.7	Hot plate and tail flick (IV)	57
4.7.8	Tremor measurement (IV).....	57
4.7.9	Electroshock convulsions and zolpidem (II)	58
4.7.10	Motor training (I, II, III, IV).....	58
4.7.11	Statistical testing.....	58
5	RESULTS AND DISCUSSION.....	61
5.1	Changes in pharmacological sensitivity caused by the $\gamma 2I77$ mutation.....	61
5.1.1	Abolition of zolpidem and DMCM high-affinity effects.....	61
5.1.2	Residual low affinity effects of zolpidem and DMCM.....	62
5.2	Lack of synaptic inhibition in PC- $\Delta\gamma 2$ mice does not cause motor deficits.....	63
5.2.1	Phenotype of PC- $\Delta\gamma 2$ mice	63
5.3	Cell-specific modulation of motor coordination in PC- $\gamma 2$ -swap mice	64
5.4	Lack of synaptic inhibition in Pv- $\Delta\gamma 2$ mice causes wide-ranging behavioral alterations	65
5.4.1	Phenotype of Pv- $\Delta\gamma 2$ mice	66
5.4.2	Increased pharmacological sensitivity of Pv- $\Delta\gamma 2$ mice	66
5.4.3	Increased GABA-insensitive binding in Pv- $\Delta\gamma 2$ mice.....	67
5.5	Partially restored synaptic inhibition in Pv- $\Delta\gamma 2$ -partial rescue mice.....	68
5.5.1	Restoration of the $\gamma 2F77$ subunit to reticular thalamic nucleus and molecular layer of cerebellum	69
5.5.2	Improvement of motor function and spatial learning	69
5.5.3	Altered pharmacological responses of Pv- $\Delta\gamma 2$ -partial rescue mice	70
6	GENERAL DISCUSSION.....	72
6.1	Earlier models of cell-type specific modulation.....	72
6.2	The zolpidem method.....	77
6.3	Benefits of the $\gamma 2I77$ as a background line	78
6.4	Static versus dynamic modulation of Purkinje cells	79
6.5	Lack of synaptic inhibition in Pv-cells causes wide-ranging behavioral alterations partially reversed by the restoration of the wild-type $\gamma 2F77$ subunit.....	80
7	CONCLUSIONS	83
8	ACKNOWLEDGEMENTS	85
9	REFERENCES	87
10	ORIGINAL PUBLICATIONS	102

ABSTRACT

Neurons can be divided into various classes according to their location, morphology, neurochemical identity and electrical properties. They form complex interconnected networks with precise roles for each cell type. GABAergic neurons expressing the calcium-binding protein parvalbumin (Pv) are mainly interneurons, which serve a coordinating function. Pv-cells modulate the activity of principal cells with high temporal precision. Abnormalities of Pv-interneuron activity in cortical areas have been linked to neuropsychiatric illnesses such as schizophrenia. Hippocampal Pv-neurons are involved in the generation of network oscillations, and when their activity level is altered, disruption of oscillatory function ensues. Cerebellar Purkinje cells are known to be central to motor learning. They are the sole output from the layered cerebellar cortex to deep cerebellar nuclei. Purkinje cells are inhibited by Pv-positive interneurons located in the molecular layer and excited by granule cells and inferior olivary neurons. There are still many open questions about the precise role of Pv-neurons and Purkinje cells, many of which could be answered if one could achieve rapid, reversible cell-type specific modulation of the activity of these neurons and observe the subsequent changes at the whole-animal level.

The aim of these studies was to develop a novel method for the modulation of Pv-neurons and Purkinje cells *in vivo* and to use this method to investigate the significance of inhibition in these neuronal types with a variety of behavioral experiments in addition to tissue autoradiography, electrophysiology and immunohistochemistry. The GABA_A receptor $\gamma 2$ subunit was ablated from Pv-neurons and Purkinje cells in four separate mouse lines. Pv- $\Delta\gamma 2$ mice had wide-ranging behavioral alterations and increased GABA-insensitive binding indicative of an altered GABA_A receptor composition, particularly in midbrain areas. PC- $\Delta\gamma 2$ mice experienced little or no motor impairment despite the lack of inhibition in Purkinje cells. In Pv- $\Delta\gamma 2$ -partial rescue mice, a reversal of motor and cognitive deficits was observed in addition to restoration of the wild-type $\gamma 2F77$ subunit to the reticular nucleus of thalamus and the cerebellar molecular layer. In PC- $\Delta\gamma 2$ -swap mice, zolpidem sensitivity was restored to Purkinje cells and the administration of systemic zolpidem evoked a transient motor impairment. On the basis of these results, it is concluded that this new method of cell-type specific modulation is a feasible way to modulate the activity of selected neuronal types. The

importance of Purkinje cells to motor control supports previous studies, and the crucial involvement of P_v-neurons in a range of behavioral modalities is confirmed.

LIST OF ORIGINAL PUBLICATIONS

- I** Leppä E, Vekovischeva OY, Lindén AM, Wulff P, Oberto A, Wisden W, Korpi ER. (2005) Agonistic effects of the β -carboline DMCM revealed in GABA(A) receptor γ 2 subunit F77I point-mutated mice. *Neuropharmacology* 48: 469-478.
- II** Leppä E, Lindén AM, Rabe H, Vekovischeva OY, Wulff P, Lüddens H, Wisden W, Korpi ER. (2011) Actions of two GABA_A receptor benzodiazepine-site ligands that are mediated via non- γ 2-dependent modulation. *European Journal of Pharmacology* 666: 111-121.
- III** Wulff P, Goetz T, Leppä E, Lindén AM, Renzi M, Swinny JD, Vekovischeva OY, Sieghart W, Somogyi P, Korpi ER, Farrant M, Wisden W. (2007) From synapse to behavior: rapid modulation of defined neuronal types with engineered GABA_A receptors. *Nature Neuroscience* 10: 923-929.
- IV** Leppä E, Lindén AM, Vekovischeva OY, Rantanen V, Toppila E, Höger H, Sieghart W, Wulff P, Wisden W, Korpi ER (2011) Removal of GABA_A receptor γ 2 subunit from parvalbumin neurons causes wide-ranging behavioral alterations. [PLoS ONE, accepted for publication]

ABBREVIATIONS

5-HT _{3A}	5-hydroxytryptamine (serotonin) receptor type 3A
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
ANOVA	analysis of variance
BAC	bacterial artificial chromosome
CA	Cornu ammonis of the hippocampus
CCK	cholecystokinin
c-Fos	a protein encoded by the FOS gene
CNS	central nervous system
DCN	deep cerebellar nuclei
DMCM	methyl 6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate
EBOB	ethynylbicycloorthobenzoate
EC ₅₀	concentration producing a half-maximal effect
EDTA	ethylenediaminetetra-acetic acid
eGFP	enhanced green fluorescent protein
GABA	γ -aminobutyric acid
GABA _A	γ -aminobutyric acid receptor type A
GABA _B	γ -aminobutyric acid receptor type B
GABA _C	γ -aminobutyric acid receptor type C
GAD	glutamic acid decarboxylase
GIS	GABA-insensitive
HEK-293	human embryonic kidney cell line
Hz	Hertz
L7Cre	Purkinje-cell specific promoter
LTD	long-term depression
IPSC	inhibitory postsynaptic current
mIPSC	miniature inhibitory postsynaptic current
MIST	molecules for inactivation of synaptic transmission
MLI	molecular layer interneurons
mRNA	messenger ribonucleic acid
NMDA	N-methyl-D-aspartic acid
PC	Purkinje cell
PC- γ 2-swap	mouse line with the GABA _A receptor γ 2F77 subunit returned to Purkinje cells
PC- $\Delta\gamma$ 2	mouse line with the GABA _A receptor γ 2 subunit removed from Purkinje cells
PPI	pre-pulse inhibition
Pv	parvalbumin
PvCre	mouse line with Cre recombinase expressed in parvalbumin-cells
Pv- $\Delta\gamma$ 2	mouse line with the GABA _A receptor γ 2 subunit removed from parvalbumin-cells
Pv- $\Delta\gamma$ 2-	
partial rescue	mouse line with the GABA _A receptor γ 2F77 subunit returned to some parvalbumin-cells
Ro 15-1788	flumazenil, a benzodiazepine antagonist
Ro 15-4513	8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid ethyl ester, a benzodiazepine inverse agonist
SHIRPA	SmithKline Beecham-Harwell-Imperial College-Royal London Hospital-phenotype assessment, a mouse behavioral screen
TBPS	<i>t</i> -butylbicyclophosphorothionate
THIP	tetrahydroisoxazopyridine
WT	wild-type
γ 2I77	a mouse line with an I77 point mutation in the γ 2 of the GABA _A receptor

1 INTRODUCTION

The appropriate functioning of the central nervous system is based on the cooperation of billions of neurons, which are the smallest units of independent action in the brain and spinal cord. There are hundreds, if not thousands, of different types of neurons, all with their special niche defined by their location, size, connections to other neurons and the electrical and chemical characteristics that allow them to communicate with each other. Networks are formed by the creation of neural circuits, in which certain neurons govern the activity of others by modulating the amount and timing of excitatory or inhibitory inputs that the principal cells receive. Usually these regulating neurons are small, inhibitory interneurons, which have connections with up to hundreds of principal neurons, and so are ideally suited for the coordination of large neuronal assemblies.

A class of inhibitory interneurons that use the inhibitory γ -aminobutyric acid (GABA) as their primary transmitter, the parvalbumin (Pv)-positive interneurons, have been shown to represent a fundamental component of information processing in many brain areas, and thus to influence many aspects of central nervous system function, ranging from regulation of the level of anxiety to effects on memory and learning. Disruptions in the functioning of Pv-cells have been implicated in neurological and neuropsychiatric illnesses such as schizophrenia and epilepsy. Although Pv-positive interneurons have been a focus of intensive study in recent years, there are nevertheless still many aspects of their roles that need further clarification.

Purkinje cells of the cerebellum have long been known to play an important part in motor learning and memory. They are connected through several well-recognized pathways to other cell types in the layered cerebellar cortex, and are the sole output from the cerebellar cortex to deep cerebellar nuclei. However, there still remain unanswered questions about the role of Purkinje cells and the significance of the molecular layer interneurons, which inhibit Purkinje cells. Many of these questions could be revealed if it were possible to selectively modulate the activity of Purkinje cells and subsequently observe the consequences of modulation at the behavioral level.

An ideal way to pinpoint the significance of a type of neuron is to examine the network and determine when specific neurons are active, and when they are not. To this end, various fast, reversible ways to modulate the activity of neurons have been developed, with varying

success. The method of modulation should cause minimal disturbance to the system at large and to produce measurable changes at the network level, so that they can be monitored in electrophysiological or behavioral experiments.

The present studies have been aimed at producing a method by which the selected neuronal type, parvalbumin-positive interneurons and cerebellar Purkinje cells are made uniquely sensitive to inhibitory modulation with the sedative-hypnotic zolpidem and the convulsant methyl 6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate (DMCM). The behavior and sensitivity to zolpidem and DMCM of five lines of transgenic mice have been carefully assessed to measure the effect of activation or inactivation of Pv-positive neurons and Purkinje cells. By measuring the effect of the genetic alterations at the behavioral level it was hoped to attain a comprehensive picture of the significance of these selected cell types to diverse behavioral modalities.

2 REVIEW OF THE LITERATURE

2.1 Neuronal networks

The central nervous system (CNS) is organized at several structural levels. The gross anatomical division of cerebrum and cerebellum divides further into fore-, mid- and hindbrain and subsequently into smaller areas like the thalamus, amygdala and basal ganglia. These areas in their turn contain discrete nuclei with definite morphological, neurochemical and electrophysiological features, which can be characterized by various neuroscientific methods to reveal their function in the CNS as a whole. Inside the nuclei and between them neurons and glial cells form networks that govern the organized activation of brain areas in response to internal and external stimuli. Neurons respond to their environment by chemical and electrical processes, which allow them to change their internal state and to relay information to other neurons. The mechanisms of ion channel activity and synaptic transmission, which are typical of neurons, have been extensively investigated and can be influenced in various ways, both experimentally and in clinical settings. The challenges in understanding the significance of various brain regions, and ultimately of the different cell types, to the brain as a whole lie in identifying the discrete parts of the networks and selectively regulating their activity. Much of the earliest work regarding neuronal circuits was conducted in the neuronal system of the sea snail *Aplysia californica*, which offers the advantages of very large (up to 1 mm diameter), easily manipulated neurons. In addition their numbers are significantly fewer than in the mammalian brain (10^6 vs. 10^{12} neurons) (reviewed in Kandel and Schwartz, 1982). In such simple systems, the basic principles of neuronal activity, such as the molecular mechanisms of learning, can be examined and subsequently extrapolated to more complicated vertebrate, mammalian organisms. Kandel's pioneering work provided the first evidence for the role of protein phosphorylation in regulating synaptic strength (reviewed in Klein and Kandel, 1978).

Information processing in the brain is a distributed phenomenon not restricted to multiple isolated areas, but requiring the cooperation of large assemblies of neurons in spatially separate regions (reviewed in Uhlhaas et al., 2008). They are connected through the activity of projection neurons originating from one area, with axons reaching across long distances to influence the activity in their target region (Lee and Tepper, 2007). Small, nonprojecting

interneurons serve important local functions, most often inhibiting the activity of their principal cells (reviewed in Rudy et al., 2011). The roles of principal cells and interneurons are basically similar in all brain regions, but the circuits they form range from very simple two-cell assemblies to intricate interconnected formations with dozens of different neuron types all contributing to the control of activity. The most detailed information on the circuit level so far has been gained in areas with a definite laminar structure, such as the hippocampus (reviewed in Freund and Buzsaki, 1996), the neocortex (reviewed in Freund and Katona, 2007) and the cerebellum (Briatore et al., 2010). Knowledge about the exact circuitry controlling the activity of different brain regions is essential to understanding CNS processes and thus for our ability to influence them in pathological conditions. Many neurological and neuropsychiatric illnesses have been shown to afflict distinct neuronal types, e.g. dopaminergic cells in Parkinson's disease and cholinergic neurons in Alzheimer's disease. If one wishes to understand the mechanisms behind these processes, detailed information must be obtained on the neurons in question. In many brain areas however, this knowledge is incomplete and requires further study. To pinpoint the roles of specific cell types, various strategies have been developed. The earliest approaches relied on the removal of entire brain structures by surgery (Grusser-Cornehls et al., 1999) or ablation of areas by the injection of toxins (Steiner et al., 1997). Such methods, though important in their period, are no longer considered specific enough for the goal of clarifying the significance of single neurons. Further strategies are needed to reveal the role of neuron classes to the function of the whole central nervous system. The opportunity to reversibly modulate the activity of selected neuronal types in awake, behaving animals would provide important new insights into this phenomenon.

2.1.1 Feedforward and feedback inhibition

The two main types of neuronal inhibition are feedforward and feedback inhibition. Though the mechanisms of initiation and synaptic transmitter release are the same in both types, they serve different functions in the circuit. In the former type, an inhibitory neuron, usually an interneuron, receives excitatory input and releases an inhibitory transmitter, usually GABA. The activity of the target neuron, either a principal cell or another interneuron, is reduced for as long as transmitter release from the interneuron continues or the target receptor becomes desensitized and inactivates (Pouille and Scanziani, 2001). In feedback inhibition, the source of interneuron excitation is the principal cell itself. Thus when the principal cell is activated,

it subsequently activates the interneuron, which curtails the activity of the principal cell. This type of inhibition serves to limit the duration and the spread of activity among principal cells, as well as lateral inhibition between interneurons (reviewed in Bartos et al., 2011). Depending on its cytoarchitecture and afferent connections, an inhibitory neuron may provide dynamically either feedforward, feedback or both types of inhibition within a network. The mapping of neural circuits is facilitated by using simple model organisms like the nematode *Caenorhabditis elegans*, in which all of the 302 cells, including neurons, of the adult hermaphrodite animal are known and characterized. The investigation of neural structures is further aided by the transparency of this animal, and several complete neuronal circuits have been identified. Additionally, by ablating selected neurons with a microlaser beam, the functional significance of each of the neurons in the network can be elucidated in turn (reviewed in deBono and Maricq, 2005). In larger, more complex organisms, different strategies must be utilized to map the neural circuitry underlying behavior, with particular emphasis on the challenges in identifying the cell types of interest.

2.2 Interneurons

Interneurons are a ubiquitous and varied class of neurons with several typical characteristics: small size, local connectivity and specialized roles in CNS circuitry. The function of interneurons is the regulation of the activity of principal cells: depending on the exact connectivity, this can mean the modulation of input via the control of dendritic excitability, as in the example of inhibitory double-bouquet interneurons (Kawaguchi and Kubota, 1998), or the control of axonal output, as in chandelier type interneurons (reviewed in DeFelipe, 1999). The actions of interneurons are most often inhibitory, although in some brain areas also excitatory interneurons exist (Woodruff et al., 2009). Additionally, during developmental stages (reviewed in Wang and Kriegstein, 2009) as well as in disease states like epilepsy (Isomura et al., 2003), the actions of the main inhibitory neurotransmitter GABA, utilized by the majority of interneurons, may become excitatory in interneurons and principal cells alike. Some invertebrate species like nematodes utilize GABA as an excitatory neurotransmitter in neuromuscular junctions (Beg and Jorgensen, 2003). However, in the majority of organisms GABA is considered as an inhibitory transmitter, and the scope of this review deals mainly with its inhibitory properties.

In many regions, principal cells, e.g. cortical pyramidal cells outnumber inhibitory interneurons by a factor of 10 or more (reviewed in Berghuis et al., 2004), but the interneurons nevertheless exert a powerful regulatory influence on network coordination, since each interneuron can possess connections to many, in some cases hundreds, of principal cells (Fino and Yuste, 2011). As parts of interconnected networks, interneurons may receive excitatory or inhibitory innervation locally or from adjacent brain areas and in their turn exert either excitatory (Woodruff et al., 2009) or inhibitory influences not only on their principal cells, but also other interneurons (reviewed in Klausberger, 2009). Principal cells can also reduce the activity of their respective interneurons via retrograde synaptic signaling via the release of inhibitory substances like cannabinoids (Wilson and Nicoll, 2001). The many types and connections of both interneurons and principal cells can result in massively complex networks, the intricacies of which are only slowly being unraveled with intense study involving many different disciplines, e.g. *in vivo* animal models, the identification of interneurons via cytochemical markers, electrophysiological characterization of firing properties, and even purely computational approaches.

In certain brain areas with relatively simple and well-recognized neuronal circuitry, particularly in the hippocampus and to some extent also the cerebral cortex, the establishment of classes of interneurons with clearly defined characteristics and modes of action has advanced the furthest (reviewed in Rudy et al., 2011). Interneurons can be divided into groups according to several criteria, although no one single method of classification has yet been proved to hold across all known types of interneurons in all brain areas. The most commonly used definitive properties are transmitter identity, localization, connectivity pattern, morphology, neurochemistry and electrophysiological character. More information is still needed on the different roles of the interneuronal types on network function. The targeted regulation of activity of single neuronal types would be one way to gain insights into their significance in various brain areas.

2.2.1 Classification of GABAergic interneurons

Most interneurons are inhibitory and their main neurotransmitter is GABA; GABAergic interneurons are indeed currently the most thoroughly researched interneuron type. Their great diversity has led to various classification schemes according to brain area and chemical as well as electrical properties. The roles of all the identified subclasses of

GABAergic interneurons are far from clear. Other transmitters are also utilized by some types of interneurons. Cholinergic interneurons of the dorsal and ventral parts of the striatum may play a role in the pathophysiology of movement disorders involving the basal ganglia (Pisani et al., 2007), glycinergic interneurons of the spinal cord take part in the pattern generator arrays of complex movement (Berkowitz et al., 2010) and dopaminergic interneurons of the olfactory bulb participate in the processing of olfactory information (reviewed in Cave and Baker, 2009). GABAergic interneurons are ubiquitously expressed throughout the CNS, in areas ranging from the spinal cord to the olfactory bulb. Several attempts have been made to establish definite types of GABAergic interneurons with uniform characteristics; most success so far has been achieved in the laminar hippocampal and cortical structures. Cortical GABAergic interneurons have been grouped into at least three classes on the basis of the expression of cellular marker proteins: I) somatostatin, II) the serotonin 5-HT_{3A} receptor and III) parvalbumin (discussed in the following sections; reviewed in Rudy et al., 2011), although other classifications with more main subtypes have also been proposed (reviewed in Lewis et al., 2005).

Somatostatin positive cells, typified by the Martinotti cell, make up ~30 % of all cortical interneurons. They are most abundant in cortical layer V, although some are found also in layers II-IV. Martinotti cells innervate the distal dendrites of the axonal tuft of the pyramidal cells in layer I, with axonal collaterals also synapsing with apical and basal pyramidal dendrites (Uematsu et al., 2008). Their firing pattern is low threshold spiking, and the excitatory input they receive is facilitating, which makes them prime candidates for feedback inhibition. Further subdivisions of somatostatin positive interneurons can be constructed on the basis of their calbindin immunoreactivity.

5-HT_{3A} receptor-positive cells form the second group of cortical interneurons, also with ~30% prevalence. This cell population is rather heterogeneous and includes vasoactive intestinal peptide positive cells with morphologies varying from bitufted and bipolar to multipolar structures, displaying a diverse variety of cytochemical markers and electrophysiological properties (Lee et al., 2010). In addition to serotonin, 5-HT_{3A} receptor-positive interneurons can be excited by acetylcholine acting on nicotinic receptors, irrespective of their cortical layer, morphology or firing profile. Based on their location in the superficial cortical layers II and III, 5-HT_{3A} positive neurons have been postulated to provide feedforward or feedback inhibition in relation to long-range cortico-cortical inputs (Petreanu et al., 2009).

Neurogliaform cells are relatively well-known 5-HT_{3A} positive cells with small, round somas and radially extending dendrites. They are unusual in that they share electrical synapses with other types of interneurons in addition to each other; usually electrically connected interneurons only relate to neurons of the same type (Olah et al., 2007). This is proposed as a way to connect multiple parallel interneuron networks and so to promote network synchronization. Another interesting feature of neurogliaform cells is the localization of synaptic vesicles to sites not adjacent to postsynaptic junctions, which may be evidence that they participate in tonic extrasynaptic transmission (Olah et al., 2009).

Additional types of cortical GABAergic interneurons are calbindin- or calretinin-positive double bouquet cells, which innervate distal dendrites of pyramidal cells (Kawaguchi and Kubota, 1998), and calretinin-positive Cajal-Retzius cells, which form synapses in the axonal tuft of the pyramidal cell (Kirmse et al., 2007). In the hippocampal CA1 area alone, 12 different types of GABAergic interneurons have recently been identified (reviewed in Klausberger, 2009), which testifies to the multiform character of this class of cell.

2.2.2 Parvalbumin-positive interneurons

Parvalbumin (Pv)-positive interneurons in cortical and hippocampal areas synapse on the soma or proximal dendrites of the pyramidal cell, or in some cases on the axon initial segment (Gonzalez-Burgos 2005). They have a typical fast-spiking firing pattern: they fire sustained, high-frequency trains of brief action potentials and have little spike frequency adaptation. These neurons have very low input resistance and fast membrane time constants, which make them ideal for developing fast synaptic responses (Gonzalez-Burgos et al., 2005). Fast spiking cortical interneurons exert a powerful and temporally precise influence on their target cells. They are under strong excitatory control from the thalamocortical inputs of layer IV and intracortical inputs of all six layers, all of which creates a narrow time window of feedforward inhibition to achieve temporal summation of the excitatory input and spike generation of principal cells (Goldberg et al., 2008). Fast-spiking interneurons typically contain many glutamatergic AMPA receptors and consequently are able to follow high-frequency repetitive stimulation with high fidelity, which enables them to mediate the rapid changes conveyed by their numerous glutamatergic inputs (Cruikshank et al., 2007).

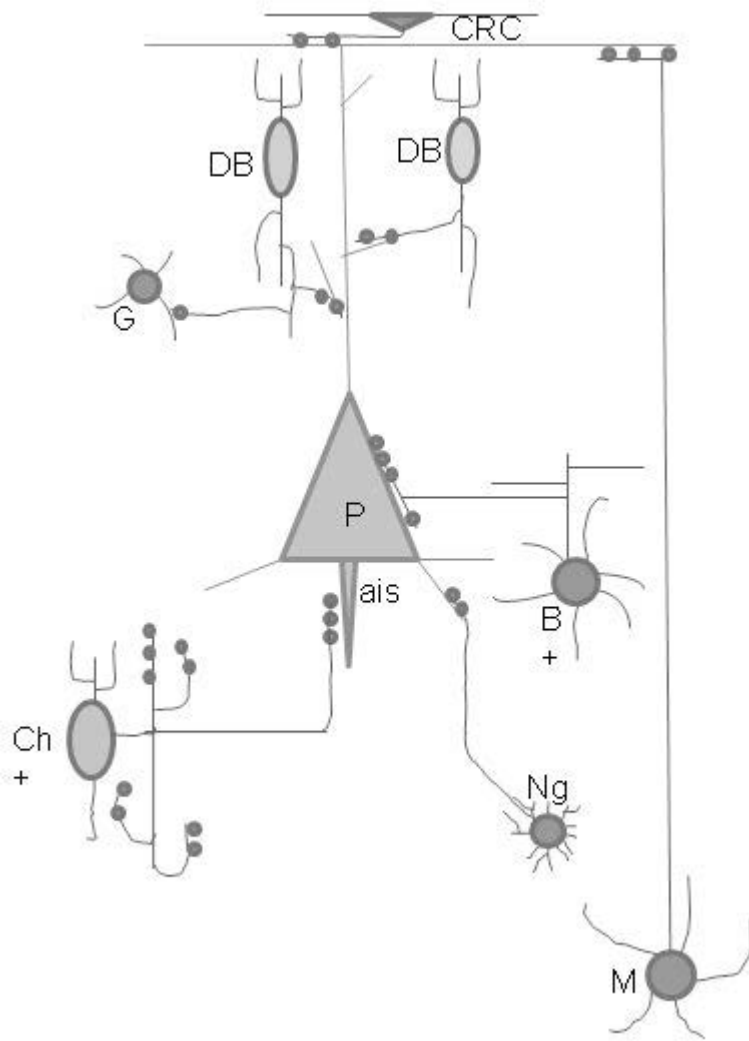


Figure 1. Interneuron types in the cerebral cortex. P pyramidal cell. DB double bouquet cell, Ch chandelier cell, Ng neurogliaform cell, M Martinotti cell, B basket cell, CRC Cajal-Retzius cell, ais axon initial segment, G other GABAergic neurons. + parvalbumin-positive cell. (Modified from Lewis et al., 2005).

The two most important types of cortical GABAergic Pv-interneurons are basket cells and chandelier cells. Basket cells reside in layer III, close to the pyramidal cells, synapsing on their soma and proximal dendrites. The axons of several basket cells converging on the perisomatic area of the pyramidal cell form a basket-like structure. They are likely to be the prevalent inhibitory influence in the neocortex (reviewed in Freund and Katona, 2007). Basket cells have been shown to innervate also other basket cells, in addition to pyramidal cells, while chandelier cells only synapse with pyramidal cells (Tamas et al., 1998). Two types of basket cells are recognized, the Pv-positive and the cholecystokinin (CCK)-positive. The latter have slower kinetics of activation and inactivation suited to the integration of excitatory signals (Glickfeld and Scanziani, 2006) and are thought to participate in the fine-tuning of network activity and perhaps the processing of information concerning mood,

emotional and motivational types of physiological status. One of the most essential tasks of the Pv-positive basket cells is the generation and control of network oscillations (Galarreta and Hestrin, 2001). CCK-positive cells can be retrogradely inhibited by the pyramidal cells via cannabinoid signaling (so-called depolarization-induced suppression of inhibition), but Pv-positive cells cannot (Wilson and Nicoll, 2001). This difference in the ability of the pyramidal cells to curtail one form of inhibition but not the other is intriguing and is likely to be connected to the different roles of the two types of basket cells. Pv-positive basket cells have a rather rigid mode of action that cannot be modulated as easily and in as many ways as the CCK-positive cells, as befits a system that has a clockwork-like oscillatory function. On the other hand, hippocampal Pv-positive basket cells are presynaptically modulated by μ opioid (Drake and Milner, 2002) and M_2 muscarinic (Hajos et al., 1998) receptors; the activation of these receptors results in reduction of the firing of basket cells. M_2 antagonists increase gamma oscillations via disinhibition of basket cells (Palhalmi et al., 2004). Experiments with selective activation or inhibition of basket cells would give further information on the role of these specialized neurons in the regulation of oscillatory functions in information processing.

Hippocampal Pv-interneurons are distinct from other interneurons in that region in that they receive much less GABAergic afferent input; only 6 % of their synaptic input is inhibitory (Gulyas et al., 1999). In contrast, they receive an extensive excitatory input via ~15000 synaptic connections from all layers of the hippocampus: feedforward excitation from Schaffer collaterals and entorhinal fibers, thalamic afferents from the nucleus Reunienis in addition to feedback excitation from CA1 recurrent collaterals (reviewed in McBain and Fisahn, 2001).

In addition to conventional chemical synapses, both hippocampal and cortical basket cells are connected to each other via gap junctions, transcellular channels located in their dendrites. Gap junctions are formed by specialized proteins called connexins, and they allow direct bidirectional electrical contact and exchange of signals between neurons. They are an extremely rapid, powerful method for achieving synchronization of a group of cells (Fukuda, 2009). Gap junctions permit the formation of interconnected assemblies well suited to the control of large pyramidal cell populations to gamma frequency oscillations. It has been shown that gap junctional connections amplify the magnitude of gamma oscillations, though they do not appear to affect their generation (reviewed in Nakazawa et al., 2011). Like most

interneurons expressing gap junctions, except for the neurogliaform cells, Pv-positive basket cells only form gap junctions with cells of the same neuronal subtype (Blatow et al., 2003). It has been estimated that each Pv-positive basket cell is connected to 20-50 other basket cells, either synaptically or via gap junctions (Amitai et al., 2002).

The role of Pv-positive chandelier cells differs from that of basket cells and seems to be somewhat controversial. Chandelier cells are located in laminar structures like the neocortex and hippocampus, but are missing from the cerebellar layers, in addition to being found in the amygdala, which does not consist of layers (reviewed in Howard et al., 2005). They are most abundant in cortical layers II/III, but much fewer in number than Pv-positive basket cells (Inda et al., 2007). They have a characteristic branched structure and they form synapses specifically on the axon initial segment of the pyramidal cell, with vertical axonal varicosities called cartridges focused on the axon initial segment in a linear manner. A single axonal cartridge innervates a single axon initial segment; one axon initial segment may harbor as many as five synapses with chandelier cell cartridges from a single or several chandelier cells (reviewed DeFelipe, 1999). Chandelier cells do not innervate other interneurons, and do not receive inputs on their own axon initial segments. They are target-specific according to their localization: visual cortex chandelier cells prefer cortico-cortical pyramidal cells, while in the entorhinal cortex layer II/III, they prefer pyramidal cells that project to the hippocampus (Arellano et al., 2002). The innervation targeting chandelier cells is less clear, but is likely to show similar specificity. In layers II/III of mouse somatosensory cortex, chandelier cells receive an excitatory input within the same layer in addition to layer Va, whereas the inhibitory input originates from layers I and II/III. As logically follows from the synaptic localization on the axon initial segment, chandelier cells have a powerful influence on the initiation of the action potential. Surprisingly, despite being GABAergic, they have been postulated to have an excitatory effect on the pyramidal cells, facilitating the generation of an action potential. Three points have been raised to support this proposal: glutamatergic disynaptic EPSPs can be recorded after a single spike in a chandelier cell, the GABA reversal potential in the chandelier cell-pyramidal cell synapse is above the resting potential of the neuron, and the KCC2 potassium-chloride cotransporter is missing from the axon initial segment of the pyramidal cell, which could lead to less chloride extrusion and account for the observed elevated GABA reversal potential (reviewed in Woodruff et al., 2010). The possibility of excitation would not preclude an inhibitory function of the chandelier cell, the mode of action being dependent on the membrane potential of the postsynaptic cell, and

consequently the state of excitation of the whole network would govern the mode of action of the chandelier cell. The lack of specific markers to separate chandelier cells from Pv-positive basket cells has been an obstacle in investigating their function.

In addition to basket and chandelier cells, another type of Pv-interneuron called the multipolar bursting cell, has been localized to the border between layers I and II, with synapses at the distal dendrites of the pyramidal cell. Its firing properties differ from the two better known interneuron types in that after initially firing a few bursts, it pauses and follows with a train of low-frequency tonic spikes, and is able to undergo paired-pulse facilitation, unlike basket and chandelier cells. The prevalence and functional significance of the multipolar bursting cell are not known at present (Blatow et al., 2003).

The hippocampus contains additional types of GABAergic Pv-positive inhibitory interneurons. Unlike the soma-focused basket cells, oriens-lacunosum moleculare cells synapse on the distal dendrites, whereas bistratified cells focus their synapses on both the basal and apical dendrites of the pyramidal cell (reviewed in McBain and Fisahn, 2001). These cell types are likely to have functions very different from the basket or chandelier cells. A mouse line with a genetic ablation of the glutamatergic AMPA receptor GluR-A subunit in Pv-cells was found to have impaired spatial working memory and disrupted exploratory behavior in addition to displaying alterations in hippocampal oscillations (Fuchs et al., 2007). In a mouse line with genetic removal of the glutamatergic NMDA receptor from Pv-cells, electrophysiological recordings from the hippocampal CA1 area revealed a disruption in the oscillations, with altered theta frequency and increased gamma frequency activity as well as deficits in certain forms of memory and learning, but no changes in open field exploratory behavior (Korotkova et al., 2010). In a later report with the same mouse line, a decreased habituation of the startle reflex, a simplistic form of learning, was observed, although the startle reflex itself, as well as the pre-pulse inhibition of startle, was normal. Further disruptions in associative fear learning and an overloading of working memory were reported, although spatial reference memory in the Morris Water Maze paradigm was normal (Carlen et al., 2011). These studies underline the importance of the excitation of Pv-interneurons in hippocampal-dependent phenomena at both the electrophysiological level (oscillations) as well as their resulting whole animal-counterparts, i.e. behavioral experiments conducted in awake, moving animals. These examples of disrupted function of Pv-cells due to decreased excitation would benefit if it were possible to undertake a reversal of the setting:

i.e. investigate the effects of decreased inhibition of Pv-cells. A model with impaired inhibition in Pv-cells would offer an important opportunity to observe the activity of the network in the presence of increased inhibition toward the principal cells.

Though the neocortex and hippocampus are the most thoroughly researched areas with regard to Pv-interneuron function, many other areas also harbor GABAergic Pv-positive interneurons. The basolateral division of the amygdala contains Pv-cells with a fast-spiking firing pattern and synapse localization in the soma and proximal dendrites of the principal cells, as well as an axo-axonic cell reminiscent of the cortical chandelier cell, in that its influence can be excitatory (reviewed in Spampanato et al., 2011). The Pv-cells of the basolateral amygdala show increased expression of the immediate early gene *c-Fos*, a marker of neuronal activity, after the systemic administration of anxiogenic compounds, such as benzodiazepine inverse agonists (Hale et al., 2010), highlighting the importance of Pv-cells in the amygdala in the regulation of anxiety-related behaviors. The basolateral amygdala is a well-known substrate of anxiety in both animal models of anxiety and in human anxiety disorders. In the spinal cord, Pv-interneurons are present in several regions including the ventral horn. They may be used as an early marker of the large-diameter 1a fibers that synapse with motoneurons. Some Pv-cells of the spinal cord may be involved in polysynaptic pathways to motoneurons, while some may be Renshaw cells, which provide recurrent inhibition to motoneurons (Dekkers et al., 2002). In the striatum, only 3-5% of cells are GABAergic Pv-interneurons, but they nevertheless exert a powerful inhibitory influence on the activity of the projection neurons. A single Pv-interneuron makes contact with many projection neurons and thus may have a coordinating function. Striatal Pv-interneurons appear to play a role in the motor symptoms observed in an animal model of idiopathic dystonia (Gernert et al., 2000).

There are several Pv-positive interneuron types in the molecular layer of the cerebellum (Mittmann et al., 2005). The function of these basket and stellate cells, like that of Pv-interneurons in other brain areas, is to impose a precise temporal control over the output of their principal cells, the Purkinje cells. Parallel fibres originating from granule cells activate the molecular layer interneurons, which rapidly modulate the activity of Purkinje cells in a feed-forward manner (Mittmann et al., 2005). Molecular layer interneurons are highly interconnected and form a network capable of mediating very fast responses to regulate the intrinsically active Purkinje cells (Kondo and Marty, 1998). Pv-positive fast-spiking

interneurons form a part of an inhibitory feedforward circuit also in the red nucleus (Liu et al., 2002). The fairly uniform characteristics of Pv-positive interneurons across different brain areas emphasize that this cell type is an important component of inhibitory control and network coordination. However, many of the details of Pv-cell function still await clarification. Ways to specifically modulate their activity *in vivo* in behaving animals would be of great value in understanding their functional roles in the regulation of activity in different brain areas.

2.2.3 Coordinating function and Pv-interneurons: network oscillations

Oscillations are rhythmic fluctuations in the extracellular field potential of neurons, which have been observed in a variety of brain areas, including the olfactory bulb, thalamus, hippocampus and cortex. They are thought to be crucial to the maintenance of the temporal precision of pyramidal cell firing, and require the recruitment of interneuron and pyramidal cell populations into neuronal assemblies, which encode sensory and other kinds of data into temporally sequential representations. The functions of the various types of oscillations have not been fully established, but they are believed to support the transfer and processing of information within and between brain regions (Hartwich et al., 2009), and to assemble the features detected in different cortical areas to a unified perceived object (Whittington et al., 1995). Oscillations are divided according to frequency into several classes, the most important being theta (4-12 Hz), gamma (30-80 Hz) and fast ripple oscillations (120-200 Hz) (reviewed in Benes and Berretta, 2001). When the appropriate type of interneurons are excited, they generate rhythmic, synchronized activity within the interneuron network and inhibit pyramidal cells in a coordinated manner. The pyramidal cells can be excited only in the phase of the oscillation cycle when inhibition is at its lowest. Thus pyramidal cell activation is a self-terminating circuit: activated pyramidal cells excite interneurons, which, after a monosynaptic delay inhibit the pyramidal cells in a feedback loop. Pyramidal cells are the main source of excitation for the interneurons, and when pyramidal cells are inhibited, the activity of the interneurons becomes reduced, thus permitting the next firing phase of the pyramidal cells (reviewed in Fries et al., 2007).

There are several reasons why Pv-positive basket cells are thought to be responsible for the generation of gamma oscillations: they are relatively numerous, e.g. in the hippocampus accounting for ~20 % of all GABAergic interneurons; they are abundantly interconnected to

other basket cells via synapses and gap junctions (Gulyas et al., 1999); one basket cell can innervate large numbers of pyramidal cells; they have fast, non-adapting kinetics and a high intrinsic resonance frequency ideal for the generation of gamma oscillations (Pike et al., 2000), and finally, their localization at the perisomatic region of the pyramidal cell represents a prime position for the distribution of the gamma oscillations generated in the basket cell network to the pyramidal cells. Pv-positive basket cells are also very active during gamma oscillations; they fire at a rate of ~1 per gamma cycle, precisely phase-locked to the oscillations (Mann et al., 2005). Gamma oscillations occur during all behavioral states. They superimpose upon, and are modulated by slower theta activity, and are believed to offer a temporal framework for binding and processing of information (Csicsvari et al., 2003).

Theta oscillations are thought to be the “online state” of the hippocampal network, and they occur during spatial navigation, learning, memory formation and retrieval, as well as in REM sleep (reviewed in Klausberger, 2009). It has been shown that spatial information can be encoded into the firing of a hippocampal place neuron in such a way that the cell undergoes phase precession during the movement of the animal in the so-called place field of the neuron, a defined visual area to which the neuron responds. During the ~1 s duration of movement across the place field, 7-12 theta cycles are produced, and the principal cell fires at an earlier and earlier phase of the cycle toward the end of the movement. The more powerful the excitation of the pyramidal cell, the earlier in the cycle it fires (Senior et al., 2008). Interneurons contribute to the theta rhythm as well as the gamma rhythm. The output of chandelier cells is markedly increased during high network activity, and they may be recruited to curtail excess excitation. Animals in which theta oscillations have been ablated, have impaired performance in tasks measuring spatial and nonspatial working memory (reviewed in Uhlhaas et al., 2008), evidence of the fundamental role of theta oscillations in this type of memory.

Ripple oscillations occur during resting, slow-wave sleep and consummatory behavior as well as the replay and consolidation of memories (Racz et al., 2009). The contribution of Pv-positive interneurons to ripple oscillations has been demonstrated in transgenic mice in which the excitatory drive from pyramidal cells to the interneurons has been reduced (Racz et al., 2009). Other types of oscillations remained unchanged in these mice, but ripple oscillations were facilitated, implying that the excitation from pyramidal cells to Pv-interneurons is a key regulating factor in reducing ripple activity.

2.2.4 Other parvalbumin-positive neuronal types

Although GABAergic Pv-positive interneurons in various brain areas are probably the most thoroughly researched cell type expressing the parvalbumin protein, other GABAergic Pv-positive cell populations have also been identified. Projections from the globus pallidus to the striatum consist mainly of GABAergic inhibitory neurons, of which 5-19% are Pv-positive, depending on the pallidal subregion (Kita and Kita, 2001). These pallidar projection neurons regulate the activity of striatal projection neurons by innervating striatal GABAergic interneurons. In the substantia nigra pars reticulata, GABAergic Pv-positive cells inhibit dopaminergic neurons via local axon collaterals, in addition to projecting long distances to other sites, e.g. thalamus and the striatum. They may be the main source of intranigral inhibition. Consequently, afferent inhibition of these intranigral GABAergic neurons results in disinhibition of the dopamine cells (Lee and Tepper, 2007). A population of GABAergic Pv-positive neurons projects from the medial septum-diagonal band of Broca region to the hippocampus, where it participates in the generation of theta oscillatory rhythm (Simon et al., 2006). Discrete nuclei within the auditory brainstem also contain Pv-positive neurons, some of which project between the nuclei and some to more distant regions (Fredrich et al., 2009). Cerebellar Purkinje cells are another important Pv-positive cell type (Schmidt et al., 2007) with a widely recognized role in the control of movement and motor learning.

It is important to note that also neurons harboring transmitters other than GABA contain parvalbumin. These neurons may still express GABA_A receptors and thus be modulated by GABAergic afferents or local inhibition, which considerably extends the variety of Pv-positive neurons affected by GABAergic transmission. Pv-positive pyramidal cells projecting from the retrosplenial and somatosensory cortices to caudal parts of the striatum are for the most part glutamatergic, with the exception of a small GABAergic subpopulation (Jinno and Kosaka, 2004). Thalamocortical projections originating from the anteroventral nucleus of thalamus are positive for parvalbumin, but negative for the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) (Danos et al., 1998). The hypothalamic mammillary bodies also contain Pv-positive, GAD-negative, glutamate/aspartate neurons projecting to the anterior thalamus and the midbrain (Bernstein et al., 2007). In the rodent entorhinal cortex, a small number of pyramidal projection neurons are Pv-positive (Wouterlood et al., 1995), as is also the case in the rodent auditory cortex (de Venecia et al., 1998).

2.2.5 Significance of Pv-positive neurons in pathophysiology

Given their ubiquitous influence on the precision of principal cell firing and the contribution to overall network coordination, it is not surprising that deficits in the functioning of Pv-positive interneurons have far-ranging consequences on information processing in many brain areas. Schizophrenia is one of the illnesses where GABAergic functioning is abnormal and where Pv-positive interneurons play a significant role (reviewed in Daskalakis et al., 2007). An overall reduction in cortical inhibitory tone and synchronized activity has been detected in the brains of schizophrenic patients (reviewed in Benes and Berretta, 2001). The number of Pv-immunoreactive interneurons is decreased in the prefrontal cortex (Beasley and Reynolds, 1997) as well as in all hippocampal subfields (Zhang and Reynolds, 2002) of schizophrenic patients. These studies have not clarified whether the Pv-positive neurons are simply destroyed, whether they have not developed appropriately in the first place, or whether the production of GABA or the expression of parvalbumin has been disrupted, although in the study of (Hashimoto et al., 2003) it was proposed that in most cases the last possibility may occur. A decrease in the number of axonal cartridges formed by chandelier cells on the axon initial segments of pyramidal cells was observed in the prefrontal cortex of schizophrenic patients, pointing to alterations in the output control of these pyramidal cells (Woo et al., 1998). Since the expression of the Ca^{2+} -buffering parvalbumin protein begins relatively late in postnatal life, in addition to Pv-positive cells displaying a fast-firing phenotype and expressing many glutamatergic NMDA receptors, it has been speculated that these neurons are especially vulnerable to excitotoxic injury during development (reviewed in Jones, 2010). In addition to cell death, excitotoxicity can also cause synaptic and dendritic atrophy (reviewed in Woo et al., 2010). Cortical networks are still undergoing plastic preprogrammed changes as late as during adolescence, with oscillatory activity which is governed by Pv-positive interneurons playing a role in the maturation process (reviewed in Uhlhaas and Singer, 2010). Schizophrenia usually appears in adolescence or early adulthood, and it is conceivable that early damage to Pv-interneurons may ultimately manifest as a lack of appropriate coordinative function during this reorganization period, leading to clinical illness.

In schizophrenia as in many other neuropsychiatric disorders, Pv-positive interneurons are the only class of interneurons affected, other types of interneurons apparently being protected by their different chemical and electrophysiological properties. One of the hallmarks of schizophrenia which encompasses several aspects of cognitive abnormalities, such as

attentional deficits and sensory disintegration, is impairment of working memory. Working memory involves the prefrontal cortex, requires coordination between multiple brain regions and has been found to be associated with memory-related theta (Schmiedt et al., 2005) and gamma (Fries et al., 2001) oscillatory activity. EEG recordings taken during working memory tasks provide evidence for the involvement of gamma oscillations in the maintenance of information in working memory, and possibly even for a role in long-term memory, as there appears to be a relationship between the amount of gamma activity during memory acquisition and the efficiency of the recall phase (reviewed in Uhlhaas et al., 2008). Indeed gamma frequency and also other types of oscillations were reduced in EEG recordings taken from schizophrenic patients during working memory tasks (reviewed in Jones, 2010). Reductions in gamma oscillations have also been observed in various animal models of schizophrenia, where deficits in sensorimotor gating are the prevalent cognitive symptom, in conjunction with a decrease in Pv-positive interneurons in the entorhinal cortex (Cunningham et al., 2006). The disruption of rhythmic oscillatory activity throughout various brain areas would be a logical mechanism to account for the typical symptomatology of schizophrenia: a disconnection of cognitive, emotive and affective processes. The fact that the function of one of the key players of oscillation, the Pv-positive interneurons, is impaired by a decrease in the parvalbumin content and the altered connectivity to principal neurons in the areas thought to be most crucial to cognition, the cortex and the hippocampus (as discussed in the previous section), adds weight to the link between disrupted oscillations and schizophrenia. An interesting approach to the dissection of the significance of Pv-cells to oscillations would be to isolate their function by either specifically decreasing or increasing their activity and observing the possible alterations in different oscillatory frequencies.

Epilepsy is another category of neurological illness that crucially depends on the balance of inhibition and excitation of the CNS. The various types of epilepsy all have one feature in common, i.e. there are episodes of uncoordinated paroxysmal electrical activity, which are generated either in specific epileptic foci or more generally throughout the brain. Various experimental models of focal epilepsy have led to the hypothesis that neocortical basket and chandelier cells along with their axon terminals are destroyed in epilepsy, resulting in deficient inhibitory control of pyramidal cells (reviewed in DeFelipe, 1999). Pv-positive interneurons in the hippocampal dentate gyrus are selectively damaged in patients with temporal lobe epilepsy as well as in an animal model of temporal lobe epilepsy (Zhou et al., 2009; Gill et al., 2010). The excitatory drive to cortical interneurons is decreased in a model

of epileptic cortical dysplasia, suggesting that deficient inhibition may lead to overexcitation of pyramidal cells and thus to seizure activity (Zhou et al., 2009). Pyramidal cells are the most important source of cortical efferent axons and intracortical collaterals, and as such, are fundamental in the spreading of epileptiform activity (Ribak, 1985). In the hippocampal dentate gyrus, many granule cells, which have a principal cell function similar to cortical pyramidal cells, are destroyed in epilepsy, and the few survivors are densely innervated by both perisomatic basket formations and an increased number of chandelier cell axonal cartridges (Arellano et al., 2004), perhaps due to axonal sprouting. It has been proposed that understanding how chandelier cells govern the axonal output of pyramidal cells is essential if one wishes to explain the appearance of epileptic discharges in hippocampal and cortical tissue. Some controversy exists as to the epilepsy-induced changes in chandelier cell function: in some studies a loss of chandelier cell synapses on the axon initial segment has been observed, but in others the numbers of axonal cartridges have even increased (Wittner et al., 2005). Increased synaptic contact between chandelier cells and principal cells could lead to pathological hypersynchrony in the firing of principal cells. Intriguingly, in temporal lobe epilepsy, where there are sclerotic changes in the hippocampus, the function of Pv-interneurons has in some cases been found to be excitatory (Cohen et al., 2002), perhaps due to denervation-induced alterations in chloride ion transporters and membrane reversal potentials.

Schizophrenia and epilepsy are the most thoroughly researched diseases with respect to Pv-positive cells. Other neurological disorders where Pv-positive cells, both interneurons and projection neurons have been implicated, are Parkinson's disease and multiple sclerosis. In Parkinson's disease, the function of pallidar inhibitory Pv-positive neurons projecting to the subthalamic nucleus is disrupted, leading to disinhibition of the subthalamic nucleus dopaminergic neurons and finally excessive inhibition of the neocortex and brainstem motor nuclei (reviewed in Berghuis et al., 2004). In an animal model of multiple sclerosis, Pv-positive interneurons within the striatum have been specifically destroyed by a yet unknown mechanism, and in multiple sclerosis patients, the function of cortical Pv-interneurons has been found to be compromised (Rossi et al., 2010). The involvement of Pv-interneurons in the altered oscillatory activity and cognitive deficits in autism has also been proposed (Gogolla et al., 2010).

2.3 Cerebellar circuits and Purkinje cells

The cerebellum is a relatively well-characterized structure with regard to its layered neuronal architecture and the connections its various cell types make with each other. Its main domain of influence has traditionally been thought to be the control of movement and motor learning, including gait, posture and balance, but more recent research has also proposed roles for cerebellum in anxiety and neuropsychiatric disorders, such as schizophrenia (reviewed in Baldacara et al., 2008). The cerebellum receives afferent connections from a variety of CNS structures. Information is gathered from the cerebral cortex through relay nuclei in the brainstem, including the olivary and pontine nuclei and these signals arrive via the middle cerebellar peduncle. Information from the spinal cord and the brainstem itself enters via the inferior cerebellar peduncle (Kiyohara et al., 2003). The main outputs of the cerebellum target the cerebral motor cortex and the brainstem via deep cerebellar nuclei (Bengtsson et al., 2004). The central units of cerebellar processing are the Purkinje cells (PC), which are situated in the cerebellar cortex in a monolayer between the white matter and the granule cell layer. PCs receive inhibitory innervation from molecular layer interneurons (MLI), mainly basket and stellate cells, and excitatory innervation from granule cells and inferior olivary neurons (reviewed in Ito, 2000). As in the cerebral cortex and other forebrain areas, cerebellar basket cells produce dense interconnected formations around the somata of principal cells, in addition to forming a plexus around the axon initial segment. Stellate cells innervate the dendritic regions of PCs (Briatore et al., 2010). MLIs supply feed-forward and lateral inhibition of PCs and thus control their firing rate, timing and spread of action potentials.

Forty percent of all inhibitory synapses in the molecular layer are between MLIs. It has been estimated that a single PC receives ~1500 inhibitory synapses from stellate cells, but PC recurrent collaterals in their turn only rarely contact MLIs (Briatore et al., 2010). MLIs are required for cerebellum-dependent behaviors, including motor learning, as they provide a tight control of input-output relations of PCs (Kondo and Marty, 1998). In the cerebellar cortex, connections between MLIs may induce fast (150-200 Hz) oscillations of PCs to enable encoding of information, in a similar manner that inter-interneuronal connections in forebrain areas influence networks of pyramidal neurons (Maex and De Schutter, 2005). Another possible function for connectivity between MLIs is that the reciprocal interaction represses inhibition during periods of strong parallel fiber activity to return the network to

baseline level. This would induce the stabilization of the firing rate of the interneuronal network and enable the maintenance of the network in optimal operational range to respond to the subsequent input (Briatore et al., 2010).

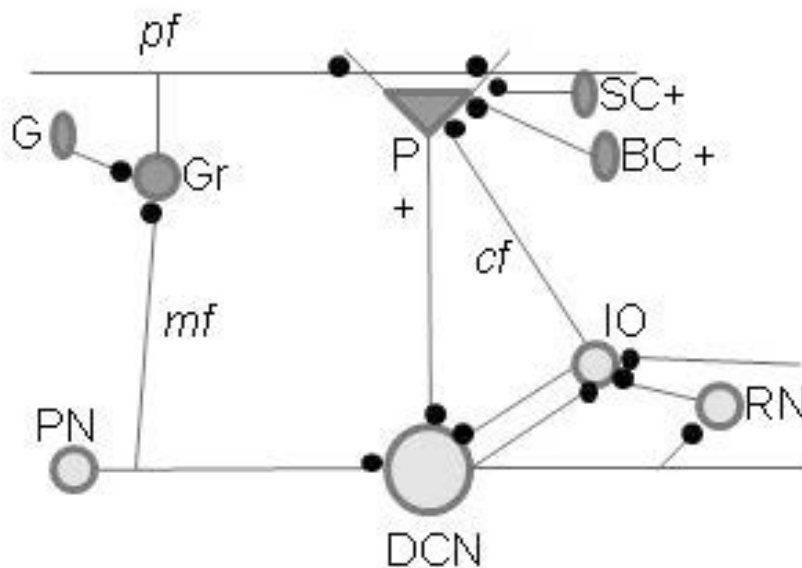


Figure 2. Cerebellar circuits. P Purkinje cell, SC stellate cell, BC basket cell, Gr granule cell, *pf* parallel fiber, *mf* mossy fiber, *cf* climbing fiber, G Golgi cell, IO inferior olive, RN red nucleus, PN precerebellar nuclei, DCN deep cerebellar nuclei. + parvalbumin-positive cell. Black dots denote synapses. (Modified from Ito, 2000).

PCs receive excitatory innervation from the axons of granule cells, which are in turn innervated by mossy fibers from extracerebellar nuclei. Granule cell axons, called parallel fibers, synapse on the dendrites of PCs, with up to 175000 connections per single PC (Isope and Barbour, 2002). In contrast to this high convergence, climbing fibers from the inferior olive, the other important source of excitatory innervations to PCs, usually contact only one PC, although the number of junctions between a climbing fiber and a PC may be as high as 26000 (reviewed in Ito, 2000). The exact mechanism of cerebellar learning has received much attention, and already decades ago (Crepel and Krupa, 1988) it was proposed that climbing fibre activity reduced the power of the parallel fibre-PC synapse in a phenomenon called cerebellar long-term depression (LTD), leading to long-lasting alterations in the circuit (Welsh et al., 2005). Depression of the excitatory influence of parallel fibers onto PCs causes decreased activity of PCs, which in turn disinhibits deep cerebellar nuclei, which are under inhibitory control by PCs (reviewed in Uusisaari and De Schutter, 2011).

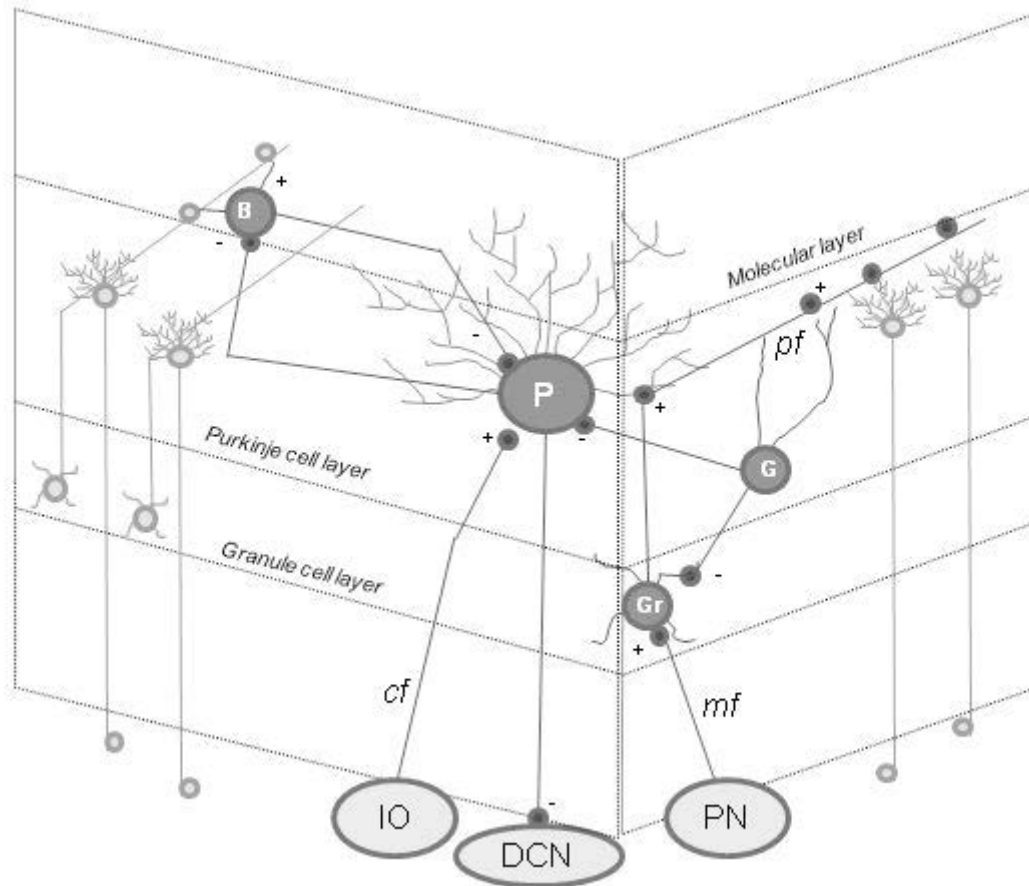


Figure 3. Layers of the cerebellar cortex. P Purkinje cell, B basket cell, Gr granule cell, G Golgi interneuron, IO inferior olive, DCN, deep cerebellar nuclei, PN precerebellar nuclei, *pf* parallel fiber, *cf* climbing fiber, *mf* mossy fiber. + excitatory synapse, - inhibitory synapse. (Modified from Wisden et al., 1996).

2.3.1 Cerebellar mutant mice

A number of cerebellar mutant mouse lines have been described and found to be of use in characterizing the function of cerebellar circuits and cell types [e.g. the *pcd* (Mullen et al., 1976), *wv*, "weaver" (Lane et al., 1977), *tg^{la}*, "leaner" (Herrup and Wilczynski, 1982), *nr*, "nervous" (Landis, 1973), *sg*, "staggerer" (Sidman et al., 1962) and *Lc*, "lurcher" (Caddy and Biscoe, 1979) mouse lines]. Most of these have focused on the role of PCs in the control of movement. These mutations cause the PCs to be wholly or partially destroyed, leading to degeneration of both efferent (deep cerebellar nuclei) and afferent (granule cells, olivary nuclei) targets due to lack of innervation and target deprivation, in addition to sizeable decreases in cerebellar volume (reviewed in Grusser-Cornehls and Baurle, 2001). In some mutants, the cells primarily affected are the granule cells, but reciprocal destruction of PCs follows, pointing to a mutual dependence and interconnectedness during development.

Depending on the exact mechanism and time point of PC and/or granule cell destruction, the mutant mice exhibit a variety of movement and gait abnormalities, ranging from mild ataxia to inability to perform independent locomotion (Baurle et al., 1998). The degree of destruction of PCs is not linear with symptom severity, as mice with near complete loss of PCs may suffer from milder deficits than those animals with ~50% of PCs surviving. This phenomenon points to downstream alterations and compensatory mechanisms and some degree of inhibition being restored to deep cerebellar nuclei in mice with complete destruction of PCs, most likely by a compensatory increase of calcium-binding proteins, which does not appear to occur before 90% of PCs are lost (Baurle et al., 1998). In mice with a significant amount of surviving PCs, the signaling from PCs to deep cerebellar nuclei is apparently so profoundly disorganized that the removal of the whole cerebellum actually improves the motor status of the mice (Grusser-Cornehls et al., 1999). Another problem inherent in these cerebellar mutant models is that the mutations are not necessarily restricted to the cerebellum; there may also be alterations in other brain areas, which confounds the interpretation of the observed defects. If it were possible to rapidly and reversibly modulate the activity of selected cerebellar cell types, e.g. the Purkinje cells *in vivo*, this would give valuable insights to the real-time involvement of the output of the cerebellar cortex to the deep cerebellar nuclei.

2.4 The GABA_A receptor

γ -Aminobutyric acid (GABA) is the most important inhibitory transmitter in the mammalian brain. It is synthesized in the nerve terminals of GABAergic neurons from glutamate by the two isoforms of glutamic acid decarboxylase (GAD) and it is stored in vesicles to await release into the synaptic cleft. When an action potential causes GABA be released into the synapse, the transmitter binds to its receptors in the synaptic membrane of the postsynaptic neuron (reviewed in e.g. Farrant and Nusser, 2005). After it has exerted its effects, GABA's actions are terminated by uptake into the presynaptic neuron or glial cells, or it can be metabolized via GABA transaminase. In addition to synaptic transmission, low concentrations of GABA drifting out of the synapse can activate extrasynaptically located GABA_A receptors. A low, ambient GABA concentration of below 300 nM is constantly maintained in the extracellular environment and contributes to tonic, extrasynaptic inhibition (Santhakumar 2006).

The effects of GABA are mediated through three types of receptors: GABA_A, a ligand-gated ion-channel; GABA_B, a G-protein coupled receptor and GABA_C, an ion channel chiefly restricted to the retina. Of these three, the GABA_A receptor is the most widespread; it is expressed ubiquitously in almost all brain areas and in the spinal cord, in addition to peripheral tissues, in a variety of different cell types. The presence of GAD is a useful neuronal marker for visualizing the location of GABAergic neurons. Due to its fundamental role in the inhibitory/excitatory balance of the CNS, GABA plays a role in a variety of neurological and psychiatric illnesses (reviewed in e.g. Gonzalez-Burgos et al., 2010; Macdonald et al., 2010). Many pathophysiological states, ranging from insomnia, anxiety and epilepsy to schizophrenia, have been shown to be linked to disturbances or abnormalities in the GABA system, whether from genetic alterations in the structure of the channel proteins or reduced amount of the transmitter in brain areas controlling integration of sensory information.

The GABA_A receptor is a member of the Cysteine-loop ligand-gated ion channel family, a anion channel protein consisting of 5 subunits (Lo et al., 2008). It is targeted to the surface membrane of the postsynaptic neuron at or near the synapse and upon agonist binding, it opens to allow the flow of Cl⁻ or other anions either into or out of the neuron to hyperpolarize or depolarize the cell. The direction of the current depends on the physiological state of the network, outflow occurring during development (reviewed in Wang and Kriegstein, 2009) and certain pathophysiological states, e.g. some forms of epilepsy (Isomura et al., 2003). The exact three-dimensional structure of the receptor has not yet been directly established, but good approximations have been obtained through comparisons with the acetylcholine binding protein (Brejc et al., 2001), a structure with close homology to the GABA_A receptor. Each subunit of the receptor weighs ca 50-60 kDa and comprises an N-terminal extracellular domain, a transmembrane domain and a cytoplasmic domain. Agonist binding takes place in the extracellular portion, at the interface of α and β subunits (Ramerstorfer et al., 2011), where a conformational change is triggered, leading to the opening of the channel gate in the transmembrane domain (Lo et al., 2008).

2.5 GABA_A receptor subunits

Nineteen different subunits of the GABA_A receptor have been identified so far, divided into 8 subunit classes: α (1-6), β (1-3), γ (1-3), δ , ϵ , θ and π , in addition to ρ 1-3, this final form mainly

expressed in the retina, where they create GABA_C receptors. They share a protein sequence homology of 70-80% within the subunit class and 30-40% between classes (reviewed in e.g. Macdonald and Olsen; 1994, Olsen and Sieghart, 2009). Some subunits, like $\alpha 1$, are ubiquitously expressed, while others, e.g. $\gamma 1$, have a distinct, restricted regional expression pattern. The generation of knockout mouse lines with ablations of specific subunits (discussed in later sections) has provided many revelations on the significance of the subunits to the rules of receptor assembly and function. The presence of two α subunits, along with two β subunits, is essential for the formation of functional GABA_A receptors, as the two GABA binding pockets are situated at the interface between an α and a β subunit.

2.5.1 The α subunits

The six types of α subunits fundamentally define the affinity of the GABA_A receptor for benzodiazepines and furthermore differentiate between the variety of effects of benzodiazepines. The amino acid H101, present in subunits $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ confers benzodiazepine sensitivity. In $\alpha 4$ and $\alpha 6$, there is an arginine at position 101, which renders these subunits insensitive to benzodiazepines (Wieland et al., 1992). $\alpha 1$ is the dominant α subunit, expressed in almost all areas of the CNS, in interneuronal subtypes as well as principal cells (Briatore et al., 2010). The $\alpha 2$ and $\alpha 3$ subunits have a more discrete pattern of expression, and their expression is generally at a lower level as compared to the $\alpha 1$ subunit (Pirker et al., 2000). The $\alpha 2$ subunit is linked to emotional behaviors due to its location in the amygdala (Low et al., 2000), and $\alpha 3$ may have relevance in absence epileptic symptoms due to its enrichment in the reticular nucleus of thalamus, an area known to regulate thalamocortical oscillations (Schofield et al., 2009). The $\alpha 4$ subunit is found in the thalamus and hippocampal granule cells, where it is usually coexpressed with the δ subunit and comprises an essential component of extrasynaptic, tonically active GABA_A receptors (Belelli et al., 2005, Jia et al., 2008). It also confers a heightened sensitivity to neurosteroids (Belelli et al., 2002). The $\alpha 5$ subunit has its greatest expression in the hippocampus, where it may take part in extrasynaptic transmission (Caraiscos et al., 2004). It is believed to be involved in cognitive tasks; $\alpha 5$ knockout mice have improved performance in memory tasks (Collinson et al., 2002). The $\alpha 6$ subunit is only expressed in the cerebellum and cochlear nuclei, where it confers unique pharmacological properties, such as greater sensitivity to GABA and insensitivity to benzodiazepines (Korpi et al., 1999). It is an essential component of cerebellar tonic extrasynaptic transmission (Brickley et al., 2001).

2.5.2 The β , δ , θ , ϵ and π subunits

A β subunit is required for the formation of functional GABA_A receptors and it is involved in the surface expression, agonist binding and anesthetic sensitivity of the GABA_A receptor. The type of the β subunit may affect the subcellular localization of the receptor: whether it is targeted to the dendrites, cell body or axon of the neuron (Connolly et al., 1996), as well as the affinity of the receptor for certain anesthetic and anticonvulsant medications, such as etomidate and loreclezole (Wingrove et al., 1994; Hill-Venning et al., 1997), in addition to binding the benzodiazepine inverse agonist DMCM (Stevenson et al., 1995) and furosemide (Korpi et al., 1995). The δ subunit is closely associated with the $\alpha 4$ and $\alpha 6$ subunits, together with which it assembles to form mainly, though perhaps not exclusively, tonically active extrasynaptic receptors (Stell et al., 2003), although in hippocampal interneurons, the δ subunit also colocalizes with the $\alpha 1$ subunit (Glykys et al., 2007). GABA may act as a partial agonist in δ containing receptors, while other GABA site agonists, such as THIP, are full agonists (Brown et al., 2002; Saarelainen et al., 2008; Chandra et al., 2010). The physiological significance of subunits θ , ϵ and π is unclear, and there are dissimilarities between species in the brain regions where they are expressed (Sinkkonen et al., 2000; Ranna et al., 2006). Overall their expression level is very low, but they may have important roles in discrete nuclei, like the locus ceruleus and some hypothalamic areas.

2.5.3 The γ subunits

A γ subunit is required for benzodiazepine modulation of the GABA_A receptor. $\gamma 2$ confers full sensitivity, whereas the presence of $\gamma 1$ or $\gamma 3$ subunits reduces the modulation by benzodiazepine site ligands as compared to $\gamma 2$ (Herb et al., 1992; Benke et al., 1996). The $\gamma 1$ subunit has a very low overall expression level, with the greatest expression being found in the basal ganglia, basal and septal forebrain nuclei, restricted thalamic nuclei, central and medial nucleus of amygdala and the inferior olivary complex (Pirker et al., 2000). The tightly restricted expression pattern suggests discrete functions, e.g. the $\gamma 1$ subunit has been suspected to be involved in hypothalamic regulation of sexual behavior (Nett et al., 1999). The pharmacological properties of benzodiazepines are changed in $\gamma 1$ compared to $\gamma 2$ containing receptors. Zolpidem produces a weak negative modulation instead of the classical positive modulation (Nett et al., 1999), and vice versa DMCM is positively, instead of negatively modulating (Puia et al., 1991).

The $\gamma 2$ subunit is one of the most widely investigated of all the GABA_A receptor subunits due to its multiple functions, ubiquitous expression and clinical significance with regard to benzodiazepine pharmacology. There are two splice variants, $\gamma 2S$ and $\gamma 2L$, which differ by only eight amino acids. Despite the differences in their expression levels during development and some regional variation in adult animals, the two variants seem to be interchangeable (Boileau et al, 2010) and the overexpression of either one produces few phenotypic changes (Wick et al., 2000). Knockout of the $\gamma 2$ subunit leads to massive neonatal mortality, with few animals surviving beyond a couple of days and displaying severe neurological abnormalities (Günther et al., 1995). Electrophysiological experiments have confirmed that receptors without the $\gamma 2$ subunit are fully functional but have a lower GABA half-maximal effective concentration (EC₅₀) and reduced single channel conductance (Lorez et al., 2000). Their GABA sensitivity is increased, but they convey smaller currents. Considering the fact that the brain morphology and endocrinology of $\gamma 2$ knockout mice are normal, and that functional GABA_A receptors are formed and transported to the cell surface, the $\gamma 2$ subunit does not seem to be required for receptor assembly, transport, insertion into membranes, subcellular targeting or clustering of the GABA_A receptors (Günther et al., 1995). Instead it is obligatory for the development of inhibitory circuits; the time frame of death of the $\gamma 2$ knockout animals coincides with the period for synaptogenesis. The $\gamma 2$ subunit targets the GABA_A receptor to the synapse, and is required for the formation of GABAergic synapses (Essrich et al., 1998). Mice heterozygous for the presence of the $\gamma 2$ subunit are viable but display phenotypic changes, including heightened neophobia and enhanced fear learning, as well as decreased amounts of synaptic clusters of GABA_A receptors (Crestani et al., 1999). This haploinsufficiency implies that in wild-type neurons there is no excess amount of $\gamma 2$ subunit.

The $\gamma 3$ subunit is the least widely expressed of the three γ subunits. The olfactory bulb, the basal forebrain including the hypothalamus, midbrain, pons and medulla harbor the most expression of the subunit, though nonetheless at a low overall level (Pirker et al., 2000). The $\gamma 3$ subunit confers a benzodiazepine agonist sensitivity which is two orders of magnitude lower than the $\gamma 2$ subunit, though the affinities of antagonists and inverse agonists remain approximately the same (Herb et al., 1992).

2.6 GABA_A receptor assembly

The rules governing the assembly into functional receptors of the 19 known GABA_A receptor subunits have been a topic of intense study. The total number of possible combinations is exceedingly large, and even after establishing some basic rules of assembly, over 800 different theoretical subtypes still exist, though the number of naturally existing subtypes is limited by temporal and cell-type restriction of the expression of subunits available for assembly (reviewed in Olsen and Sieghart, 2009). For some receptor subtypes there is definitive proof of expression in animal brain, for others the evidence is more tentative (Table 1). There is by now universal agreement of the obligatory coassembly of two α subunits, two β subunits and an additional subunit, usually γ or δ . This knowledge has been complemented with evidence from rarer, anatomically restricted subunits that may play unique physiological roles (reviewed in e.g. Ranna et al., 2006). The prevailing GABA_A receptor subtype is thought to be $\alpha 1\beta 2\gamma 2$, with a prevalence of 43% of all GABA_A receptors in the CNS (McKernan and Whiting, 1996).

A. Confirmed

$\alpha 1\beta 2\gamma 2$	$\alpha 4\beta \gamma 2$	$\alpha 5\beta \gamma 2$	$\alpha 6\beta 3\delta$
$\alpha 2\beta \gamma 2$	$\alpha 4\beta 2\delta$	$\alpha 6\beta \gamma 2$	ρ
$\alpha 3\beta \gamma 2$	$\alpha 4\beta 3\delta$	$\alpha 6\beta 2\delta$	

B. High probability of existence

$\alpha 1\beta 3\gamma 2$	$\alpha 5\beta 3\gamma 2$	$\alpha \beta$
$\alpha 1\beta \delta$	$\alpha \beta 1\gamma / \alpha \beta 1\delta$	$\alpha 1\alpha 6\beta \gamma / \alpha 1\alpha 6\beta \delta$

C. Possible

$\rho 1$	$\alpha \beta \gamma 1$	$\alpha \beta \theta$
$\rho 2$	$\alpha \beta \gamma 1$	$\alpha \beta \pi$
$\rho 3$	$\alpha \beta \epsilon$	$\alpha x \alpha y \beta \gamma 2$

Table 1. GABA_A receptor subtypes proposed to exist *in vivo*. (Modified from Olsen and Sieghart, 2009).

2.7 Synaptic and extrasynaptic inhibition

Fast synaptic inhibition is a temporally precise inhibitory function that targets specific areas of the postsynaptic neuron. It occurs through quantal release of transmitter from the

presynaptic neuron in response to an action potential. Transmitter molecules are stored in the presynaptic terminal and are released by a mechanism of various recognized classes of proteins involved in the priming, docking and fusion of transmitter vesicles to the neuronal surface membrane to achieve release of the transmitter into the synaptic cleft (Delgado-Martinez et al., 2007). The GABA concentrations produced in this way are present in the millimolar range, with every vesicle containing several thousands of GABA molecules. The released transmitter is received into the postsynaptic density of the synaptic bouton of the postsynaptic neuron, where it may bind to various receptors and subsequently may modulate the activity of the neuron. Synaptic transmission is fast, usually on a time scale of milliseconds; the neurotransmitter effect can be terminated by the diffusion of the transmitter out of the synapse, its breakdown into metabolites, its disengagement from the receptors or by receptor desensitization (reviewed in Farrant and Nusser, 2005). In the GABAergic system synaptic receptors require the $\gamma 2$ subunit to be correctly targeted to a postsynaptic localization and anchored there by the synaptic protein gephyrin, possibly by direct protein-protein interactions (Essrich et al., 1998). Some receptors containing the $\gamma 2$ subunit can also be found located peri- or extrasynaptically, but the majority of GABA_A receptors outside synapses are of the binary $\alpha\beta$ type or $\alpha\beta\delta$ receptors (Brickley et al., 1999; Mortensen et al., 2010). The $\gamma 2$ subunit confers synaptic receptor properties missing from $\alpha\beta$ receptors: benzodiazepine sensitivity, a lower affinity for GABA and a higher conductance (Hevers and Lüddens, 1998).

The characteristics typical of extrasynaptic inhibition compared to synaptic inhibition are lower GABA EC₅₀ values, reduced current amplitudes, slower desensitization and absence of fast desensitization, in addition to increased outward rectification (Belelli et al., 2009; Bright et al., 2011). Peri- and extrasynaptic receptors are activated by high nanomolar, ambient GABA concentrations that are attributable to GABA drifting out of the synapse after fast synaptic transmission. This type of inhibition is also called tonic, to designate the stable, background nature of extrasynaptic inhibition, although it is nevertheless subject to dynamic modulation by physiological events and pharmacological treatments (reviewed in Farrant and Nusser, 2005). Tonic inhibition is believed to maintain inhibitory equilibrium during and in the absence of fast, synaptic events and so to regulate the overall GABAergic tone of many brain areas. Considering that extrasynaptic receptors may be spread throughout the surface membrane of the neuron, as opposed to the minimal surface area occupied by from tens to some hundreds of synaptic receptors, it becomes evident that extrasynaptic transmission is able to carry more charge transfer than synaptic transmission. It is estimated that there is a

threefold overall difference in charge transfer, and it follows that extrasynaptic inhibition is a fundamental element in the control of neuronal excitability. First discovered in the cerebellar granule cells (Brickley et al., 1996), it has since been shown to play many roles e.g. in the development of absence epileptic discharges in the thalamus (Belelli et al., 2009) and is involved in mediating the effects of neurosteroids and various other classes of GABAergic drugs (excluding the benzodiazepines).

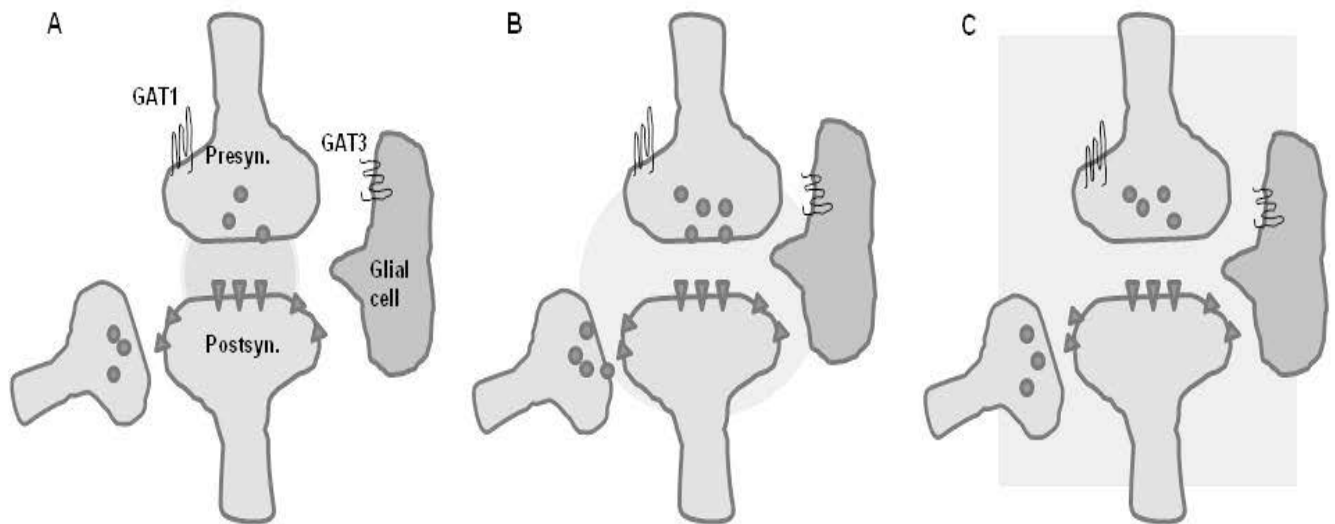


Figure 4. Synaptic and extrasynaptic transmission in a GABAergic synapse. **A** Transmitter release from a vesicle activates synaptic GABA_A receptors. **B** Release from multiple vesicles or terminals results in transmitter spillover and activates also extrasynaptic receptors. **C** Tonicly active high-affinity extrasynaptic receptors respond to low, ambient concentrations of GABA. GAT1, GAT3: GABA transporter proteins. (Modified from Farrant and Nusser, 2005).

2.8 Ligand binding sites of the GABA_A receptor

The GABA_A receptor harbors binding sites for a number of substances which can modulate its activity, many of which have marked clinical significance. Two similar agonist binding sites are situated at the interface between α and β subunits (Ramerstorfer et al., 2011) and exist in all known receptor subtypes, also in binary receptors. In addition to the natural agonist GABA, the site binds several experimental agonists such as muscimol, THIP, and isoguvacine, as well as antagonists such as bicuculline. The affinity of some agonists, e.g. muscimol and THIP, is dependent on the subunit composition of the receptor; unlike GABA, they have a higher efficacy at $\alpha 4$ and $\alpha 6$ containing receptors (Winsky-Sommerer et al., 2007). The exact sequence of conformational changes that occurs after the activation of the agonist binding site to the opening of the channel pore has been under vigorous investigation for many years, but is still not completely understood. It is currently considered most likely

that the changes proceed in a stepwise manner instead of a single hinge-or pivot-like alteration of the component parts of the channel in relation to each other (Kash et al., 2004).

The cage convulsant TBPS, as well as other members of the related group of convulsants with a cage-like chemical structure, binds to the picrotoxin-sensitive convulsant binding site in the channel pore (Korpi et al., 1996), and this can be used as a pharmacological tool for investigating the properties and functional mechanics of the GABA_A receptor. Radiolabeled [³⁵S]TBPS binds to all conformations and subtypes of the receptor and is displaced by the natural ligand GABA in a concentration-dependent manner (Luddens and Korpi, 1995). The GABA-induced displacement can be modulated bidirectionally by GABAergic substances: ligands which enhance the actions of GABA decrease [³⁵S]TBPS binding and conversely ligands which decrease the actions of GABA increase [³⁵S]TBPS binding (Maksay and Simonyi, 1986). In addition to offering a way to quantify pharmacological effects at the *ex vivo* level, [³⁵S]TBPS binding is a feasible technique to assess the levels of GABA_A receptors in various brain areas. It is a flexible method and can be used in a variety of assays, ranging from those based on recombinant receptors and homogenized whole-brain membrane preparations to experiments with frozen slices of brain tissue.

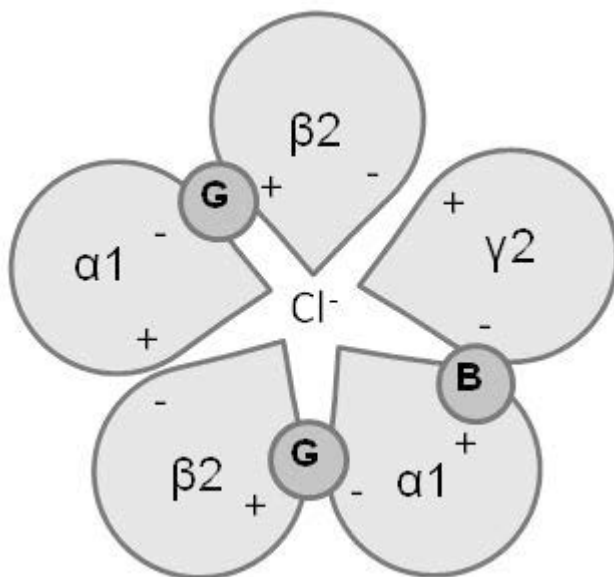


Figure 5. A topside view of the GABA_A receptor. Five subunits center around a Cl⁻ channel pore. Two GABA binding sites (G) are located between α and β subunits, and a benzodiazepine binding site (B) is formed between an α and a γ subunit.

2.8.1 The benzodiazepine site

The class of GABAergic substances with currently the most clinical significance and consequently the subject of the most research effort, is the benzodiazepines. Their spectrum of effects ranges from mild sedation to full hypnosis, giving rise to a variety of medical indications: anxiety, insomnia, status epilepticus and preoperative care etc. The allosteric benzodiazepine binding site of the GABA_A receptor is situated at the interface between the α and γ subunits. The γ 2 subunit is required for benzodiazepine sensitivity, receptors with γ 1 or γ 3 having a significantly lower or nonexistent sensitivity (Herb et al., 1992; Khom et al., 2006). When binding to this allosteric site, benzodiazepines increase the opening frequency of the channel and so increase the apparent affinity of GABA for the receptor (reviewed in Sigel and Luscher, 2011). In the absence of the natural ligand, benzodiazepines have no effect on the receptor and as such have low toxicity. The chemical variety of substances that have affinity for the benzodiazepine site is large, but they can be divided into three groups according to effect. Agonists of the site (also called positive modulators) enhance the effects of GABA and have anxiolytic, sedative-hypnotic, anticonvulsant, amnesic and muscle relaxant actions. Inverse agonists decrease the effects of GABA by lowering the opening frequency of the channel and produce the opposite effects, including anxiogenesis, heightened vigilance and convulsions. Antagonists have no intrinsic effect on the activity of the receptor even at high concentrations, but negate the effects of both agonists and inverse agonists (reviewed in Olsen and Sieghart, 2009).

The benzodiazepine binding site has been highly conserved during evolution, with several amino acids in the α subunit, in addition to at least two in the γ 2 subunit, having been identified as being essential for its structure. Despite the similarities in the sequences of α subunits, it has been possible to develop subtype selective ligands of the benzodiazepine site, which have significantly more affinity for or efficacy in only a distinct subset of GABA_A receptors (Savic et al., 2010). These have provided valuable insights into the influence of the receptor subtype on the pharmacological profile of the receptor. Pharmacological and genetic experiments have established that sedation/hypnosis and anticonvulsive activities are largely mediated through the α 1 subunit (Crestani et al., 1999), anxiolysis and muscle relaxation through α 2/ α 3 (Low et al., 2000) and amnesia through the α 5 subunit (Collinson et al., 2002). Subunits α 4 and α 6 are insensitive to benzodiazepines due to the presence of an arginine instead of histidine at position 101 (Dunn et al., 1999). A number of the benzodiazepine site

ligands with some subtype selectivity have shown either clinical or experimental utility. Zolpidem is a widely used sedative-hypnotic with high affinity for $\alpha 1$ subunit containing receptors (Crestani et al., 2000). L-838,417 and TPA023 are relatively new compounds with equal affinity for all of the benzodiazepine sensitive α subunits ($\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$), but efficacy only at $\alpha 2/\alpha 3$ containing receptors (Scott-Stevens et al., 2005; Ator et al., 2010). L-655,708 has an inverse agonist action at $\alpha 5$ containing receptors and has been shown to enhance cognition (Martin et al., 2009). These subtype targeted substances are selectively aimed at producing only some of the many effects of classical benzodiazepines, avoiding others which are perceived as unwanted, even harmful, depending on the treatment modality.

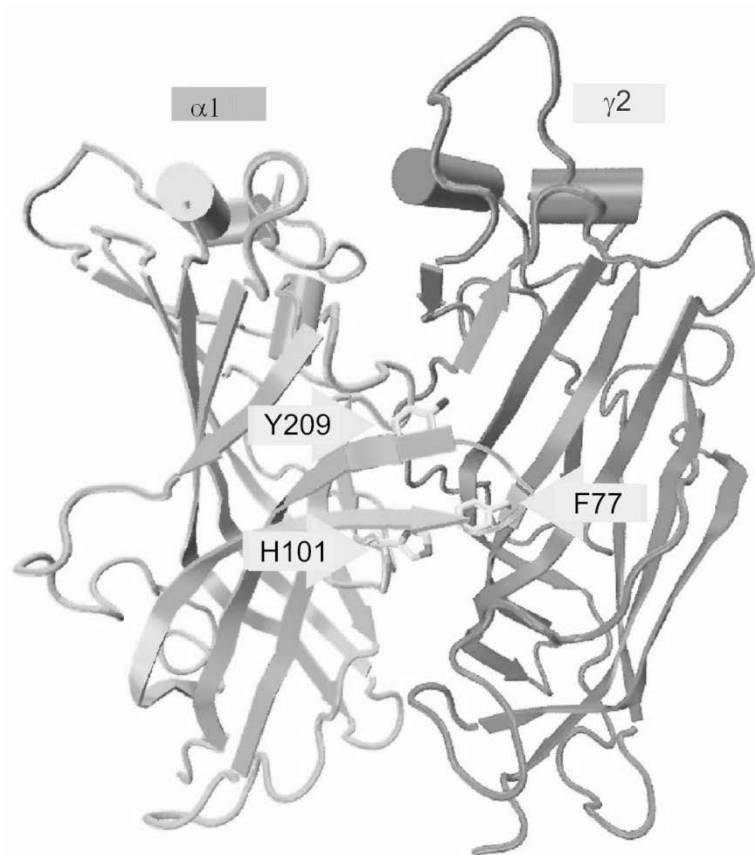


Figure 6. A homology model of the benzodiazepine binding site of the GABA_A receptor: the extracellular domains of $\alpha 1$ and $\gamma 2$ subunits indicating the ligand binding pocket. The bottom of the figure corresponds to the C-terminal end of the extracellular domain. Amino acid residues $\alpha 1$ H101, $\alpha 1$ Y209 and $\gamma 2$ F77 are highlighted. (Reproduced from Ogris et al., 2004 with permission).

2.8.2 The loreclezole site

Loreclezole is a broad-spectrum anticonvulsant which binds to a β subunit dependent site on the GABA_A receptor. The actions of loreclezole do not require the presence of the $\gamma 2$ subunit, which is needed for benzodiazepine effects, and as such it is likely to be present in all

GABA_A receptors, also those with the binary $\alpha\beta$ composition. Receptors with $\beta 2/\beta 3$ are more sensitive to loreclezole than receptors with $\beta 1$ with about 300-fold difference in affinity (Wingrove et al., 1994). Two of the key amino acids present in this site are N289 and N265 in the channel lining TM2 region; when the asparagine is mutated to serine, there is a marked loss of sensitivity (Wingrove et al., 1994; Stevenson et al., 1995). One of the other substances that has been shown to share the same binding site is the β -carboline structured convulsant, DMCM. Though better recognized as a full inverse agonist of the benzodiazepine binding site with high nanomolar affinity, DMCM has low micromolar affinity *in vitro* (Stevenson et al., 1995) and *ex vivo* (Makela et al., 1997) also at the loreclezole site, where its effects are GABA-potentiating. The phenomenon was first investigated in cerebellar $\alpha 6$ subunit containing receptors, which are naturally insensitive to effects mediated via the benzodiazepine site, and the results have subsequently been confirmed with recombinant receptors. One interesting question is whether this agonistic effect could also be produced *in vivo* in conscious animals without concurrently invoking the strong inverse agonism of DMCM. In addition to DMCM, other β -carbolines like β -CCE also have affinity for this low-affinity site. The report of Walters et al., (2000) speculated that even classical benzodiazepines like diazepam may have some affinity to the low micromolar site, in addition to their more traditional effects. This could be a possible mechanism for the anesthetic effects of diazepam at high doses, and also makes the low affinity site an interesting target for the development of novel GABAergic substances. Since it is likely to be present also in binary, extrasynaptically located receptors, substances binding there would also affect tonic inhibition.

2.8.3 Other binding sites

Barbiturates, a class of drugs previously widely used for sedative and hypnotic and anti-convulsant purposes, have two binding sites in the GABA_A receptor, an allosteric modulatory one, where barbiturates increase the open time of the channel, but do not increase the opening frequency or probability, and a directly activating one, where at high concentrations, they are able to activate the channel by themselves (Serafini et al., 2000). Amino acid residues in the β subunit are known to be essential for the actions of barbiturates (Serafini et al., 2000). Neuroactive steroids also have both modulatory and directly activating effects at the receptor and are assumed to have two separate binding sites. In addition, there are reports of certain neurosteroids having a negative modulatory effect, perhaps via yet another binding site (Akk

et al., 2001). Volatile anesthetics, e.g. halothane, were previously believed to exert their inhibitory effect on the CNS via a nonspecific modulation of the lipid bilayer of the neuronal membrane, but they have since been shown to have a specific binding site in the GABA_A receptor (Werner et al., 2011). Intravenous anesthetics, like etomidate and propofol also have their own binding/effector site, which is common to the anticonvulsant loreclezole. Other minor binding sites in the GABA_A receptor, such as the sites for zinc, lanthanum and furosemide, though not clinically relevant, can be used in experimental approaches to investigate the properties of the receptor (Korpi and Luddens, 1997).

Ethanol is a clinically and epidemiologically significant substance of abuse, which binds to several targets in the CNS, among them the GABA_A receptor. Much recent work has focused on its multiple binding sites in the GABA_A receptor, especially a γ 2 subunit-dependent site and a recently reported δ subunit-dependent one (Hancher 2006). Some controversy has existed as to the mechanism of action of the partial benzodiazepine inverse agonist Ro 15-4513 in reversing the receptor-mediated effects of ethanol, and as such the importance of the γ 2 subunit as a site of action of ethanol. Currently it seems more likely that at least the sedation caused by ethanol is mediated through the γ 2-dependent and not the δ -dependent binding site (Linden 2011).

2.9 The γ 2I77 point mutation

One of the amino acids present in the benzodiazepine binding site is phenylalanine (F)77 of the γ 2 subunit. Point mutation studies have established that changes in this position, interestingly analogous to F64 in the GABA binding site of the α subunit (Buhr et al., 1997), affect the binding of benzodiazepines. F77L, F77Y, F77W and F77I mutations have been created and shown to exhibit reduced affinities for a number of benzodiazepine-type substances to varying degrees depending on their chemical structures (Buhr et al., 1997). Classical nonselective benzodiazepines like diazepam and flunitrazepam mostly retain their affinities, but the affinity of some ligands like zolpidem, is reduced, as well as the affinity of the inverse agonist DMCM. In all of these mutations, the affinity of the receptor for GABA remains largely the same as in F77 containing receptors, and the coupling of the benzodiazepine site to the agonist site is intact, which implies that the functionality of the receptor has been preserved despite the point mutation (Buhr et al., 1997; Wingrove et al.,

1997). The I77 mutation is particularly interesting, as there is an isoleucine instead of phenylalanine at position 77 in the $\gamma 1$ subunit, which is insensitive to DMCM and zolpidem (Puia et al., 1991). The I77 mutation renders the $\gamma 2$ subunit insensitive to triazolam, clonazepam, zolpidem, flumazenil, Ro 15-4513, DMCM and other β -carbolines (Buhr et al., 1997; Wingrove et al., 1997; Cope et al., 2004).

A mouse line with the $\gamma 2I77$ point mutation was generated and an electrophysiological, pharmacological and phenotypic analysis was performed (Cope et al., 2004). The $\gamma 2I77$ mice were healthy and viable, they bred normally and had no phenotypic abnormalities in the SHIRPA behavioral screen. Furthermore, their gross brain morphology was unaltered. The expression levels of most GABA_A receptor subunits in $\gamma 2I77$ brains were normal, but there was an overall 15% decrease in $\gamma 2$ subunit levels. The ability of zolpidem and also flumazenil to displace [³H]flunitrazepam binding was strongly reduced in $\gamma 2I77$ brains, with only a slight reduction in the ability of flurazepam to displace the radioligand (Cope et al., 2004). There were no differences in baseline synaptic currents of $\gamma 2I77$ mice, but zolpidem was unable to enhance the peak amplitude, weighted decay, rise time or charge transfer of averaged miniature postsynaptic currents (mIPSCs), or the total current and frequency of mIPSCs in the cerebellar Purkinje cells, of $\gamma 2I77$ mice, a cell type where both $\alpha 1$ and $\gamma 2$ subunits are expressed (Cope et al., 2004). Zolpidem was administered to awake, freely moving $\gamma 2I77$ mice in behavioral tests. Zolpidem at 3 mg/kg did not produce sedation in the staircase chamber in $\gamma 2I77$ mice, whereas control mice were clearly sedated and failed to climb any stairs. In a rotarod test, a cumulative dosing of 1-30 mg/kg zolpidem did not impair the performance of $\gamma 2I77$ mice. The effects of flurazepam on the rotarod performance of $\gamma 2I77$ mice were unaltered compared to wild-type mice (Cope et al., 2004). The effects of zolpidem were also strongly reduced in the hippocampus of $\gamma 2I77$ mice, although slight residual effects were found to be present in that area (Cope et al., 2005). Ogris et al., (2004) performed an extensive study on the effects of a total of 24 substances from various classes of benzodiazepine binding site ligands on [³H]flunitrazepam binding in a membrane preparation of $\gamma 2I77$ brains and confirmed that the $\gamma 2I77$ mutation selectively affected only a few ligands acting through the benzodiazepine binding site. The greatest reductions in the ability to displace [³H]flunitrazepam binding were found with zolpidem, flumazenil, the triazolopyridazine CI 218872 and the β -carboline DMCM (Ogris et al., 2004). The $\gamma 2I77$ mouse line would be an interesting tool to further test *in vivo* the reductions in the effects of benzodiazepine ligands, as well as the residual effect of zolpidem observed by (Cope et al.,

2005) and the positive modulating effect of DMCM observed *in vitro* by (Stevenson et al., 1995).

3 AIMS OF THE STUDY

The purpose of these studies was to develop a method for the fast, reversible modulation of specific neuronal populations and to use that method to clarify the significance of selected neuron types to the overall function of the CNS. Pv-positive neurons and cerebellar Purkinje cells form integral parts of complex networks for the control of diverse behavioral modalities, the details of which are incompletely known. By modulating the activity of one cell type of the network in genetically altered mouse models, new information on the roles of the particular cell types to the network and behavior of the animal could be obtained. In addition, it was wished to use the background $\gamma 2I77$ mouse line to explore the possible residual actions of GABA_A receptor benzodiazepine binding site ligands at other, low affinity binding sites uncovered by the point mutation in the $\gamma 2$ subunit of the receptor. Both *in vivo* and *ex vivo* methods were used to achieve these specific aims:

1. To investigate whether the convulsant, GABA_A receptor inverse agonist DMCM and the subtype-selective hypnotic zolpidem have residual activity in $\gamma 2$ subunit I77 point mutant mice mediated through non-benzodiazepine sites of action
2. To analyze the significance of the removal of the GABA_A receptor $\gamma 2$ subunit from cerebellar Purkinje cells on mouse behavior
3. To modulate cerebellar Purkinje cells in a fast and reversible manner to reveal their significance on mouse motor coordination
4. To analyze the significance of the removal of the GABA_A receptor $\gamma 2$ subunit from Pv-positive neurons on mouse behavior
5. To modulate Pv-positive neurons in a fast and reversible manner to reveal their significance on mouse behavior

4 MATERIALS AND METHODS

4.1 Experimental animals

All animal experiments were approved of by the Southern Finland Provincial Government and the Institutional Animal Use and Care committee of the University of Helsinki. All efforts were made to minimize the number and suffering of animals used. C57BL/6J (n=35), wild type $\gamma 2F77$ (n=22), heterozygous $\gamma 2F77/\gamma 2I77$ (n=4), $\gamma 2I77$ (n=196), Pv- $\Delta\gamma 2$ (n=16), Pv- $\Delta\gamma 2$ -partial rescue (n=36), PC- $\Delta\gamma 2$ (n=26) and PC- $\gamma 2$ -swap (n=22) mice of both sexes were used. In all behavioral experiments the individual who observed and recorded the behavior was blind to the genotype of the animals

4.1.1 Generation of mouse lines

The genetically engineered mouse lines were generated by collaborators as described below in the original references: For the $\gamma 2I77$ line, bacterial artificial chromosome (BAC) mediated recombination was performed in mouse embryonic stem cells to replace the wild type F77 codon with the I77 codon (Cope et al., 2004). For the Pv- $\Delta\gamma 2$ line, mice with loxP-site flanked GABA_A receptor $\gamma 2I77$ subunits were crossed with mice expressing Cre recombinase in Pv-positive cells; correct expression of transgene was ascertained with immunohistochemistry for parvalbumin and *in situ* hybridization probes for parvalbumin and the $\gamma 2$ subunit (Wulff et al., 2009a). For the Pv- $\Delta\gamma 2$ -partial rescue line, two transgenes were co-integrated by mixing and co-injection of two BACs into pro-nuclei of mouse embryonic stem cells; the resulting mouse line was crossed with mice expressing the loxP-flanked $\gamma 2I77$ subunits. Expression of the transgene was ascertained with *in situ* hybridization (IV). For the PC- $\Delta\gamma 2$ line, mice with loxP-flanked GABA_A receptor $\gamma 2I77$ subunits were crossed with mice expressing Cre recombinase in cerebellar Purkinje cells (III). For the PC- $\gamma 2$ -swap line, a triple cross between loxP-flanked $\gamma 2I77$ subunit mice, mice expressing Cre recombinase in Purkinje cells, and mice with green fluorescent protein (GFP)-tagged wild type F77 subunits expressed in Purkinje cells was performed. Expression of transgene was ascertained with *in situ* hybridization and immunohistochemistry (III).

4.2 Culturing and transfection of HEK-293 cells

Human embryonic kidney (HEK-293) cells are a widely used, feasible method of expressing many types of proteins in the cellular surface membrane. They can be utilized e.g. in the electrophysiological analysis of ion channel activity and the functions of transport proteins, in addition to autoradiographic experiments.

HEK-293 cells were passaged and replated on plastic dishes, which were filled with medium and supplemented with sodium bicarbonate, glutamine, penicillin-streptomycin and fetal calf serum. Cultures were maintained at 37°C in a humidified atmosphere for 2-3 days. Transfection with recombinant rat GABA_A receptors was carried out as described in detail in (Korpi and Lüddens, 1993). For the identification of transfected cells for electrophysiological recordings peGFP-N1 vector was added. HEK-293 cells were used for ligand binding autoradiography and electrophysiological experiments (II).

4.3 Ligand autoradiography and binding assays

GABA_A receptor ligands labeled with a radioactive isotope can be used to visualize the amount and location of the receptor in the brain. The ionophore, convulsant binding site is recognized by several radioligands, e.g. the picrotoxin-sensitive [³⁵S]*t*-butylbicyclophosphorothionate ([³⁵S]TBPS), a cage convulsant which binds inside the Cl⁻ channel pore. [³⁵S]TBPS binding can be modulated with GABAergic substances: compounds which enhance the effects of GABA decrease [³⁵S]TBPS binding whereas, conversely, compounds which decrease GABA effects enhance [³⁵S]TBPS binding. The assay provides a good way to evaluate the actions of various substances at the receptor level; in addition to bidirectional modulation there is also good regional resolution (Maksay and Simonyi, 1986; Makela et al., 1997).

Another radioligand useful in these studies is [³H]Ro 15-4513, which labels the flumazenil-sensitive benzodiazepine binding site of the GABA_A receptor. Its binding is dependent on the presence of the wild-type γ 2 subunit and it can be displaced competitively with other ligands which bind to the same site. The autoradiographic method can be used for homogenized brain samples or frozen cryostat sections. Although cell structure is lost by freezing, the receptors as well as gross anatomy remain intact (Sieghart et al., 1987; Uusi-Oukari, 1992).

4.3.1 [³⁵S]TBPS autoradiography (I, II, IV)

Cryostat sections were preincubated in an ice-water bath in an EDTA-containing preincubation buffer solution, after which the sections were dipped in the incubation buffer solution. The final incubation took place with [³⁵S]TBPS in the incubation buffer at room temperature for 90 min. Nonspecific binding was determined with picrotoxinin. After the incubation, the sections were washed with wash buffer, dipped in distilled water and dried in air flow at room temperature. The sections were then exposed with [¹⁴C]-plastic standards to imaging plates or to radiosensitive films (Makela et al., 1997).

4.3.2 [³H]Ro 15-4513 autoradiography (II, IV)

Cryostat sections were preincubated in an ice-water bath in incubation buffer. The incubation took place with [³H]Ro 15-4513 in the incubation buffer in the dark at 4°C for 60 min in plastic slide mailers. Nonspecific binding was determined with flumazenil. After incubation, the sections were washed with ice-cold incubation buffer, dipped in distilled water and dried under air flow at room temperature. The sections were then exposed with [³H]-plastic standards to imaging plates or to radiosensitive films (Makela et al., 1997).

4.3.3 [³H]EBOB binding assay (II)

Resuspended cell membranes were incubated in buffer solution. GABA was diluted in buffer and loreclezole in dimethylsulfoxide. The zolpidem and DMCM stock solutions were dissolved in ethanol. Non-specific binding was determined by picrotoxinin. After incubation at room temperature, the assay mixtures were rapidly diluted with buffer, filtered and washed. Filters were immersed in scintillation fluid and the radioactivity determined in a liquid scintillation counter using external standardization (Yagle et al., 2003).

4.3.4 Quantification from films (I, II, IV)

Representative images from autoradiography films were scanned using a scanner and suitable software. For quantification of binding densities, the scanned images were then analyzed. Binding densities for each brain area were referenced to the [³H]- or [¹⁴C]-standards,

converted to radioactivity levels estimated for gray matter areas (nCi/mg), and given as means \pm SEM (Makela et al., 1997).

4.4 *In situ* hybridization (III, IV)

In situ hybridization is a protocol for visualizing radiolabeled proteins. It utilizes the construction of specific probes for known genomic sequences and the reaction of the probes with the target to produce a quantifiable signal, which is subsequently analyzed.

In situ hybridization with [³⁵S]-labeled oligonucleotide probes for γ 2 subunit exon 4, eGFP and Cre recombinase was performed as described (Wisden et al., 1992). Images were generated from 10 week exposures to X-ray film. To assess non-specific labeling of the sections, each labeled oligonucleotide was hybridized to brain sections with a 100-fold excess of unlabeled oligonucleotide (Duncan et al., 1995).

4.5 Immunohistochemistry (III, IV)

Immunohistochemistry is a method for detecting the presence of proteins of interest by the immunological reaction of antibodies to their targets. The antibodies are raised against their target proteins in a different animal species and incubated with tissue samples. Fluorescent and dye labels are used to visualize the signal. The results are qualitative rather than quantitative.

4.5.1 Enhanced green fluorescent protein (eGFP) imaging and β -galactosidase staining (IV)

Free-floating sagittal sections were washed in buffer, permeabilized and blocked by incubation in normal goat serum at room temperature and subsequently incubated with a rabbit polyclonal anti-eGFP primary antibody. Incubated slices were washed, incubated with a Cy3-conjugated goat anti-rabbit IgG, and subsequently washed. Slices were rinsed briefly, mounted on slides, embedded in embedding medium, cover-slipped, and analyzed using an upright fluorescent microscope. For β -galactosidase staining, free-floating sections were incubated in 5-bromo-4-chloro-3-indolyl- β -galactoside (Mellor et al., 1998).

4.5.2 Triple labeling (III)

For triple-immunofluorescence, free-floating sections were incubated in blocking solution. The sections were then incubated in primary antibodies: affinity purified rabbit antibody to the $\alpha 1$ subunit, guinea pig antiserum to eGFP and sheep antibody to GAD. On the following day, the sections were rinsed thoroughly and then incubated in donkey anti-rabbit Cy5, donkey anti-sheep Cy3, and donkey anti-guinea pig Alexa 488. Finally, the sections were rinsed and mounted. The method specificity was tested by incubating sections in one primary antibody with the full set of three secondary antibodies and images were acquired at all wavelengths (Zezula et al., 1991).

4.5.3 c-Fos expression (I, II)

Brain sections were immunostained as described earlier (Linden et al., 2004). After paraformaldehyde fixation and hydrogen peroxide treatment, sections were incubated in blocking buffer, after which they were incubated overnight at 4°C in incubation buffer containing goat polyclonal anti-c-Fos antibody and on the next day, after several washes, with a biotinylated rabbit anti-goat IgG. Visualization was performed using avidin-horseradish peroxidase and diaminobenzidine-nickel sulfate. Photomicrographs of sections were captured using a digital camera under a light microscope. An image-analysis software was used to measure mean optic density levels within the hippocampal dentate gyrus.

4.6 Electrophysiology

Electrophysiology deals with the electrical properties of neurons and other cells, and is a field of various methods aimed at analyzing the electrical activity and properties of cells of interest. The available techniques range from the investigation of single ion channels on the cell surface to the mapping of whole networks of brain regions. Activity can be studied in artificial environments like ion channels expressed in cultured cell lines, in tissue slices from the brains of animals or even in alive, behaving animals.

4.6.1 Recordings from HEK 293 cells (II)

Two days after transfection coverslips with HEK 293 cells were placed in a recording chamber mounted under a fluorescence microscope and perfused with a recording solution. Transfected cells were identified by their eGFP fluorescence. Membrane currents in these cells were studied in the whole-cell configuration of the patch-clamp technique (Rabe et al., 2007). Data analysis was performed using the appropriate programs. The amplitudes of peak currents were measured from recorded traces.

4.6.2 Recordings from brain slices (III)

Adult mice were anaesthetized and decapitated. The brains were removed and dissected in cold oxygenated slicing solution, after which parasagittal slices were cut from the cerebellar vermis. After incubation, individual slices were transferred to a submerged recording chamber. Neurons were directly visualized under infrared differential interference contrast optics. Whole-cell patch-clamp recordings were performed (III).

4.7 Behavioral studies

The animal models of altered neuronal function used in these studies are based on the genetic manipulation of receptor properties. To confirm the changes at the cellular and receptor level, *ex vivo* methods like immunohistochemistry and autoradiography are most useful. However, the end point was at the whole animal level; the behavior of the mutant mouse reveals the degree of the success achieved with the model. To this end a wide array of behavioral paradigms were used, ranging from the SHIRPA screen which assesses gross phenotypical changes, to more sophisticated tests like prepulse inhibition of the acoustic startle reflex, which is aimed at quantifying a specific deficit in sensorimotor integration. By not limiting the approach to only a few modalities it was hoped to gain a comprehensive picture of the phenotypes of these mutant mouse lines, and ultimately of the effect of the mutations on the networks which control these behaviors.

4.7.1 SHIRPA screen (IV)

Basic behavioral and physiological characterization of phenotype was performed using a modified version (Vekovischeva et al., 2004) of the primary observational screen described in the SHIRPA protocol (http://empress.har.mrc.ac.uk/browser/?sop_id=10_002_0). In the screen, animals were visually observed both in a confined space and moving freely. Their motor coordination and sensory perception were assessed and they were handled to provoke mild reactions. Simple measurements like body weight and body temperature were taken. Any abnormalities were compared to control mice screened at the same time under similar conditions. The animals were naïve to handling at the time of SHIRPA testing.

4.7.2 Elevated plus-maze (I, IV)

To investigate the basal anxiety level of the mice, an elevated plus-maze test was performed. The test utilizes the conflict between the innate tendency of rodents to explore their surroundings and the natural aversion they exhibit towards open, brightly lit spaces. The total time spent in and the entries made into the brightly lit open arms and the arms enclosed by the walls of the cross-shaped apparatus provide a measure of the anxiety state of the animals. In a state of heightened anxiety there is a tendency to avoid the open arms and stay in the enclosed arms; vice versa in a state of decreased anxiety.

The test was performed after the primary SHIRPA screen, before any other tests. The apparatus was made of grey plastic and elevated from the floor level. It consisted of a central platform, from which two open arms and two enclosed arms extended (Lister, 1987). The mice were placed individually on the central platform facing an open arm and allowed free exploration of the maze with their behavior being recorded using an Ethovision video tracking system with a charge-coupled device video camera above the plus maze (Saarelainen et al., 2008).

4.7.3 Open field exploration (II)

The open field test can be used to assess the overall locomotor activity of rodents, as well as the effects of substances that affect the alertness level of animals. An alert animal placed in an open field for the first time will explore the arena. If the animal has been administered

stimulants, like amphetamine, it will move more compared to an unmedicated control animal, which can be detected automatically by a video camera and quantified by the attached software. In the case of sedative substances, locomotor activity is decreased. Deficits in motor coordination may also lead to a decreased level of locomotion (Carola et al., 2002).

Mice naïve to the open field apparatus were placed individually in the center of an open arena. The mice were allowed free exploration of the arena and their behavior was recorded via a camera. The position and movements of the center of the animal's surface area were analyzed automatically with the Ethovision software (Linden et al., 2008). The number of rearing events was recorded manually from a monitor by an observer blind to genotype and pharmacological treatment. When pharmacological testing was conducted, a minimum wash-out period of three days was kept between treatments.

4.7.4 Startle reflex and prepulse inhibition (IV)

The acoustic startle reflex is a measure of an animal's reactivity to sudden, unexpected noises. On perceiving a loud noise, mice blink and flinch; the amplitude of the flinch is measured. The startle paradigm is primarily used to evaluate hearing, but various other factors, e.g. the anxiety state of the animal, may also have a bearing on the result. Disturbances in the oligosynaptic startle reflex circuit of the brainstem may also reflect in this paradigm (Plappert and Pilz, 2002). When a slight acoustic prepulse stimulus precedes the actual stimulus, the amplitude of the startle response is decreased in healthy mice. This is taken as an indication of sensorimotor gating, the ability of the brain to screen irrelevant stimuli from perception. Deficits in sensorimotor integration are a hallmark symptom in schizophrenic human patients, and many animal models of schizophrenia reproduce these deficits (Geyer and Braff, 1987; Andreasen et al., 2006). The predictive validity of the prepulse inhibition test in disease models is seen in its responsiveness to antipsychotics and psychotomimetics (Duncan et al., 2006).

The acoustic startle response and prepulse inhibition were measured using an automated startle system (Brody et al., 2004; Heldt et al., 2004). In an illuminated and sound-attenuated chamber, a small cage was mounted above a piezoelectric sensor. Background noise and acoustic stimuli were delivered through speakers in the ceiling of the chambers. First, acoustic startle responses to stimuli of different intensities were determined. An acclimation

period was followed by seven blocks of trials containing seven stimuli of different intensities in a pseudorandom order.

After startle testing, prepulse inhibition was analyzed in trials, in which the startle stimulus was preceded by a prepulse stimulus. Antipsychotics and psychotomimetics were administered to investigate whether they could modulate prepulse inhibition. The percent prepulse inhibition was calculated from the formula: %PPI = [(amplitude of startle pulse alone – amplitude of startle pulse when preceded by a prepulse) / amplitude of startle pulse alone] x 100.

4.7.5 Morris water maze (IV)

Spatial reference memory, a hippocampal-dependent aspect of cognition, can be assessed in an environment where the animal learns to navigate to a given target by using external visual cues. Mice were trained for 4 days to find a platform submerged below the surface of a pool of water. Visual markers were placed on the walls of the experiment room to facilitate spatial learning. The escape latency, swimming velocity and total distance were recorded. One day after training, a probe trial was performed in which the platform was removed and the time the mice spent in the platform quadrant as well as the number of visits to the former platform position and the swimming velocity were recorded (modified from Florian and Roulet, 2004).

4.7.6 Fear conditioning (II)

A fear conditioning test utilizes the ability of a noxious stimulus, e.g. an electrical shock, to elicit a forceful memory trace. During the conditioning session, where two shocks are administered signaled by a cue sound, the animal acquires a freezing response when the cue is sounded, in anticipation of the shock. The acquisition of the memory trace can be bidirectionally modulated with substances affecting the different phases of memory formation (Sieve et al., 2001).

On the conditioning day, mice were injected i.p. with 20 mg/kg DMCM or vehicle. Fifteen min post injection the animals were placed individually in test chambers, where they were allowed free exploration, after which a cue tone was created from wall-mounted speakers, co-

terminating with a shock through the grid floor. The cue-shock pairing was repeated once. After a final exploration period, the mice were removed from the chambers and returned to home cages. On the testing day, the mice were placed in the test chambers and freezing to context was scored. After this, the context was altered and the mice were re-tested for freezing in the presence and absence of the cue tone. The amount of freezing from both conditioning and testing days was collected by an infrared video camera and automatically analyzed (Sieve et al., 2001).

4.7.7 Hot plate and tail flick (IV)

Pain can be divided to several different modalities, and accordingly different paradigms are used to test nociception. Two tests measuring thermal pain were utilized, the hot plate and the tail flick. Both tests are dependent on the ability of the mouse to react to a painful stimulus by physical movement. While the hot plate test measures a supraspinal reflex, the tail flick test is dependent on a spinal nociceptive reflex (Davidova et al., 2009; Kubo et al., 2009). The tail flick test was chosen to supplement the hot plate test also because of the motor deficits observed in Pv- $\Delta\gamma 2$ and Pv- $\Delta\gamma 2$ -partial rescue mice.

During the hot plate test, the latency to react was scored visually when the mouse rapidly moved or licked its hindpaw or jumped. In the tail flick test, reaction latency was scored as the time before the tail withdrawal reflex. After DMCM administration, analgesic responses were measured (Linden et al., 2007).

4.7.8 Tremor measurement (IV)

Tremor can be either innate or induced by pharmacological substances (Milner et al., 1995; Handforth et al., 2010). Its two basic dimensions are frequency, which denotes the amount of oscillation in a given time period, and amplitude, which reveals the energy spent in tremor. Innate tremor is caused by a variety of factors, e.g. disturbances in the function of cerebellar circuits.

The tremor measurement apparatus was developed in-house and consisted of a piezoelectric weight sensor with three independent measurement stations. On the test, day baseline

measurements were performed, after which mice were injected with harmaline, and tremor was measured.

4.7.9 Electroshock convulsions and zolpidem (II)

Electroshock-induced convulsions are known to produce a robust expression of the immediate early gene c-Fos in various brain areas. c-Fos expression is a marker of neuronal activity, and as such, its expression can be modulated with substances that increase or decrease the actions of GABA. In order to study the inhibitory actions of the non-classical benzodiazepine zolpidem in hippocampal neurons, mice were given a short electroshock after saline or zolpidem administration. Electroshock-induced convulsions were produced by delivering current pulses via ear-clip electrodes from an electric unit. The animals received a single electroshock, after which they were decapitated. The brains were collected for analysis of c-Fos expression (Linden et al., 2004).

4.7.10 Motor training (I, II, III, IV)

Motor coordination is a complex function dependent on various brain areas, which affect different components of motor learning and performance (reviewed in Ito, 2000). One of the most important sites is thought to be the cerebellum, in which relatively well-defined networks govern the activity of output neurons in the deep cerebellar nuclei (reviewed in Uusisaari and De Schutter, 2011). Disturbances in cerebellar circuit function may affect the learning of motor tasks. Motor coordination can also be modulated with substances that act on the cerebellum. Mice were trained to stay on a rotating rod for three minutes and to walk along a thin wooden beam to their home cages (Korpi et al., 1999; Linden et al., 2008). After the animals had acquired the required motor skills, in order to study pharmacological sensitivities to GABAergic substances, they were injected with the test compounds and performance evaluated on the rotarod and the walking beam. Latency to fall from the rotarod and time to cross the beam were measured.

4.7.11 Statistical testing

Mouse lines and pharmacological or behavioral treatments were compared with repeated measures ANOVA, two-way ANOVA, one-way ANOVA, Newman-Keuls, Dunnett, Mann-

Whitney U, Wilcoxon signed rank, Fisher or Student's t-test. The variance analyses were used to compare two or more treatment groups (genotype or pharmacological treatment, with single or multiple time points, drug doses or drug concentrations), and the *post hoc* test was chosen according to the type of data utilized. When only two groups were compared, the Student's t-test or Wilcoxon signed rank test was used.

study	mouse line	method	aim of method
I	$\gamma 2I77$	c-Fos expression convulsion test locomotor activity rotarod horizontal wire elevated plus maze staircase test	effects of DMCM effects of DMCM effects of DMCM effects of DMCM effects of DMCM effects of DMCM effects of DMCM
II	$\gamma 2I77$	[³ H] Ro 15-4513 autoradiography [³⁵ S] TBPS autoradiography [³ H] EBOB autoradiography electrophysiology in HEK-293 cells open field rotarod walking beam fear conditioning c-Fos expression	effects of DMCM and zol effects of DMCM and zol effects of DMCM and zol effects of DMCM and zol effects of DMCM and zol effects of zol effects of zol effects of DMCM effects of zol
III	PC- $\Delta\gamma 2$, PC- $\gamma 2$ -swap	<i>in situ</i> hybridization immunohistochemistry whole-cell electrophysiology rotarod walking beam	expression of transgene expression of transgene activity of Purkinje cells, effects of zol effects of zol effects of zol
IV	Pv- $\Delta\gamma 2$, Pv- $\Delta\gamma 2$ -pr	<i>in situ</i> hybridization immunohistochemistry [³ H] Ro 15-4513 autoradiography [³⁵ S] TBPS autoradiography SHIRPA screen elevated plus maze acoustic startle pre-pulse inhibition of startle Morris water maze rotarod walking beam tremor hot plate tail flick motor pharmacology	expression of transgene expression of transgene expression of $\gamma 2$ subunit composition of GABA _A receptors basic phenotype anxiety level auditory reactivity sensorimotor gating spatial navigation motor coordination motor coordination tremor activity nociception nociception effects of DMCM and zol on motor coordination

Table 2. The main methods of the four studies in this thesis according to the transgenic mouse lines used.

5 RESULTS AND DISCUSSION

5.1 Changes in pharmacological sensitivity caused by the $\gamma 2I77$ mutation

As previously described, transgenic mice with the $\gamma 2I77$ point mutation were phenotypically normal and showed no behavioral anomalies compared to wild-type (WT) littermate control mice (Cope et al., 2004). The GABA_A receptor levels of $\gamma 2I77$ mice were similar to WT mice, as indicated by unaltered basal binding levels in [³⁵S]TBPS autoradiography (**I**, Fig. 1). Thus the I77 point mutation in the $\gamma 2$ subunit does not appear to alter synaptic clustering or the formation of functional GABA_A receptors. However, as predicted on the basis of earlier work done on recombinant receptors (Stevenson et al., 1995; Thomet et al., 1999), the sensitivity of $\gamma 2I77$ receptors to the non-benzodiazepine structured substances zolpidem (an $\alpha 1$ -selective agonist) and DMCM (an inverse agonist) was radically reduced.

5.1.1 Abolition of zolpidem and DMCM high-affinity effects

The $\gamma 2I77$ point mutation affects the benzodiazepine binding site in the interface between the α and γ subunits and prevents the conventional effects of zolpidem and DMCM (Buhr et al., 1997). In $\gamma 2I77$ mice, unlike in WT mice, zolpidem at 3-6 mg/kg doses produces no sedative effects (Cope et al., 2004). A high 30 mg/kg dose of zolpidem did not prevent electroshock-induced increase in the expression of the immediate early gene c-Fos, a marker of neuronal activity, in the hippocampus of $\gamma 2I77$ mice (**II**, Fig. 4). The inactivity of zolpidem at the benzodiazepine binding site of the GABA_A receptor of $\gamma 2I77$ mice was also indicated in autoradiographic assays of $\gamma 2I77$ mouse brains by the inability of zolpidem to displace [³H]Ro 15-4513, a benzodiazepine site ligand (**II**, Fig. 1), and the lack of positive modulating effects to the binding of [³⁵S]TBPS, a convulsant site ligand suitable for the measurement of GABA_A receptor levels (**II**, Fig. 2B). Due to the disruption of the benzodiazepine binding site, DMCM did not exert its characteristic inverse agonistic action in $\gamma 2I77$ mice: it did not elicit convulsions even at very high (60 mg/kg) doses, nor did administration of DMCM cause increased expression of c-Fos in the hippocampus of $\gamma 2I77$ mice (**I**, Fig. 4). DMCM up to 60 mg/kg did not increase locomotor activity in $\gamma 2I77$ mice in a staircase chamber or in an open field test (**I**, Table 2), while in WT mice, even at very low doses, DMCM is an anxiogenic and increases locomotion (Stephens et al., 1987; Savic et al., 2006).

5.1.2 Residual low affinity effects of zolpidem and DMCM

The lack of effects mediated through the benzodiazepine binding site in $\gamma 2I77$ mice allowed the manifestation of residual activity of zolpidem and DMCM. These residual effects are smaller in magnitude and do not appear *in vivo* in WT animals as they are masked by the robust benzodiazepine site effects.

A high 40 mg/kg dose of zolpidem decreased locomotor activity in an open field test in $\gamma 2I77$ mice (**II**, Fig. 5A), and a 60 mg/kg dose impaired motor coordination in a rotarod test (**II**, Fig. 5B). The impairment could not be reversed by flumazenil pretreatment, strongly implying that this residual effect was not being mediated through zolpidem binding to the benzodiazepine binding site. Zolpidem had no effect on the so-called loreclezole site, a low affinity positive modulator binding site dependent on the type of the β subunit and independent on the presence of a $\gamma 2$ subunit (**II**, Fig. 3). Other possible sites for the residual zolpidem actions include neuronal Ca^{2+} channels (Taft and DeLorenzo, 1984; Takahashi et al., 1999). The residual activity was displayed also in *ex vivo* conditions: a high 10 μM concentration of zolpidem slightly displaced [3H]Ro 15-4513 benzodiazepine binding and modulated [^{35}S]TBPS binding in a similar manner to GABAergic positive modulators (**II**, Fig. 1A,C; Fig. 2B).

DMCM also had residual effects in $\gamma 2I77$ mice, mediated through a binding site previously discovered in recombinant receptors (Stevenson et al., 1995; Thomet et al., 1999), which it shares with the antiepileptic loreclezole (Wingrove et al., 1994). DMCM positively modulated the GABA_A receptor and decreased locomotor activity in the open field test (**II**, Fig. 6), likely via the induction of mild sedation. 20 mg/kg DMCM also produced an anxiolytic-like effect in $\gamma 2I77$ mice (**I**, Table 2), as reflected by an increased amount of entries into and time spent in the open arms of an elevated plus maze. In the plus maze test, no decreased locomotion was observed, possibly due to inherent differences between the open field and elevated plus maze paradigms and also slight background strain effects between the different batches of $\gamma 2I77$ mice used. DMCM modulated fear learning in $\gamma 2I77$ mice, surprisingly enhancing the ability of a foot shock to produce a freezing response (**II**, Fig. 7). Positive modulators of the GABA_A receptor are typically amnesic (Makkar et al., 2010); the memory-enhancing effect of DMCM may have resulted from an altered emotional

state during the acquisition phase of the experiment, as mild anxiety (in contrast to panic) has been shown to promote learning (Sieve et al., 2001).

5.2 Lack of synaptic inhibition in PC- $\Delta\gamma 2$ mice does not cause motor deficits

PC- $\Delta\gamma 2$ mice were created by excising the $\gamma 2$ subunit selectively from cerebellar Purkinje cells via Cre/loxP mediated recombination. The globally zolpidem and DMCM-insensitive $\gamma 2I77$ mouse line, with the $\gamma 2$ subunits additionally flanked by loxP recombination sites, was crossed with the L7Cre line, in which Cre recombinase is expressed selectively in cerebellar Purkinje cells from postnatal day 6, guided by the L7 promoter (Barski et al., 2000). The rationale for this was twofold: firstly it was wished to investigate the effect of a long-term lack of inhibition to the Purkinje cells from molecular layer interneurons, and secondly it was planned to contrast this static lack of inhibition produced with transgenic means to cell-type specific rapid, reversible modulation of inhibition. Purkinje cells are inhibited mainly by stellate and basket cells, with little to no inhibition from recurrent axon collaterals of Purkinje cells themselves in the adult mouse (Wulff et al., 2009b). The resulting mice (PC- $\Delta\gamma 2$ mice) lacked the $\gamma 2$ subunit in Purkinje cells and consequently had no synaptic inhibition in this neuronal type. As proof of this, a lack of mIPSCs in Purkinje cells was demonstrated by whole-cell recordings from PC- $\Delta\gamma 2$ mice (III, Fig. 4A).

5.2.1 Phenotype of PC- $\Delta\gamma 2$ mice

Despite the lack of inhibition to Purkinje cells, PC- $\Delta\gamma 2$ mice displayed no phenotypical abnormalities and, perhaps surprisingly, little or no motor impairment. Their working memory, spatial memory, basal anxiety level, nociception, startle reflex and pre-pulse inhibition of startle were found to be normal compared to that of control mice. They learned to perform the rotarod (III, Figure 5A) and walking beam (Figure 7) tasks with only slight, initial impairment. Although PC- $\Delta\gamma 2$ mice had no deficits in performing learned motor tasks, they have been found to have strong impairment in the consolidation of motor memories in paradigms measuring the optokinetic and vestibulo-ocular reflexes (Wulff et al., 2009b). The conclusion from experiments performed with PC- $\Delta\gamma 2$ mice is that synaptic inhibition of Purkinje cells is indispensable for certain types of cerebellar learning, but not for others. The type of feedforward inhibition ablated from the Purkinje cells of PC- $\Delta\gamma 2$ mice may play a

role in memory functions also in other brain areas, such as the amygdala and hippocampus (Wulff et al., 2009b).

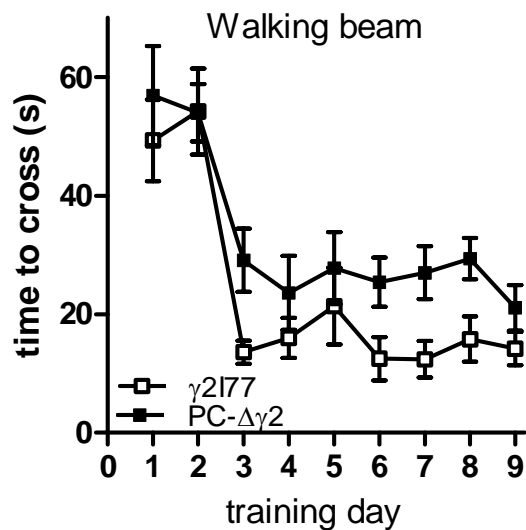


Figure 7. Motor training of PC- $\Delta\gamma 2$ mice on the walking beam. Mice were trained during 9 days to walk on a 1 m-long, 0.8 cm-diameter beam to home cage and the latency (s) to reach the cage was recorded. Daily averages \pm SEM are depicted. $n(\gamma 2I77)=5$, $n(\text{PC-}\Delta\gamma)=5$.

5.3 Cell-specific modulation of motor coordination in PC- $\gamma 2$ -swap mice

In order to achieve selective control over cerebellar Purkinje cells in the intact animal, wild-type $\gamma 2F77$ subunits were restored to the cerebellum via triple crossing $\gamma 2I77\text{lox}$ mice, L7Cre mice and a mouse line in which eGFP-tagged $\gamma 2F77$ subunits were expressed in Purkinje cells. The aim was to have uniquely zolpidem- and DMCM-sensitive cells in an otherwise insensitive background, enabling the targeted modulation of this cell type by systemic pharmacological exposure. The resulting PC- $\gamma 2$ -swap mice had GFP-immunoreactivity in their Purkinje cell somata and proximal dendrites, confirming the restoration of $\gamma 2F77$ subunits to the GABA_A receptors in Purkinje cells (III, Fig. 3). Whereas the PC- $\Delta\gamma 2$ mice lacked mIPSCs in Purkinje cells, in PC- $\gamma 2$ -swap mice the mIPSCs had returned to levels comparable with $\gamma 2I77$ control mice (III, Fig. 4A). Zolpidem increased the amplitude and decay of mIPSCs in PC- $\gamma 2$ -swap Purkinje cells, unlike in $\gamma 2I77$ controls, implying that there had been restoration of fully functional $\gamma 2$ subunits (III, Fig. 4B). In contrast to the unaltered basal motor behavior of PC- $\Delta\gamma 2$ mice, where the lack of inhibition to Purkinje cells is static, when PC- $\gamma 2$ -swap mice were administered cumulative doses (3+3+3 mg/kg, rotarod) or a single 12 mg/kg dose (walking beam) of zolpidem, an impairment of motor performance was

observed (III, Fig. 5B,C). Already the first 3 mg/kg dose on the rotarod produced the maximal effect, with no increase in efficacy with the following doses, indicating that a ceiling effect was reached. The impairment was significant and clearly noticeable, but smaller in magnitude compared to WT mice, which are completely incapacitated by 3 mg/kg zolpidem in similar conditions (Cope et al., 2004).

The response of PC- γ 2-swap mice to DMCM did not differ from γ 2I77 controls (Figure 8). This was rather unexpected, since the restoration of the γ 2F77 subunit did render the Purkinje cells sensitive to low doses of zolpidem. Possibly the proper functioning of Purkinje cells suffers more from rapid inhibition, causing a decreased inhibitory load to deep cerebellar nuclei (DCN), the main efferent target of Purkinje cells, than from an increase in inhibition to the DCN resulting from excitation of Purkinje cells by DMCM acting as an inverse agonist through the benzodiazepine binding site. The lack of clear motor impairment in PC- $\Delta\gamma$ 2 mice also indicates that excessive inhibition from Purkinje cells to DCN does not severely compromise the motor output of the cerebellum.

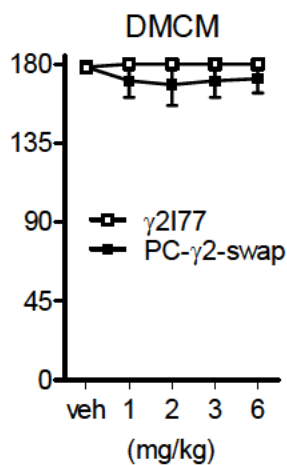


Figure 8. Motor pharmacological sensitivity of PC- γ 2-swap mice to DMCM.

5.4 Lack of synaptic inhibition in Pv- $\Delta\gamma$ 2 mice causes wide-ranging behavioral alterations

A mouse line with a selective ablation of the γ 2 subunit in parvalbumin-positive neurons (Pv- $\Delta\gamma$ 2 mice) was generated in a similar manner to the PC- $\Delta\gamma$ 2 line. γ 2I77lox mice were crossed with mice in which Cre recombinase was targeted to Pv-cells through the use of the Pv-promoter (Fuchs et al., 2007). Consequently the γ 2 subunit was excised via Cre/loxP mediated recombination from Pv-positive neurons (Wulff et al., 2009a).

Examination at the mRNA level revealed the absence of the $\gamma 2$ signal in areas rich in Pv-positive cells (reticular nucleus of thalamus, inferior colliculus and cerebellum), whereas areas in which Pv-cells are in a minority (hippocampus, neocortex) no change in overall $\gamma 2$ levels was detected (Wulff et al., 2009a). The gross anatomical CNS morphology of Pv- $\Delta\gamma 2$ mice was unaltered. Pv- $\Delta\gamma 2$ mice, like their Purkinje-cell targeted counterparts, lacked synaptic inhibition in the selected cell type, the Pv-positive neurons. Wulff et al., (2009a) investigated miniature IPSCs in hippocampal parvalbumin-positive interneurons of Pv- $\Delta\gamma 2$ mice and found them to be absent, confirming the ablation of the $\gamma 2$ subunit, which targets the GABA_A receptor to the synapse. They also investigated hippocampal oscillations, a network function essential to many types of information processing, conveyed by Pv-positive interneurons, and found them to be impaired. Although gamma oscillations were intact in Pv- $\Delta\gamma 2$ mice, theta oscillations were disrupted as well as the phase coupling between theta and gamma oscillations (Wulff et al., 2009a).

5.4.1 Phenotype of Pv- $\Delta\gamma 2$ mice

Basic phenotyping was first performed on the Pv- $\Delta\gamma 2$ mice. The mice were noticeably smaller than $\gamma 2I77$ control mice and did not gain weight over time as much as their controls. Their gait and posture were abnormally flat, although not sufficiently to impair basic locomotive behavior. Nevertheless they learned certain motor tasks, such as the rotarod and walking beam, incompletely and never reached the level of $\gamma 2I77$ controls (IV, Fig. 7A,B). Further testing of the deficits observed in the behavioral screen (IV, Table 1) revealed that Pv- $\Delta\gamma 2$ mice also had an increased startle reflex, decreased pre-pulse inhibition of startle (IV, Fig. 5), a decreased level of anxiety (IV, Fig. 4), decreased thermal nociceptive sensitivity (IV, Fig. 8), impaired spatial learning (IV, Fig. 6) and abnormal expression of tremor.

5.4.2 Increased pharmacological sensitivity of Pv- $\Delta\gamma 2$ mice

The motor pharmacological responses of Pv- $\Delta\gamma 2$ mice were altered as compared to $\gamma 2I77$ mice. Their sensitivity to the motor impairment caused by cumulative administration of flurazepam was increased (Figure 9). The increased sensitivity to flurazepam could be caused by the interaction of a heightened inhibitory load to the principal cells in a variety of brain areas resulting from increased activity of Pv-interneurons lacking synaptic inhibition, and the

inhibitory effect of flurazepam on the GABA_A receptors of the principal cells themselves. As expected, on the rotarod the sensitivity of Pv-Δγ2 mice to the inverse agonist DMCM was unaltered, but in tests measuring thermal nociception, 3 mg/kg DMCM produced an analgesic effect in Pv-Δγ2 mice (IV, Fig. 8B). Administration of 20 mg/kg zolpidem to Pv-Δγ2 mice resulted in motor impairment on the rotarod (IV, Fig. 7C). The reason for this unexpected sensitivity is unclear, but may be related to the residual sensitivity of the background γ2I77 line to zolpidem (II), possibly caused by zolpidem inhibiting neuronal Ca²⁺ channels (Taft and DeLorenzo, 1984; Takahashi et al., 1999). The lack of impairment by residual effects of zolpidem in the control γ2I77 mice was likely due to the excessive facility of the 5→20 rpm rotarod task, which was the highest speed at which the Pv-Δγ2 mice could reliably perform. However, the residual motor effect was seen in γ2I77 mice with a more difficult rotarod task at high zolpidem doses (II, Fig. 5B).

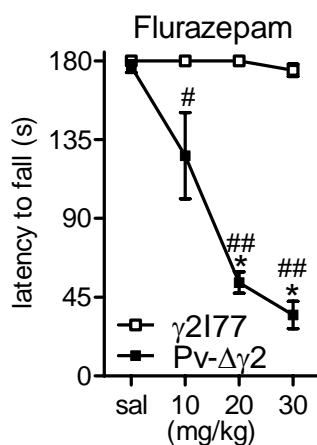


Figure 9. Motor pharmacological sensitivity of Pv-Δγ2 mice to flurazepam. *, p<0.05, for the significance of the difference compared to saline or vehicle (one-way ANOVA and Dunnett's *post hoc* test). #, ##, ### p<0.05, p<0.01, p<0.001, respectively, for the difference between mouse lines, two-way ANOVA and Newman-Keuls *post hoc* test.

5.4.3 Increased GABA-insensitive binding in Pv-Δγ2 mice

[³⁵S]TBPS autoradiography was used to investigate the amount and composition of GABA_A receptors in Pv-Δγ2 mice. GABA reduces the binding of this convulsant site ligand to the receptor, and changes in the efficacy of GABA may indicate changes in receptor subunit composition. In Pv-Δγ2 mice, the effect of 2 μM GABA was increased in several midbrain areas, including the substantia nigra pars reticulata, periaqueductal gray area and raphe nucleus. Interestingly, largely in these same areas (though not completely overlapping), GABA-insensitive (GIS) binding was increased as compared to control γ2I77 mice (Figure 10; IV, Fig. 3A, Supplementary Table 1). In GIS binding, high 1 mM concentrations of GABA are not able to completely displace [³⁵S]TBPS from the receptor. This kind of binding

is attributed to binary GABA_A receptors composed of α and β subunits, and is a hallmark of this mainly extrasynaptic receptor subtype (Sinkkonen et al., 2004). On the basis of these *ex vivo* findings, it is plausible that in Pv- $\Delta\gamma 2$ mice, in the absence of synaptic inhibition, an upregulation of binary extrasynaptic $\alpha\beta$ receptors has taken place to compensate for the imbalance in excitation and inhibition in Pv-positive cells.

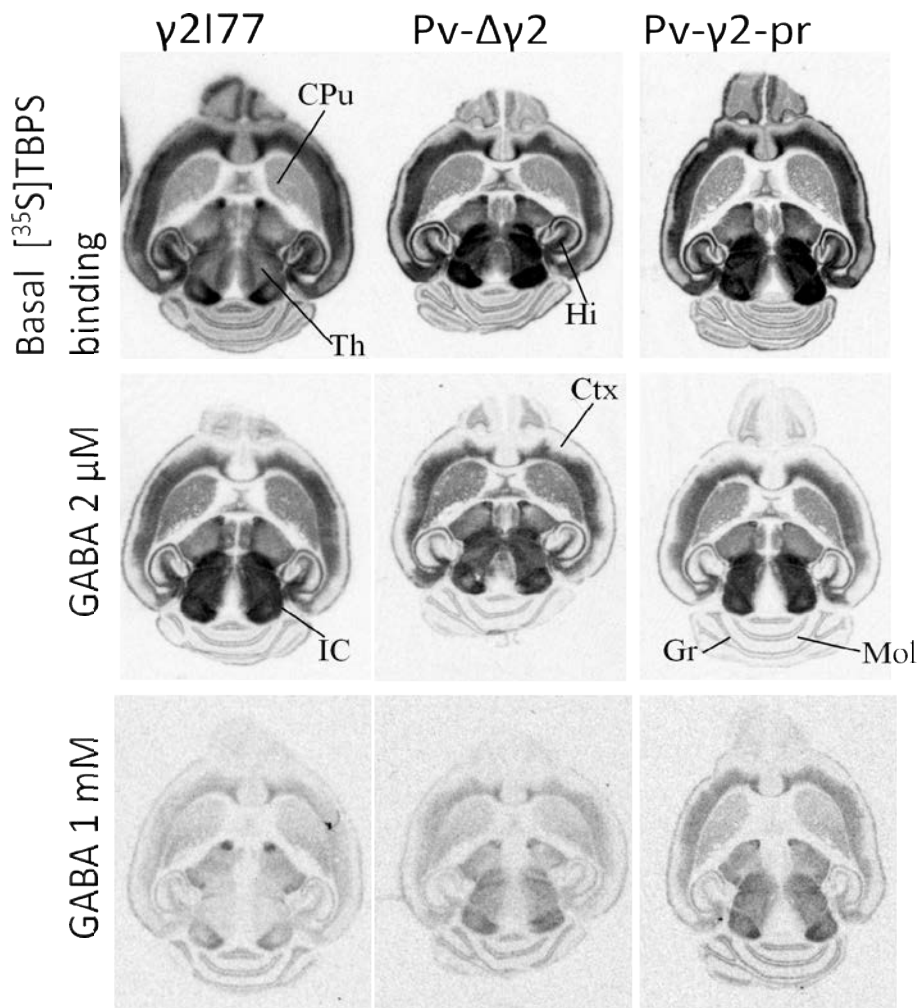


Figure 10. Basal [³⁵S]TBPS binding and the effect of 2 μ M and 1 mM GABA in $\gamma 2I77$, Pv- $\Delta\gamma 2$ and Pv- $\Delta\gamma 2$ -partial rescue mice, aged 3-6 months (bregma level -2.60 mm). Ctx, cortex; CPu, caudate putamen; Hi, hippocampus; Th, thalamus; IC, inferior colliculus; Gr, cerebellar granule cell layer; Mol, molecular layer.

5.5 Partially restored synaptic inhibition in Pv- $\Delta\gamma 2$ -partial rescue mice

In an attempt to replicate the cell-type selective modulation of activity achieved in PC-swap mice (IV), Pv- $\Delta\gamma 2$ -partial rescue mice were produced. To simplify the otherwise extensive breeding scheme, which would have included the production and crossing of three separate mouse lines, two transgenes were co-integrated to produce the Pv- $\Delta\gamma 2$ -partial rescue mice.

The mixing and co-injection into pro-nuclei of two bacterial artificial chromosome transgenes will often result in their integration into the same genomic position. This strategy ultimately resulted in mice with an ablation of the $\gamma 2$ subunit in Pv-positive cells and a restoration of the $\gamma 2F77$ subunit at detectable levels to the reticular thalamic nucleus and cells in the cerebellar molecular layer. This unfortunately incomplete restoration nevertheless provided an opportunity to compare Pv- $\Delta\gamma 2$ and Pv- $\Delta\gamma 2$ -partial rescue mice and to elucidate the effects on the various behavioral modalities of the restored synaptic inhibition in these restricted areas.

5.5.1 Restoration of the $\gamma 2F77$ subunit to reticular thalamic nucleus and molecular layer of cerebellum

In the $\gamma 2I77$ background line, the mutated benzodiazepine binding site of the GABA_A receptor only weakly binds several ligands, including [³H]Ro 15-4513, a partial inverse agonist used for the visualizing of the benzodiazepine binding site (Makela et al., 1997). In Pv- $\Delta\gamma 2$ -partial rescue mice, the restoration of the $\gamma 2F77$ subunit could clearly be seen as the increased signal in the reticular nucleus of thalamus and the cerebellar molecular layer (**IV**, Fig. 2A). The binding levels in these areas were 55% and 16%, respectively, from those of wild-type homozygous $\gamma 2F77$ mice. The restoration of $\gamma 2F77$ subunit protein to Pv-cells in other brain areas cannot be excluded, since despite their importance to network functions, Pv-cells are a minor neuronal type in many areas (e.g. neocortex, hippocampus), and the autoradiographic and *in situ* hybridization methods used here are not suitable for the detection of such low-level signals.

5.5.2 Improvement of motor function and spatial learning

The clearest difference between Pv- $\Delta\gamma 2$ and Pv- $\Delta\gamma 2$ -partial rescue mice was the reversal of motor deficits. While Pv- $\Delta\gamma 2$ had a noticeably abnormal gait (belly and tail held low to the ground, flattened body posture) the movements of Pv- $\Delta\gamma 2$ -partial rescue mice appeared normal, although they were slightly hyperactive, as seen in their increased locomotion in several behavioral experiments. Pv- $\Delta\gamma 2$ -partial rescue mice performed better on the rotarod and learned to stay on a rod accelerating from 5 to 30 rpm in contrast to the lower maximal speed of 5 to 20 rpm used with Pv- $\Delta\gamma 2$ mice. The cognitive abilities of Pv- $\Delta\gamma 2$ -partial rescue mice were improved in the Morris water maze paradigm as compared to Pv- $\Delta\gamma 2$ mice; this

being indicated by their lower escape latencies during training, and the increased number of visits to the former platform position in the probe trial (**IV**, Fig. 6). However, some of the other phenotypic and behavioral abnormalities (lower body weight, tremor, increased startle reflex, decreased pre-pulse inhibition of startle, decreased anxiety level and decreased thermal nociception) apparent in Pv- $\Delta\gamma 2$ mice were also present in Pv- $\Delta\gamma 2$ -partial rescue mice. The ablation of the $\gamma 2$ subunit from Pv-cells produced so many and such wide-ranging alterations that it is unrealistic to attempt to pinpoint them to any single brain structure or area, and likewise the restoration of the $\gamma 2F77$ subunit to only a few, restricted areas could not reverse the majority of the deficits produced by the lack of the $\gamma 2$ subunit in Pv-cells.

5.5.3 Altered pharmacological responses of Pv- $\Delta\gamma 2$ -partial rescue mice

Surprisingly, Pv- $\Delta\gamma 2$ -partial rescue mice did not show an increased sensitivity to zolpidem compared to $\gamma 2I77$ control mice, and still did not exhibit the increased sensitivity to zolpidem manifested in Pv- $\Delta\gamma 2$ mice. Although this is likely to be a consequence of the normalization of synaptic inhibition in Pv-cells, the exact mechanism is unclear. It is possible that the impaired motor performance of Pv- $\Delta\gamma 2$ mice together with the residual sensitivity of the background $\gamma 2I77$ line produced an exceptional sensitivity to zolpidem in Pv- $\Delta\gamma 2$ mice. The situation may have been reversed in Pv- $\Delta\gamma 2$ -partial rescue mice, in which the baseline motor performance was improved and so may have rendered them less prone to disturbance by the slight residual effects of zolpidem. The motor sensitivity of Pv- $\Delta\gamma 2$ -partial rescue mice to flurazepam was also normalized as compared to Pv- $\Delta\gamma 2$ mice. Presumably the abnormally strong inhibitory drive from Pv-cells toward principal cells was reduced by the restored synaptic inhibition of Pv-cells to the level of $\gamma 2I77$ control mice and thus the flurazepam administration did not cause a stronger impairment in motor function than that detected in the control mice (Figure 11). Finally, the administration of a single, low dose of DMCM to Pv- $\Delta\gamma 2$ -partial rescue mice resulted in motor impairment on the rotarod, an impairment which could be partially reversed by flumazenil pretreatment (**III**, Fig. 7C). This can be taken as another confirmation of the restoration of the $\gamma 2F77$ subunit. When Pv-cells are disinhibited by the inverse agonist action of DMCM in GABA_A receptors, their inhibitory load toward principal cells is increased, the situation resembling that of the baseline motor performance of Pv- $\Delta\gamma 2$ mice, where static loss of synaptic inhibition in Pv-cells results in a significant impairment.

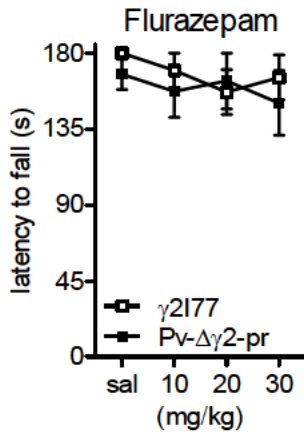


Figure 11. Motor pharmacological sensitivity of Pv-Δγ2-partial rescue mice to flurazepam.

study	mouse line	main finding
I	γ2I77	lack of DMCM-induced convulsions lack of DMCM-induced c-Fos activation anxiolysis caused by a high dose (20 mg/kg) of DMCM positive modulation of [³⁵ S]TBPS binding by 30 μM DMCM in the cerebellum
II	γ2I77	sedation caused by high-dose (40 mg/kg) zolpidem motor impairment on the rotarod and walking beam by high-dose zolpidem sedation caused by high-dose (20 mg/kg) DMCM modulation of fear learning by high-dose (20 mg/kg) DMCM
III	PC- Δγ2 PC- γ2-swap	lack of mIPSCs in Purkinje cells lack of motor impairment on the rotarod restoration of mIPSCs to Purkinje cells modulation of synaptic currents by zolpidem modulation of motor performance on the rotarod by low-dose (3-9 mg/kg) zolpidem
IV	Pv-Δγ2 Pv- Δγ2-pr	wide-ranging behavioral alterations (motor functions, anxiety, spatial learning, startle reflex, pre-pulse inhibition of startle, nociception) increased sensitivity to flurazepam, pregnanolone and zolpidem increased GIS binding restoration of γ2F77 subunit to reticular thalamic nucleus and cerebellar molecular layer improvement of motor function and spatial learning increased sensitivity to DMCM

Table 3. The main findings of the four studies in this thesis according to the transgenic mouse lines used.

6 GENERAL DISCUSSION

Cell-type specific modulation of selected components of neural circuits has been a long-time goal in the quest toward understanding the meaning of different neuronal types to the whole of the CNS. By isolating a single part of a circuit and modulating its function independently of the other components, one can gather essential information on its role. The studies in this thesis have aimed at developing a rapid, reversible method of regulating the activity of specific cell types *in vivo* in awake, behaving animals, and subsequently attributing the significance of these cell types to various behavioral modalities through the use of pharmacological tools. A transgenic approach was used to engineer a cell-type selective sensitivity to both an inhibitory and an excitatory systemically administered compound in a brain which should be otherwise insensitive to these pharmacological agents. Purkinje cells were chosen as a target for the validation of the method due to ease of access of their reasonably well-defined cerebellar circuitry. After confirming the feasibility of the method, it was further used to investigate the importance of Pv-positive cells in mouse emotional, cognitive and motor behavior.

6.1 Earlier models of cell-type specific modulation

Previous work on selective modulation of neuronal activity has utilized a variety of different methods. One fundamental, widely used strategy of modulating neuronal function has been the genetic ablation of target proteins, e.g. neurotransmitter receptors, uptake proteins or synthesizing enzymes (Günther et al., 1995, Stork et al., 2000, Salichon et al., 2001). However, as few such targets are restricted to one cell type or brain area only, additional techniques for the restriction of the desired effect to the cell type of interest are required. The next generation of genetic manipulations may be driven by cell-type specific promoters to affect only a subset of neurons. One limitation of this approach is the number of suitable promoters available; although promoters for cerebellar Purkinje cells (Barski et al., 2000), parvalbumin-positive cells (Fuchs et al., 2007), hippocampal CA3 neurons (Nakazawa et al., 2002) and olfactory neurons (Isles et al., 2001) have been described. Through the use of these promoters, a number of alterations in the properties of the cells in question can be achieved, from ectopic expression of protein (Wisden et al., 2002) to knock-out of subunits of inhibitory or excitatory receptors (Homanics et al., 1997; Lyon et al., 2011). Several ways of

excising and introducing proteins are known. In the present studies the Cre/loxP technique was used, where the Cre recombinase enzyme removes a target flanked by loxP recombination sites (Lakso et al., 1992, Meyer et al., 2002). The Cre recombinase was driven by cell-type specific promoters in the L7Cre mouse line (Purkinje cells (Barski et al., 2000)) and the PvCre mouse line (Pv-positive neurons (Fuchs et al., 2007)) which were then crossed with the background GABA_A γ 2I77 line to remove the γ 2 subunit in the target cell types. In this way static knockout mice were generated, where the absence of synaptic inhibition begins at the time of L7- (after postnatal day 6 (Barski et al., 2000)) and Pv- (after postnatal day 7 (Fuchs et al., 2007)) promoter expression. Ablation of a protein is not the only way to remove its influence. In other studies, the tetracycline (Gossen and Bujard, 1992) and mifepristone-inducible (Pierson et al., 2000) techniques have been used to silence the activity of target genes by activation or inactivation of transcription. Area-specific silencing of activity can be achieved through stereotaxic injections of inhibitory substances (Jasnow and Huhman, 2001).

Cre recombinase and similar methods of excision of genetic material can also be guided to selected brain areas, instead of specific cell types, via injection of viral vectors to the brain. Important considerations when using viral vectors are the selectivity of the virus to neuronal cells, rate of infection, efficacy of transfection, expression level of the delivered transgene following transfection, and absence of viral spreading to neighboring areas. The viral delivery tactic could also be combined with cell-type specific promoters to achieve restriction of the transgene both spatially and to only a subset of neurons in the target area (Kravitz et al., 2010). Covering of large areas with viral delivery is challenging due to the limited area of diffusion of the vector. Targeting of cell types to a wide, but sparse distribution is not feasible with this method (Boyden et al., 2005; Gradinaru et al., 2010). It has been suggested that in animal species not amenable to standard transgenic techniques, viral delivery of transgenes to modulate activity in specific areas or cell types could prove very useful (Tan et al., 2006).

Other strategies of silencing the activity of cells range from the expression of tonically active, inward-rectifying potassium channels (Nadeau et al., 2000) to ivermectin-activated Cl⁻ channels (Slimko et al., 2002) and membrane-tethered toxins, which inactivate target channels in the neurons to which they become attached to (Ibanez-Tallon et al., 2004). Such permanent modulations of cell excitability may render the neurons vulnerable to apoptosis (Nadeau et al., 2000) and thus may not be suitable for *in vivo* experiments. In the study of

(Coward et al., 1998) a system was developed where genetically engineered G-protein coupled receptors were activated selectively by synthetic ligands.

All of these approaches, particularly those involving deletions of essential proteins, that rely on genetic modifications during the embryonic stages, run the risk of unexpected compensatory changes, as was also the case in our studies of the Pv- $\Delta\gamma 2$ and Pv- $\Delta\gamma 2$ -partial rescue mice. However, the background $\gamma 2I77$ mutation used in our studies was phenotypically inert. In contrast to static genetic alterations, a rapid time scale and reversibility of modulation are key factors in the success of the cell-type specific modulation scheme, if the method is to stand the ultimate test of real-time regulation of neuronal activity at the whole animal level, in awake, behaving animals. In the work of Karpova et al., (2005) a fairly rapid induction of modulation (20-30 min) was achieved with molecules for inhibition of synaptic transmission (MIST), the transgenically introduced dimerization-caused inactivation of synaptic proteins, but termination of the effects still took over 24 hours. In addition, in this study no electrophysiological proof of actual synaptic events was obtained from transgenic animals, although the induction of motor impairment during training and performance of the rotarod task was observed (Karpova et al., 2005). Still more rapid timescales (several minutes) were achieved in another study with the introduction by viral vector of the *Drosophila*-derived allatostatin receptor, which couples to the mammalian inward rectifying K⁺ GIRK channel (Lechner et al., 2002) and subsequent application of allatostatin topically to brain surfaces (Tan et al., 2006). However, as the diffusion of allatostatin in the cerebral tissue was modest, areas distant from the cerebral surface and the ventricles would be unreachable with this method. Systemic administration would not be feasible, as allatostatin does not cross the blood-brain barrier (Tan et al., 2006). One advantage of the allatostatic-receptor system, as well as the MIST system described by Karpova et al., (2005) is its good specificity, since allatostatin and MIST do not affect cells without the introduced receptor or dimerization molecule, respectively.

An interesting new branch of research, optogenetics, is a potentially groundbreaking way to modulate target neurons with pinpoint accuracy. This relies on the expression of light-sensitive channelrhodopsin-2, a *Chlamydomonas* alga-derived cation channel activated by certain wavelengths of light (Boyden et al., 2005). When the protein is introduced either through viral delivery or with transgenic methods into neurons, which are subsequently illuminated, modulation of the activity is achieved within seconds (Boyden et al., 2005,

Gradinaru et al., 2010). Lin et al., (2011) used optogenetics to reversibly modulate hypothalamic areas to induce and abolish aggressive behavior, and Tye et al., (2011) provided proof for a centromedial amygdaloid circuit in the regulation of anxiety. The light-activated channelrhodopsin-2 is an elegant way of regulating the actions of neurons, with ultra-fast induction and termination of effect, but it does require both a reliable way, either genetic or a viral vector, to deliver the transgene needed for channel expression, which is as labor-intensive as in the older approaches, and also the invasive implantation of the fiber optics to provide the light source. Nevertheless, optogenetics does hold great future promise in the field of cell-type specific modulation.

Method	Specificity	Time scale	Reversibility	Reference
Promoter-driven deletion of subunit	good	permanent from embryonic stage	-	Nakazawa et al., 2002 Korotkova et al., 2010
Receptors activated solely by synthetic ligands (RASSL)	poor	na	+	Coward et al., 1998
Membrane-tethered toxins	poor	permanent	-	Ibanez-Tallon et al., 2004
Overexpression of tonically active K ⁺ channels	poor	permanent	-	Nadeau et al., 2000
Cl ⁻ channels activated by ivermectin	good	several hours	+	Slimko et al., 2002
Infusion of muscimol to central amygdala	poor	minutes to hours	+	Jasnow&Huhman, 2001
Promoter-driven toxic ablation of olfactory neurons	good	permanent	-	Isles et al., 2001
Molecules for inhibition of synaptic transmission (MIST)	good	I: 20-30 min T: >24 h	+	Karpova et al., 2010
Ectopic allatostatin-receptor expression	good	minutes	+	Lechner et al., 2002 Tan et al., 2006
Optogenetics	good	seconds	+	Lin et al., 2011 Tye et al., 2011
Selective zolpidem- and DMCM modulation of Pv- and Purkinje cells	good	seconds	+	III, IV

Table 4. Methods of cell type- or area specific modulation of neuronal activity.

6.2 The zolpidem method

The present approach relied on a strategy with no invasive steps necessary at any phase, by placing the appropriate genetic manipulations in place through crossbreeding of the suitable mouse lines. A similar strategy of cell-type specific knockout of a receptor subunit was recently used by Korotkova et al., (2010); they ablated the mandatory NR1-subunit of the glutamatergic NMDA receptor in Pv-cells, which resulted in cognitive deficits in the mice due to disturbances in hippocampal oscillatory function. For the relatively straightforward goal of simply knocking out the GABA_A receptor γ 2 subunit in selected cells, the genetic manipulations could have been performed with two transgenic mouse lines (the floxed line and the Cre carrier line), but to reach the ultimate target, rapid and reversible modulation of activity, a different background line was needed. From previous work on recombinant receptors, it was known that the γ 2 subunit could be altered in such a way that the sensitivity to several benzodiazepine binding site substances, e.g. the sedative-hypnotic zolpidem and the inverse agonist convulsant DMCM would be abolished (Buhr et al., 1997; Wingrove et al., 1997). The γ 2I77 point mutation was introduced to a transgenic mouse line without affecting the sensitivity to other GABAergic substances or producing phenotypic changes (Cope et al., 2004). This mouse line was used for the ablation of the γ 2 subunit in the target neurons i.e. the cerebellar Purkinje cells and parvalbumin-positive neurons. Static knockouts of the γ 2 subunit, used for comparison with the rapid modulation model, were achieved, leading to loss of synaptic inhibition in Purkinje cells and Pv-cells. The final step in the cell-type specific modulation was to substitute the wild-type γ 2F77 subunit for the γ 2I77 subunit in the target cells. This was accomplished as planned in the PC- γ 2-swap mouse line, but only incompletely in the Pv- $\Delta\gamma$ 2-partial rescue line. However, the overall aim of modulation of specific neurons through systemic administration of pharmacological substances was achieved in both PC- γ 2-swap and (most likely) in the Pv- $\Delta\gamma$ 2-partial rescue mice.

The advantages of the present method, as established in the study of the PC- γ 2-swap mice, are 1) the accurate targeting of the modulation to cells expressing the L7 promoter, the Purkinje cells; the more specific the promoter, the better the precision. When the changes required for the selective sensitivity to pharmacological modulation are achieved through transgenic methods, one does not need to attempt invasive procedures such as stereotaxic injection of viral vectors to small, difficult-to-reach brain areas. 2) Systemic administration of the modulating substance. Again, neither invasive procedures, nor permanent implantation of

fiberoptics or intracerebral cannulas are needed, reducing the operative load on the animal and simplifying the performance of possible behavioral experiments. 3) Rapid time scale of induction and termination of effect. The kinetics of zolpidem in particular are fast, its effect appearing within minutes of intraperitoneal injection (**II**, **III**, **IV**) and reaching a maximum in 10 minutes, with a half-life of 20 minutes (Benavides et al., 1988). The effects of both zolpidem and DMCM can furthermore be reversed with the pharmacological antagonist, flumazenil, enabling the interruption of the effect at a selected time point, if that were deemed necessary in the behavioral paradigm.

Despite the success with the PC- γ 2-swap mice, the mixed results obtained with the Pv- $\Delta\gamma$ 2-partial rescue mice reveal the need for further refinements of this method. The crossbreeding of three mouse lines is labor-and time-intensive. The success of the breeding scheme cannot be easily evaluated at intermediate stages, but can only be ascertained at the end. Short cuts by co-integration of more than one construct may result in an unreliable pattern of expression of the target protein. A possible future direction for the present studies could be the viral delivery of the Cre recombinase driven by a cell-type specific promoter in the γ 2I77 mouse line to attain selective modulation in discrete brain areas. Despite the inherent challenges in the use of viral vectors, this direction of research holds great interest in investigating area-specific functions. In particular, the widespread deficits observed in Pv- $\Delta\gamma$ 2 mice merit further research to pinpoint the alterations of behavior to disruption of the function of Pv-cells to discrete areas, such as the amygdala, the basal ganglia or the spinal cord. The original aim of bidirectional modulation of activity was not fully achieved in PC- γ 2-swap or Pv- $\Delta\gamma$ 2-partial rescue mice, but could perhaps be fulfilled better in another neuronal type, where both inhibition and excitation of the neuron had a robust effect on the network.

6.3 Benefits of the γ 2I77 as a background line

In addition to the γ 2I77 line being a necessary background line and an essential intermediary toward cell-type specific modulation, it was possible to reveal residual effects of zolpidem and DMCM, main pharmacological tools in these studies, in γ 2I77 mice. Since in the studies of cell-type specific modulation, high doses of zolpidem were used, it was important to first quantify their effects in the background line, in order to be sure of the correct interpretation of the results in the Purkinje- and Pv mouse lines. The effects of high-dose (20-40 mg/kg) DMCM in γ 2I77 mice were positively modulating, in contrast to the strong inverse agonism

in wild-type mice. DMCM is known to affect a low-affinity binding site of the GABA_A receptor which is common with the anticonvulsant loreclezole (Wafford et al., 1994, Stevenson et al., 1995), and it is most likely that this site was responsible for the mild agonistic effects observed (anxiolysis, sedation, modulation of fear learning, enhancement of GABA effects in [³⁵S]TBPS tissue autoradiography). Zolpidem also manifested slight residual effects, but not through this same site. The site of these zolpidem residual properties remains to be further investigated, but may be neuronal Ca²⁺ channels, where zolpidem was previously shown to have an effect (Taft and DeLorenzo, 1984; Takahashi et al., 1999). For the first time it was possible to demonstrate the residual sedative and motor-impairing effects of high-dose zolpidem *in vivo* in the γ 2I77 mice, which are insensitive to the conventional benzodiazepine site effects of this substance. There might be some clinical significance to the residual effects of zolpidem, as Ca²⁺ channel blockers are widely used for cardiovascular indications, and interactions with high doses zolpidem could conceivably arise. The biphasic effect of DMCM is shared by other benzodiazepine site ligands, such as diazepam (Walters et al., 2000), and it would be possible to explore the structural determinants of the dual affinity to the benzodiazepine site and the loreclezole site to avoid unintentional side effects as well as for exploiting the duality in future research or clinical settings. The γ 2I77 mouse line has also proved to be useful in dissecting the molecular-level activity of ethanol on the GABA_A receptor; antagonism of ethanol effects by the inverse agonist Ro 15-4513 was absent in γ 2I77 mice, implying that the antagonism may be mediated through the benzodiazepine binding site (Linden et al., 2011).

6.4 Static versus dynamic modulation of Purkinje cells

The results obtained from the study of the Purkinje cell transgenic mouse lines revealed a contrast between static and dynamic modulation of cerebellar motor performance. Little to no motor deficits were observed in PC- $\Delta\gamma$ 2 mice. Although the motor learning process and the performance of a complex motor task i.e. the rotarod, were intact in PC- $\Delta\gamma$ 2 mice, it is important to note that there was impairment of the consolidation of memories in another type of motor experiment investigating the optokinetic and vestibulo-ocular reflexes (Wulff et al., 2009b). The possibility of compensatory changes in the synaptic composition of GABA_A receptors in PC- $\Delta\gamma$ 2 mice, acting toward a re-establishment of the balance between inhibition and excitation, cannot be excluded. In that respect, it has been postulated that there may be decreased glutamatergic currents in the Purkinje cells of PC- $\Delta\gamma$ 2 mice (Wulff et al., 2009b).

Opposite to the lack of deficits in rotarod performance in PC- $\Delta\gamma 2$ mice, in PC- $\gamma 2$ -swap mice cumulative administration of zolpidem resulted in motor impairment on the rotarod, presumably due to acute inhibition of Purkinje cell activity and the consequent disinhibition of deep cerebellar nuclei. Similar results have been presented by (Karpova et al., 2005), through the inhibition of synaptic transmission in Purkinje cells, albeit with slower kinetics of onset and termination. The administration of the inverse agonist DMCM, possibly disinhibiting the Purkinje cells, did not alter the motor behavior observed in the PC- $\gamma 2$ -swap mice. This resembles the situation in the PC- $\Delta\gamma 2$ mice, where there is a static loss of synaptic inhibition and presumably consequent disinhibition of Purkinje cells. On the basis of this comparison between static and dynamic modulation of Purkinje cell activity *in vivo*, it appears that the output of the Purkinje cells to the deep cerebellar nuclei is more prone to interference with increased than decreased inhibition of Purkinje cell activity.

6.5 Lack of synaptic inhibition in Pv-cells causes wide-ranging behavioral alterations partially reversed by the restoration of the wild-type $\gamma 2F77$ subunit

The major alterations in behavior and GABA_A receptor composition we observed in Pv- $\Delta\gamma 2$ mice confirm the essential role of Pv-positive cells in a wide variety of brain areas and behavioral modalities. A lack of synaptic inhibition in Pv-cells of Pv- $\Delta\gamma 2$ mice was established earlier by (Wulff et al., 2009a). The present experiments with [³⁵S]TBPS binding point to a compensatorially increased amount of extrasynaptic receptors of the low-conductance binary $\alpha\beta$ subtype, the hallmark of which is increased GABA-insensitive binding, observed particularly in midbrain areas (substantia nigra pars reticulata, periaqueductal gray area and raphe nucleus) of Pv- $\Delta\gamma 2$ mice. Based on these behavioral findings (motor and cognitive impairment, increased startle reflex, decreased pre-pulse inhibition of startle, a decreased level of anxiety, decreased thermal nociception, and altered GABAergic pharmacological sensitivity), the putative increase in extrasynaptic transmission was not able to compensate for the lack of synaptic inhibition in Pv- $\Delta\gamma 2$ mice. The loss of afferent inhibition to Pv-cells likely disinhibits their activity and consequently increases the inhibitory load toward principal cells. In the diverse areas where Pv-cells form an essential part of networks for cognitive processing, this altered level of inhibition is would be predicted to cause severe disturbances. Indeed, the coupling of gamma and theta oscillations is known to be disrupted in the hippocampus of Pv- $\Delta\gamma 2$ mice (Wulff et al., 2009a), which can

be a major factor in the cognitive deficits observed in the water maze performance of Pv- $\Delta\gamma 2$ mice. Interestingly, Korotkova et al., (2010) found that ablation of the glutamatergic NMDA subunit selectively from Pv-cells impaired some forms of memory, in addition to changes in oscillatory activity. Pv- $\Delta\gamma 2$ mice also had a decreased level of theta oscillations (Wulff et al., 2009a), which may be connected to their decreased level of anxiety. In a mouse model with an ablation of the 5-HT_{1A} receptor, both increased anxiety and increased theta oscillations were observed (Gordon et al., 2005). Benzodiazepines tend to decrease theta oscillations (Zhu and McNaughton, 1995). Both of these observations together with the present results of decreased anxiety and decreased theta oscillations in Pv- $\Delta\gamma 2$ mice provide support for a role for hippocampal Pv-neurons in the regulation of anxiety. Apparently the network function of Pv-cells is delicately balanced and sensitive to disturbances in both the inhibitory and the excitatory directions.

A partial restoration of synaptic inhibition in Pv- $\Delta\gamma 2$ -partial rescue mice was achieved, reflected in the reversal of motor deficits on the rotarod and walking beam tasks, as well as the improved spatial learning in the water maze. The restoration of wild-type $\gamma 2F77$ subunits was confirmed in the reticular nucleus of thalamus and the cerebellar molecular layer by [³H]Ro 15-4513 binding, a benzodiazepine binding site ligand suitable for the detection of the $\gamma 2$ subunit. The restoration in other brain areas, e.g. neocortex and hippocampus, cannot be excluded, but the autoradiographic method is not suited for detecting the low, sparsely distributed signals emitted by Pv-cells, in regions where they are in a minority. Nonetheless, [³⁵S]TBPS autoradiography experiments revealed widespread changes in GABA_A receptor composition in Pv- $\Delta\gamma 2$ -partial rescue brains fully comparable to those in Pv- $\Delta\gamma 2$ brains, implying that a major part of the cellular-level deficits still remain. At the behavioral level this is manifested by the unaltered phenotype of Pv- $\Delta\gamma 2$ -partial rescue mice compared to Pv- $\Delta\gamma 2$ mice, except for motor and cognitive behavior. This is due to the limited success of the present method to restore the $\gamma 2F77$ subunit to Pv-cells only in the reticular thalamus and the cerebellum, whereas in other areas there is a loss of $\gamma 2$ subunit and a consequent lack of synaptic inhibition, similar to that encountered in Pv- $\Delta\gamma 2$ mice. To accurately dissect which behavioral alterations of Pv- $\Delta\gamma 2$ and Pv- $\Delta\gamma 2$ -partial rescue mice were attributable to which brain areas where autoradiographic changes were detected was not within the scope of these studies. It would be interesting to target selected areas with the zolpidem method, possibly via viral delivery of Cre recombinase and insertion of the $\gamma 2F77$ subunit, to achieve in-depth information on the area-specific functions of Pv-cells.

In summary, it can be stated that the present method of cell-type specific, rapid and reversible modulation of neuronal activity is a feasible way of dissecting the role of single neuron types (Pv-positive cells or cerebellar Purkinje cells) within a network. Provided that suitable promoters are available, the method could be utilized in investigating further GABA_A receptor γ 2 subunit expressing cell types. The systemic administration and fast time course of zolpidem and DMCM allow for performance of a variety of different behavioral paradigms. However, when constructing the transgenic mouse lines, due care must be taken to ascertain the desired expression pattern of the γ 2F77 subunit in order to avoid incomplete expression and mosaicism of the receptor protein in the target cells.

7 CONCLUSIONS

1. The inverse agonist DMCM and the sedative-hypnotic zolpidem both have residual activity mediated through non-benzodiazepine binding sites at high doses in the GABA_A receptor, as revealed in the $\gamma 2$ subunit I77 point mutant mice. The inability of the benzodiazepine antagonist flumazenil to reverse the effects of zolpidem supports the involvement of a non-benzodiazepine site of action. Both DMCM and zolpidem have effects pointing toward positive modulation at the GABA_A receptor. Both substances also act as positive modulators in the [³⁵S]TBPS autoradiographic assay at high concentrations.

2. A lack of synaptic inhibition was confirmed in the Purkinje cells of PC- $\Delta\gamma 2$ mice, but this evoked little or no impairment in motor learning and performance on the rotarod test. Either the lack of synaptic inhibition is being compensated for by changes in receptor subunit composition, or the output of the cerebellar cortex to deep cerebellar nuclei is not disrupted by the elevated level of inhibition from Purkinje cells.

3. Fast, reversible inhibition of Purkinje cells was achieved by systemic, cumulative administration of zolpidem in PC- $\gamma 2$ -swap mice. This caused a transient impairment in their motor performance on the rotarod and walking beam. It is postulated that compared to the static loss of inhibition in PC- $\Delta\gamma 2$ mice, the output of the cerebellar cortex to deep cerebellar nuclei had been disrupted by the putative sudden decrease of inhibition from Purkinje cells caused by the administration of zolpidem.

4. The lack of synaptic inhibition in Pv-cells caused wide-ranging behavioral alterations in Pv- $\Delta\gamma 2$ mice. The increased GABA-insensitive binding, particularly in midbrain areas, implies that changes in GABA_A receptor subunit composition had occurred, possibly an increased amount of extrasynaptic $\alpha\beta$ subtype receptors. The observed behavioral alterations particularly in cognition and emotion support previous studies on the role of Pv-cells as being essential to network oscillations and coordination of principal cell firing e.g. in neocortex and hippocampus.

5. In Pv- $\Delta\gamma 2$ -partial rescue mice the wild-type $\gamma 2F77$ subunit was restored to the reticular nucleus of thalamus and the cerebellar molecular layer, which alleviated the cognitive and

motor impairments detected in Pv- $\Delta\gamma 2$ mice, highlighting the important role of these structures in cognition and motor control. Systemic administration of DMCM resulted in fast, reversible motor impairment in Pv- $\Delta\gamma 2$ -partial rescue mice, mediated through the benzodiazepine binding site.

8 ACKNOWLEDGEMENTS

These thesis studies were carried out in the Institute of Biomedicine, Pharmacology, in the University of Helsinki during the years 2004-2011. I am grateful for the support of the department and the opportunity to use its excellent facilities. I would like to express my sincerest gratitude to my supervisors, Professor Esa Korpi and Docent Anni-Maija Lindén for scientific inspiration and unfailing support. Their example of hard work, profound expertise in neuroscience and constant curiosity for new research themes has been a guiding light during this project. They encouraged me to continue during the inevitable moments of self-doubt and were always helpful in interpreting my sometimes cryptic results.

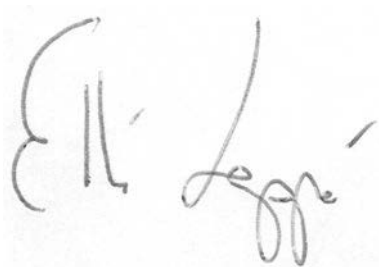
This work would not have been possible without the cooperation of a multinational consortium of researchers, of which I'd like most especially thank Professor William Wisden of the London Imperial College and Dr. Peer Wulff of the University of Aberdeen. Docent Eriika Savontaus and Professor Garry Wong are acknowledged for reviewing my thesis and providing many valuable suggestions for improvement. I thank Docent Ewen MacDonald for reviewing the language of my thesis and Professor Pertti Panula and Docent Tarja Stenberg for participating in my Supervisory Committee. I am grateful to the Sigrid Juselius Foundation and the Finnish Graduate School of Neuroscience for financial support of my thesis.

My heartfelt thanks go to all present and former members of the Korpi research group: Bjørnar Den Hollander, Lauri Halonen, Kati Hellsten, Milica Maksimovic, Salla Mansikkamäki, Tommi Möykkynen, Heidi Pehkonen, Chiara Procaccini, Martin Ranna, Saku Sinkkonen, Elena Vashchinkina, Olga Vekovischeva and Xiaomin Zheng. It wouldn't have been half as much fun without you! I'd especially like to thank Teemu Aitta-aho, Pia Bäckström and Anne Panhelainen for sharing my triumphs and sympathizing with my occasional complaining. I warmly acknowledge Aira Säisä for providing expert technical assistance and for taking me under her wing when I first arrived in the department. I also thank all the other staff and students in the department for creating a friendly, inspiring atmosphere to work in.

I want to express my gratitude to my friends Ilana, Lissu, Topi, Heli and Heikki for providing a refreshing, otherworldly counterpoint to my work, and in particular, Maarit and Satu for the

fruitful conversations on how to combine the scientific and family life. I fondly remember my earliest steps in pharmacology, taken together with my fellow students Sanna and Anne at Viikki.

I'm grateful to my parents Marja and Ilkka Leppä for care and love and for always believing in me, as well as for interest in my work. I'd like to thank my parents-in-law Mirja and Heikki Vitikka especially for childcare when my work has kept me from home. And finally I give my loving thanks to my husband Juha for his patience, understanding and every kind of support during these years, and our son Otso for being the clever little sunshine he is.

A handwritten signature in black ink, appearing to read 'Ilkka Leppä'. The signature is written in a cursive style with a large initial 'I' and a long, sweeping underline.

Helsinki, September 2011

9 REFERENCES

- Akk G, Bracamontes J, Steinbach JH (Pregnenolone sulfate block of GABA(A) receptors: mechanism and involvement of a residue in the M2 region of the α subunit. *J Physiol* 532:673-684.2001).
- Amitai Y, Gibson JR, Beierlein M, Patrick SL, Ho AM, Connors BW, Golomb D (The spatial dimensions of electrically coupled networks of interneurons in the neocortex. *J Neurosci* 22:4142-4152.2002).
- Andreasen JT, Andersen KK, Nielsen EO, Mathiasen L, Mirza NR (Nicotine and clozapine selectively reverse a PCP-induced deficit of PPI in BALB/cByJ but not NMRI mice: comparison with risperidone. *Behav Brain Res* 167:118-127.2006).
- Arellano JI, DeFelipe J, Munoz A (PSA-NCAM immunoreactivity in chandelier cell axon terminals of the human temporal cortex. *Cereb Cortex* 12:617-624.2002).
- Arellano JI, Munoz A, Ballesteros-Yanez I, Sola RG, DeFelipe J (Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain* 127:45-64.2004).
- Ator NA, Atack JR, Hargreaves RJ, Burns HD, Dawson GR (Reducing abuse liability of GABA_A/benzodiazepine ligands via selective partial agonist efficacy at α 1 and α 2/3 subtypes. *J Pharmacol Exp Ther* 332:4-16.2010).
- Baldacara L, Borgio JG, Lacerda AL, Jackowski AP (Cerebellum and psychiatric disorders. *Rev Bras Psiquiatr* 30:281-289.2008).
- Barski JJ, Dethleffsen K, Meyer M (Cre recombinase expression in cerebellar Purkinje cells. *Genesis* 28:93-98.2000).
- Bartos M, Alle H, Vida I (Role of microcircuit structure and input integration in hippocampal interneuron recruitment and plasticity. *Neuropharmacology* 60:730-739.2011).
- Baurle J, Hoshi M, Grusser-Cornehls U (Dependence of parvalbumin expression on Purkinje cell input in the deep cerebellar nuclei. *J Comp Neurol* 392:499-514.1998).
- Beasley CL, Reynolds GP (Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophr Res* 24:349-355.1997).
- Beg AA, Jorgensen EM (EXP-1 is an excitatory GABA-gated cation channel. *Nat Neurosci* 6: 1145-1152. 2003).
- Belelli D, Casula A, Ling A, Lambert JJ (The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology* 43:651-661.2002).
- Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW (Extrasynaptic GABA_A receptors: form, pharmacology, and function. *J Neurosci* 29:12757-12763.2009).
- Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (Extrasynaptic GABA_A receptors of thalamocortical neurons: a molecular target for hypnotics. *J Neurosci* 25:11513-11520.2005).
- Benavides J, Peny B, Dubois A, Perrault G, Morel E, Zivkovic B, Scatton B (In vivo interaction with central benzodiazepine (BZD) binding sites (as labelled by [³H]Ro 15-1788) in the mouse brain. Preferential affinity of zolpidem for the ω 1 (BZD1) subtype. *J Pharmacol Exp Ther* 245:1033-1041.1988).
- Benes FM, Berretta S (GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 25:1-27.2001).
- Bengtsson F, Svensson P, Hesslow G (Feedback control of Purkinje cell activity by the cerebello-olivary pathway. *Eur J Neurosci* 20:2999-3005.2004).

- Benke D, Honer M, Michel C, Mohler H (GABA_A receptor subtypes differentiated by their γ -subunit variants: prevalence, pharmacology and subunit architecture. *Neuropharmacology* 35:1413-1423.1996).
- Berghuis P, Dobszay MB, Ibanez RM, Ernfors P, Harkany T (Turning the heterogeneous into homogeneous: studies on selectively isolated GABAergic interneuron subsets. *Int J Dev Neurosci* 22:533-543.2004).
- Berkowitz A, Roberts A, Soffe SR (Roles for multifunctional and specialized spinal interneurons during motor pattern generation in tadpoles, zebrafish larvae, and turtles. *Front Behav Neurosci* 4:36.2010).
- Bernstein HG, Krause S, Krell D, Dobrowolny H, Wolter M, Stauch R, Ranft K, Danos P, Jirikowski GF, Bogerts B (Strongly reduced number of parvalbumin-immunoreactive projection neurons in the mammillary bodies in schizophrenia: further evidence for limbic neuropathology. *Ann N Y Acad Sci* 1096:120-127.2007).
- Blatow M, Rozov A, Katona I, Hormuzdi SG, Meyer AH, Whittington MA, Caputi A, Monyer H (A novel network of multipolar bursting interneurons generates theta frequency oscillations in neocortex. *Neuron* 38:805-817.2003).
- Boileau AJ, Pearce RA, Czajkowski C (The short splice variant of the γ 2 subunit acts as an external modulator of GABA_A receptor function. *J Neurosci* 30:4895-4903.2010).
- Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 8:1263-1268.2005).
- Brejck K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, Sixma TK (Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* 411:269-276.2001).
- Briatore F, Patrizi A, Viltono L, Sassoe-Pognetto M, Wulff P (Quantitative organization of GABAergic synapses in the molecular layer of the mouse cerebellar cortex. *PLoS One* 5:e12119.2010).
- Brickley SG, Cull-Candy SG, Farrant M (Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABA_A receptors. *J Physiol* 497:753-759.1996).
- Brickley SG, Cull-Candy SG, Farrant M (Single-channel properties of synaptic and extrasynaptic GABA_A receptors suggest differential targeting of receptor subtypes. *J Neurosci* 19:2960-2973.1999).
- Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M (Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 409:88-92.2001).
- Bright DP, Renzi M, Bartram J, McGee TP, MacKenzie G, Hosie AM, Farrant M, Brickley SG (Profound desensitization by ambient GABA limits activation of δ -containing GABA_A receptors during spillover. *J Neurosci* 31:753-763.2011).
- Brody SA, Dulawa SC, Conquet F, Geyer MA (Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. *Mol Psychiatry* 9:35-41.2004).
- Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (Pharmacological characterization of a novel cell line expressing human $\alpha(4)\beta(3)\delta$ GABA(A) receptors. *Br J Pharmacol* 136:965-974.2002).
- Buhr A, Baur R, Sigel E (Subtle changes in residue 77 of the γ subunit of $\alpha 1\beta 2\gamma 2$ GABA_A receptors drastically alter the affinity for ligands of the benzodiazepine binding site. *J Biol Chem* 272:11799-11804.1997).
- Caddy KW, Biscoe TJ (Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. *Philos Trans R Soc Lond B Biol Sci* 287:167-201.1979).
- Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (Tonic inhibition in

- mouse hippocampal CA1 pyramidal neurons is mediated by $\alpha 5$ subunit-containing γ -aminobutyric acid type A receptors. *Proc Natl Acad Sci U S A* 101:3662-3667.2004).
- Carlen M, Meletis K, Siegle JH, Cardin JA, Futai K, Vierling-Claassen D, Ruhlmann C, Jones SR, Deisseroth K, Sheng M, Moore CI, Tsai LH (A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol Psychiatry*.2011).
- Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P (Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behav Brain Res* 134:49-57.2002).
- Cave JW, Baker H (Dopamine systems in the forebrain. *Adv Exp Med Biol* 651:15-35.2009).
- Chandra D, Halonen LM, Linden AM, Procaccini C, Hellsten K, Homanics GE, Korpi ER (Prototypic GABA(A) receptor agonist muscimol acts preferentially through forebrain high-affinity binding sites. *Neuropsychopharmacology* 35:999-1007.2010).
- Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R (On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science* 298:1418-1421.2002).
- Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW (Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the $\alpha 5$ subunit of the GABA_A receptor. *J Neurosci* 22:5572-5580.2002).
- Connolly CN, Woollorton JR, Smart TG, Moss SJ (Subcellular localization of γ -aminobutyric acid type A receptors is determined by receptor β subunits. *Proc Natl Acad Sci U S A* 93:9899-9904.1996).
- Cope DW, Halbsguth C, Karayannis T, Wulff P, Ferraguti F, Hoeger H, Leppa E, Linden AM, Oberto A, Ogris W, Korpi ER, Sieghart W, Somogyi P, Wisden W, Capogna M (Loss of zolpidem efficacy in the hippocampus of mice with the GABA_A receptor $\gamma 2$ F77I point mutation. *Eur J Neurosci* 21:3002-3016.2005).
- Cope DW, Wulff P, Oberto A, Aller MI, Capogna M, Ferraguti F, Halbsguth C, Hoeger H, Jolin HE, Jones A, McKenzie AN, Ogris W, Poeltl A, Sinkkonen ST, Vekovischeva OY, Korpi ER, Sieghart W, Sigel E, Somogyi P, Wisden W (Abolition of zolpidem sensitivity in mice with a point mutation in the GABA_A receptor $\gamma 2$ subunit. *Neuropharmacology* 47:17-34.2004).
- Coward P, Wada HG, Falk MS, Chan SD, Meng F, Akil H, Conklin BR (Controlling signaling with a specifically designed G_i-coupled receptor. *Proc Natl Acad Sci U S A* 95:352-357.1998).
- Crepel F, Krupa M (Activation of protein kinase C induces a long-term depression of glutamate sensitivity of cerebellar Purkinje cells. An in vitro study. *Brain Res* 458:397-401.1988).
- Crestani F, Lorez M, Baer K, Essrich C, Benke D, Laurent JP, Belzung C, Fritschy JM, Luscher B, Mohler H (Decreased GABA_A-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat Neurosci* 2:833-839.1999).
- Crestani F, Martin JR, Mohler H, Rudolph U (Mechanism of action of the hypnotic zolpidem in vivo. *Br J Pharmacol* 131:1251-1254.2000).
- Cruikshank SJ, Lewis TJ, Connors BW (Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. *Nat Neurosci* 10:462-468.2007).
- Csicsvari J, Jamieson B, Wise KD, Buzsaki G (Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron* 37:311-322.2003).
- Cunningham MO, Hunt J, Middleton S, LeBeau FE, Gillies MJ, Davies CH, Maycox PR, Whittington MA, Racca C (Region-specific reduction in entorhinal gamma oscillations and parvalbumin-immunoreactive neurons in animal models of psychiatric illness. *J Neurosci* 26:2767-2776.2006).

- Danos P, Baumann B, Bernstein HG, Franz M, Stauch R, Northoff G, Krell D, Falkai P, Bogerts B (Schizophrenia and anteroventral thalamic nucleus: selective decrease of parvalbumin-immunoreactive thalamocortical projection neurons. *Psychiatry Res* 82:1-10.1998).
- Daskalakis ZJ, Fitzgerald PB, Christensen BK (The role of cortical inhibition in the pathophysiology and treatment of schizophrenia. *Brain Res Rev* 56:427-442.2007).
- Davidova A, Schreiberova A, Kolesar D, Capkova L, Krizanova O, Lukacova N (Spinal cord transection significantly influences nNOS-IR in neuronal circuitry that underlies the tail-flick reflex activity. *Cell Mol Neurobiol* 29:879-886.2009).
- de Venecia RK, Smelser CB, McMullen NT (Parvalbumin is expressed in a reciprocal circuit linking the medial geniculate body and auditory neocortex in the rabbit. *J Comp Neurol* 400:349-362.1998).
- deBono M, Maricq AV (Neuronal Substrates of complex behaviors in *C. Elegans*. *Annu Rev Neurosci* 28:451-501.2005).
- DeFelipe J (Chandelier cells and epilepsy. *Brain* 122 (Pt 10):1807-1822.1999).
- Dekkers J, Greensmith L, Navarrete R (Changes in the expression of parvalbumin immunoreactivity in the lumbar spinal cord of the rat following neonatal nerve injury. *Dev Neurosci* 24:283-293.2002).
- Delgado-Martinez I, Nehring RB, Sorensen JB (Differential abilities of SNAP-25 homologs to support neuronal function. *J Neurosci* 27:9380-9391.2007).
- Drake CT, Milner TA (μ opioid receptors are in discrete hippocampal interneuron subpopulations. *Hippocampus* 12:119-136.2002).
- Duncan GE, Breese GR, Criswell HE, McCown TJ, Herbert JS, Devaud LL, Morrow AL (Distribution of [3 H]zolpidem binding sites in relation to messenger RNA encoding the α 1, β 2 and γ 2 subunits of GABA_A receptors in rat brain. *Neuroscience* 64:1113-1128.1995).
- Duncan GE, Moy SS, Lieberman JA, Koller BH (Effects of haloperidol, clozapine, and quetiapine on sensorimotor gating in a genetic model of reduced NMDA receptor function. *Psychopharmacology (Berl)* 184:190-200.2006).
- Dunn SM, Davies M, Muntoni AL, Lambert JJ (Mutagenesis of the rat α 1 subunit of the γ -aminobutyric acid(A) receptor reveals the importance of residue 101 in determining the allosteric effects of benzodiazepine site ligands. *Mol Pharmacol* 56:768-774.1999).
- Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B (Postsynaptic clustering of major GABA_A receptor subtypes requires the γ 2 subunit and gephyrin. *Nat Neurosci* 1:563-571.1998).
- Farrant M, Nusser Z (Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* 6:215-229.2005).
- Fino E, Yuste R (Dense inhibitory connectivity in neocortex. *Neuron* 69:1188-1203.2011).
- Florian C, Roulet P (Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice. *Behav Brain Res* 154:365-374.2004).
- Fredrich M, Reisch A, Illing RB (Neuronal subtype identity in the rat auditory brainstem as defined by molecular profile and axonal projection. *Exp Brain Res* 195:241-260.2009).
- Freund TF, Buzsaki G (Interneurons of the hippocampus. *Hippocampus* 6:347-470.1996).
- Freund TF, Katona I (Perisomatic inhibition. *Neuron* 56:33-42.2007).
- Fries P, Nikolic D, Singer W (The gamma cycle. *Trends Neurosci* 30:309-316.2007).
- Fries P, Reynolds JH, Rorie AE, Desimone R (Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 291:1560-1563.2001).

- Fuchs EC, Zivkovic AR, Cunningham MO, Middleton S, Lebeau FE, Bannerman DM, Rozov A, Whittington MA, Traub RD, Rawlins JN, Monyer H (Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. *Neuron* 53:591-604.2007).
- Fukuda T (Network architecture of gap junction-coupled neuronal linkage in the striatum. *J Neurosci* 29:1235-1243.2009).
- Galarreta M, Hestrin S (Spike transmission and synchrony detection in networks of GABAergic interneurons. *Science* 292:2295-2299.2001).
- Gernert M, Hamann M, Bennay M, Loscher W, Richter A (Deficit of striatal parvalbumin-reactive GABAergic interneurons and decreased basal ganglia output in a genetic rodent model of idiopathic paroxysmal dystonia. *J Neurosci* 20:7052-7058.2000).
- Geyer MA, Braff DL (Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr Bull* 13:643-668.1987).
- Gill DA, Ramsay SL, Tasker RA (Selective reductions in subpopulations of GABAergic neurons in a developmental rat model of epilepsy. *Brain Res* 1331:114-123.2010).
- Glickfeld LL, Scanziani M (Distinct timing in the activity of cannabinoid-sensitive and cannabinoid-insensitive basket cells. *Nat Neurosci* 9:807-815.2006).
- Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I (A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci* 10:40-48.2007).
- Gogolla N, LeBlanc JJ, Quast KB, Südhof T, Fagiolini M, Hensch TK (Common circuit defect of excitatory-inhibitory balance in mouse models of autism. *J Neurodev Disord* 1:172-181.2009).
- Goldberg EM, Clark BD, Zaghera E, Nahmani M, Erisir A, Rudy B (K^+ channels at the axon initial segment dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons. *Neuron* 58:387-400.2008).
- Gonzalez-Burgos G, Hashimoto T, Lewis DA (Alterations of cortical GABA neurons and network oscillations in schizophrenia. *Curr Psychiatry Rep* 12:335-344.2010).
- Gonzalez-Burgos G, Krimer LS, Povysheva NV, Barrionuevo G, Lewis DA (Functional properties of fast spiking interneurons and their synaptic connections with pyramidal cells in primate dorsolateral prefrontal cortex. *J Neurophysiol* 93:942-953.2005).
- Gordon JA, Lacefield CO, Kentros CG, Hen R (State-dependent alterations in hippocampal oscillations in serotonin 1A receptor-deficient mice. *J Neurosci* 25:6509-6519.2005).
- Gossen M, Bujard H (Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A* 89:5547-5551.1992).
- Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K (Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* 141:154-165.2010).
- Grusser-Cornehls U, Baurle J (Mutant mice as a model for cerebellar ataxia. *Prog Neurobiol* 63:489-540.2001).
- Grusser-Cornehls U, Grusser C, Baurle J (Vermectomy enhances parvalbumin expression and improves motor performance in weaver mutant mice: an animal model for cerebellar ataxia. *Neuroscience* 91:315-326.1999).
- Gulyas AI, Megias M, Emri Z, Freund TF (Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. *J Neurosci* 19:10082-10097.1999).
- Günther U, Benson J, Benke D, Fritschy JM, Reyes G, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y, Bluethmann H, Möhler H, Lüscher B (Benzodiazepine-insensitive mice generated by targeted disruption of the $\gamma 2$ subunit gene of γ -aminobutyric acid type A receptors. *Proc Natl Acad Sci U S A* 92:7749-7753.1995).

- Hajos N, Papp EC, Acsady L, Levey AI, Freund TF (Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neuroscience* 82:355-376.1998).
- Hale MW, Johnson PL, Westerman AM, Abrams JK, Shekhar A, Lowry CA (Multiple anxiogenic drugs recruit a parvalbumin-containing subpopulation of GABAergic interneurons in the basolateral amygdala. *Prog Neuropsychopharmacol Biol Psychiatry* 34:1285-1293.2010).
- Handforth A, Homanics GE, Covey DF, Krishnan K, Lee JY, Sakimura K, Martin FC, Quesada A (T-type calcium channel antagonists suppress tremor in two mouse models of essential tremor. *Neuropharmacology* 59:380-387.2010).
- Hartwich K, Pollak T, Klausberger T (Distinct firing patterns of identified basket and dendrite-targeting interneurons in the prefrontal cortex during hippocampal theta and local spindle oscillations. *J Neurosci* 29:9563-9574.2009).
- Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA (Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci* 23:6315-6326.2003).
- Heldt SA, Green A, Ressler KJ (Prepulse inhibition deficits in GAD65 knockout mice and the effect of antipsychotic treatment. *Neuropsychopharmacology* 29:1610-1619.2004).
- Herb A, Wisden W, Luddens H, Puia G, Vicini S, Seeburg PH (The third γ subunit of the gamma-aminobutyric acid type A receptor family. *Proc Natl Acad Sci U S A* 89:1433-1437.1992).
- Herrup K, Wilczynski SL (Cerebellar cell degeneration in the leaner mutant mouse. *Neuroscience* 7:2185-2196.1982).
- Hevers W, Lüddens H (The diversity of GABA_A receptors. Pharmacological and electrophysiological properties of GABA_A channel subtypes. *Mol Neurobiol* 18:35-86.1998).
- Hill-Venning C, Belelli D, Peters JA, Lambert JJ (Subunit-dependent interaction of the general anaesthetic etomidate with the γ -aminobutyric acid type A receptor. *Br J Pharmacol* 120:749-756.1997).
- Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, Krasowski MD, Rick CE, Korpi ER, Mäkelä R, Brilliant MH, Hagiwara N, Ferguson C, Snyder K, Olsen RW (Mice devoid of γ -aminobutyrate type A receptor β 3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc Natl Acad Sci U S A* 94:4143-4148.1997).
- Howard A, Tamas G, Soltesz I (Lighting the chandelier: new vistas for axo-axonic cells. *Trends Neurosci* 28:310-316.2005).
- Ibanez-Tallon I, Wen H, Miwa JM, Xing J, Tekinay AB, Ono F, Brehm P, Heintz N (Tethering naturally occurring peptide toxins for cell-autonomous modulation of ion channels and receptors in vivo. *Neuron* 43:305-311.2004).
- Inda MC, Defelipe J, Munoz A (The distribution of chandelier cell axon terminals that express the GABA plasma membrane transporter GAT-1 in the human neocortex. *Cereb Cortex* 17:2060-2071.2007).
- Isles AR, Ma D, Milsom C, Skynner MJ, Cui W, Clark J, Keverne EB, Allen ND (Conditional ablation of neurones in transgenic mice. *J Neurobiol* 47:183-193.2001).
- Isomura Y, Fujiwara-Tsukamoto Y, Takada M (Glutamatergic propagation of GABAergic seizure-like afterdischarge in the hippocampus in vitro. *J Neurophysiol* 90:2746-2751.2003).
- Isope P, Barbour B (Properties of unitary granule cell-->Purkinje cell synapses in adult rat cerebellar slices. *J Neurosci* 22:9668-9678.2002).
- Ito M (Mechanisms of motor learning in the cerebellum. *Brain Res* 886:237-245.2000).

- Jasnow AM, Huhman KL (Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain Res* 920:142-150.2001).
- Jia F, Chandra D, Homanics GE, Harrison NL (Ethanol modulates synaptic and extrasynaptic GABA_A receptors in the thalamus. *J Pharmacol Exp Ther* 326:475-482.2008).
- Jinno S, Kosaka T (Parvalbumin is expressed in glutamatergic and GABAergic corticostriatal pathway in mice. *J Comp Neurol* 477:188-201.2004).
- Jones MW (Errant ensembles: dysfunctional neuronal network dynamics in schizophrenia. *Biochem Soc Trans* 38:516-521.2010).
- Kandel ER, Schwartz (Molecular biology of learning: Modulation of transmitter release). *Science* 218:433-443.1982).
- Karpova AY, Tervo DG, Gray NW, Svoboda K (Rapid and reversible chemical inactivation of synaptic transmission in genetically targeted neurons. *Neuron* 48:727-735.2005).
- Kash TL, Trudell JR, Harrison NL (Structural elements involved in activation of the γ -aminobutyric acid type A (GABA_A) receptor. *Biochem Soc Trans* 32:540-546.2004).
- Kawaguchi Y, Kubota Y (Neurochemical features and synaptic connections of large physiologically-identified GABAergic cells in the rat frontal cortex. *Neuroscience* 85:677-701.1998).
- Khom S, Baburin I, Timin EN, Hohaus A, Sieghart W, Hering S (Pharmacological properties of GABA_A receptors containing γ 1 subunits. *Mol Pharmacol* 69:640-649.2006).
- Kirmse K, Dvorzhak A, Henneberger C, Grantyn R, Kirischuk S (Cajal Retzius cells in the mouse neocortex receive two types of pre- and postsynaptically distinct GABAergic inputs. *J Physiol* 585:881-895.2007).
- Kita H, Kita T (Number, origins, and chemical types of rat pallidostriatal projection neurons. *J Comp Neurol* 437:438-448.2001).
- Kiyohara Y, Endo K, Ide C, Mizoguchi A (A novel morphological technique to investigate a single climbing fibre synaptogenesis with a Purkinje cell in the developing mouse cerebellum: DiI injection into the inferior cerebellar peduncle. *J Electron Microsc* (Tokyo) 52:327-335.2003).
- Klausberger T (GABAergic interneurons targeting dendrites of pyramidal cells in the CA1 area of the hippocampus. *Eur J Neurosci* 30:947-957.2009).
- Klein M, Kandel ER (Presynaptic modulation of voltage-dependent Ca²⁺-current: Mechanism for behavioral sensitization in *Aplysia californica*. *Proc Natl Acad Sci USA* 75:3512-3516.1978).
- Kondo S, Marty A (Synaptic currents at individual connections among stellate cells in rat cerebellar slices. *J Physiol* 509 (Pt 1):221-232.1998).
- Korotkova T, Fuchs EC, Ponomarenko A, von Engelhardt J, Monyer H (NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* 68:557-569.2010).
- Korpi ER, Koikkalainen P, Vekovischeva OY, Makela R, Kleinz R, Uusi-Oukari M, Wisden W (Cerebellar granule-cell-specific GABA_A receptors attenuate benzodiazepine-induced ataxia: evidence from α 6-subunit-deficient mice. *Eur J Neurosci* 11:233-240.1999).
- Korpi ER, Kuner T, Seeburg PH, Luddens H (Selective antagonist for the cerebellar granule cell-specific γ aminobutyric acid type A receptor. *Mol Pharmacol* 47:283-289.1995).
- Korpi ER, Luddens H (Furosemide interactions with brain GABA_A receptors. *Br J Pharmacol* 120:741-748.1997).
- Korpi ER, Lüddens H (Regional γ -aminobutyric acid sensitivity of t-butylbicyclophosphoro[³⁵S]thionate binding depends on γ -aminobutyric acid_A receptor α subunit. *Mol Pharmacol* 44:87-92.1993).

- Korpi ER, Seeburg PH, Lüddens H (Modulation of GABA_A receptor tert-[³⁵S]butylbicyclophosphorothionate binding by antagonists: relationship to patterns of subunit expression. *J Neurochem* 66:2179-2187.1996).
- Kravitz AV, Freeze BS, Parker PR, Kay K, Thwin MT, Deisseroth K, Kreitzer AC (Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466:622-626.2010).
- Kubo K, Nishikawa K, Ishizeki J, Hardy-Yamada M, Yanagawa Y, Saito S (Thermal hyperalgesia via supraspinal mechanisms in mice lacking glutamate decarboxylase 65. *J Pharmacol Exp Ther* 331:162-169.2009).
- Lakso M, Sauer B, Mosinger B, Jr., Lee EJ, Manning RW, Yu SH, Mulder KL, Westphal H (Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc Natl Acad Sci U S A* 89:6232-6236.1992).
- Landis SC (Changes in neuronal mitochondrial shape in brains of nervous mutant mice. *J Hered* 64:193-196.1973).
- Lane JD, Nadi NS, McBride WJ, Aprison MH, Kusano K (Contents of serotonin, norepinephrine and dopamine in the cerebrum of the "staggerer", "weaver" and "nervous" neurologically mutant mice. *J Neurochem* 29:349-350.1977).
- Lechner HA, Lein ES, Callaway EM (A genetic method for selective and quickly reversible silencing of Mammalian neurons. *J Neurosci* 22:5287-5290.2002).
- Lee CR, Tepper JM (Morphological and physiological properties of parvalbumin- and calretinin-containing γ -aminobutyric acidergic neurons in the substantia nigra. *J Comp Neurol* 500:958-972.2007).
- Lee S, Hjerling-Leffler J, Zagua E, Fishell G, Rudy B (The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. *J Neurosci* 30:16796-16808.2010).
- Lewis DA, Hashimoto T, Volk DW (Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci* 6:312-324.2005).
- Lin D, Boyle MP, Dollar P, Lee H, Lein ES, Perona P, Anderson DJ (Functional identification of an aggression locus in the mouse hypothalamus. *Nature* 470:221-226.2011).
- Linden AM, Aller MI, Leppa E, Rosenberg PH, Wisden W, Korpi ER (K⁺ channel TASK-1 knockout mice show enhanced sensitivities to ataxic and hypnotic effects of GABA(A) receptor ligands. *J Pharmacol Exp Ther* 327:277-286.2008).
- Linden AM, Greene SJ, Bergeron M, Schoepp DD (Anxiolytic activity of the MGLU2/3 receptor agonist LY354740 on the elevated plus maze is associated with the suppression of stress-induced c-Fos in the hippocampus and increases in c-Fos induction in several other stress-sensitive brain regions. *Neuropsychopharmacology* 29:502-513.2004).
- Linden AM, Sandu C, Aller MI, Vekovischeva OY, Rosenberg PH, Wisden W, Korpi ER (TASK-3 knockout mice exhibit exaggerated nocturnal activity, impairments in cognitive functions, and reduced sensitivity to inhalation anesthetics. *J Pharmacol Exp Ther* 323:924-934.2007).
- Linden AM, Schmitt U, Leppa E, Wulff P, Wisden W, Lüddens H, Korpi ER (Ro 15-4513 Antagonizes Alcohol-Induced Sedation in Mice Through α -2-type GABA(A) Receptors. *Front Neurosci* 5:3.2011).
- Lister RG (The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl)* 92:180-185.1987).
- Liu CL, Wang YJ, Chen JR, Tseng GF (Parvalbumin-containing neurons mediate the feedforward inhibition of rat rubrospinal neurons. *Anat Embryol (Berl)* 205:245-254.2002).

- Lo WY, Botzolakis EJ, Tang X, Macdonald RL (A conserved Cys-loop receptor aspartate residue in the M3-M4 cytoplasmic loop is required for GABA_A receptor assembly. *J Biol Chem* 283:29740-29752.2008).
- Lorez M, Benke D, Luscher B, Mohler H, Benson JA (Single-channel properties of neuronal GABA_A receptors from mice lacking the γ 2 subunit. *J Physiol* 527:11-31.2000).
- Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U (Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 290:131-134.2000).
- Luddens H, Korpi ER (GABA antagonists differentiate between recombinant GABA_A/benzodiazepine receptor subtypes. *J Neurosci* 15:6957-6962.1995).
- Lyon L, Borel M, Carrion M, Kew JN, Corti C, Harrison PJ, Burnet PW, Paulsen O, Rodriguez-Moreno A (Hippocampal mossy fiber long-term depression in Grm2/3 double knockout mice. *Synapse*.2011).
- Macdonald RL, Kang JQ, Gallagher MJ (Mutations in GABA_A receptor subunits associated with genetic epilepsies. *J Physiol* 588:1861-1869.2010).
- Macdonald RL, Olsen RW (GABA_A receptor channels. *Annu Rev Neurosci* 17:569-602.1994).
- Maex R, De Schutter E (Oscillations in the cerebellar cortex: a prediction of their frequency bands. *Prog Brain Res* 148:181-188.2005).
- Makela R, Uusi-Oukari M, Homanics GE, Quinlan JJ, Firestone LL, Wisden W, Korpi ER (Cerebellar γ -aminobutyric acid type A receptors: pharmacological subtypes revealed by mutant mouse lines. *Mol Pharmacol* 52:380-388.1997).
- Makkar SR, Zhang SQ, Cranney J (Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. *Neuropsychopharmacology* 35:1625-1652.2010).
- Maksay G, Simonyi M (Kinetic regulation of convulsant (TBPS) binding by GABAergic agents. *Mol Pharmacol* 30:321-328.1986).
- Mann EO, Suckling JM, Hajos N, Greenfield SA, Paulsen O (Perisomatic feedback inhibition underlies cholinergically induced fast network oscillations in the rat hippocampus in vitro. *Neuron* 45:105-117.2005).
- Martin LJ, Oh GH, Orser BA (Etomidate targets α 5 gamma-aminobutyric acid subtype A receptors to regulate synaptic plasticity and memory blockade. *Anesthesiology* 111:1025-1035.2009).
- McBain CJ, Fisahn A (Interneurons unbound. *Nat Rev Neurosci* 2:11-23.2001).
- McKernan RM, Whiting PJ (Which GABA_A receptor subtypes really occur in the brain? *Trends Neurosci* 19:139-143.1996).
- Mellor JR, Merlo D, Jones A, Wisden W, Randall AD (Mouse cerebellar granule cell differentiation: electrical activity regulates the GABA_A receptor α 6 subunit gene. *J Neurosci* 18:2822-2833.1998).
- Meyer AH, Katona I, Blatow M, Rozov A, Monyer H (In vivo labeling of parvalbumin-positive interneurons and analysis of electrical coupling in identified neurons. *J Neurosci* 22:7055-7064.2002).
- Milner TE, Cadoret G, Lessard L, Smith AM (EMG analysis of harmaline-induced tremor in normal and three strains of mutant mice with Purkinje cell degeneration and the role of the inferior olive. *J Neurophysiol* 73:2568-2577.1995).
- Mittmann W, Koch U, Hausser M (Feed-forward inhibition shapes the spike output of cerebellar Purkinje cells. *J Physiol* 563:369-378.2005).
- Mortensen M, Ebert B, Wafford K, Smart TG (Distinct activities of GABA agonists at synaptic- and extrasynaptic-type GABA_A receptors. *J Physiol* 588:1251-1268.2010).

- Mullen RJ, Eicher EM, Sidman RL (Purkinje cell degeneration, a new neurological mutation in the mouse. *Proc Natl Acad Sci U S A* 73:208-212.1976).
- Nadeau H, McKinney S, Anderson DJ, Lester HA (ROMK1 (Kir1.1) causes apoptosis and chronic silencing of hippocampal neurons. *J Neurophysiol* 84:1062-1075.2000).
- Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel MF, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA, Tonegawa S (Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* 297:211-218.2002).
- Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S, Belforte JE (GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology*.2011).
- Nett ST, Jorge-Rivera JC, Myers M, Clark AS, Henderson LP (Properties and sex-specific differences of GABA_A receptors in neurons expressing γ 1 subunit mRNA in the preoptic area of the rat. *J Neurophysiol* 81:192-203.1999).
- Ogris W, Poltl A, Hauer B, Ernst M, Oberto A, Wulff P, Hoger H, Wisden W, Sieghart W (Affinity of various benzodiazepine site ligands in mice with a point mutation in the GABA(A) receptor γ 2 subunit. *Biochem Pharmacol* 68:1621-1629.2004).
- Olah S, Fule M, Komlosi G, Varga C, Baldi R, Barzo P, Tamas G (Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature* 461:1278-1281.2009).
- Olah S, Komlosi G, Szabadics J, Varga C, Toth E, Barzo P, Tamas G (Output of neurogliaform cells to various neuron types in the human and rat cerebral cortex. *Front Neural Circuits* 1:4.2007).
- Olsen RW, Sieghart W (GABA_A receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology* 56:141-148.2009).
- Palhalmi J, Paulsen O, Freund TF, Hajos N (Distinct properties of carbachol- and DHPG-induced network oscillations in hippocampal slices. *Neuropharmacology* 47:381-389.2004).
- Petreaunu L, Mao T, Sternson SM, Svoboda K (The subcellular organization of neocortical excitatory connections. *Nature* 457:1142-1145.2009).
- Pierson TM, Wang Y, DeMayo FJ, Matzuk MM, Tsai SY, Omalley BW (Regulable expression of inhibin A in wild-type and inhibin α null mice. *Mol Endocrinol* 14:1075-1085.2000).
- Pike FG, Goddard RS, Suckling JM, Ganter P, Kasthuri N, Paulsen O (Distinct frequency preferences of different types of rat hippocampal neurones in response to oscillatory input currents. *J Physiol* 529 Pt 1:205-213.2000).
- Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* 101:815-850.2000).
- Pisani A, Bernardi G, Ding J, Surmeier DJ (Re-emergence of striatal cholinergic interneurons in movement disorders. *Trends Neurosci* 30:545-553.2007).
- Plappert CF, Pilz PK (Difference in anxiety and sensitization of the acoustic startle response between the two inbred mouse strains BALB/cAN and DBA/2N. *Genes Brain Behav* 1:178-186.2002).
- Pouille F, Scanziani M (Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* 293:1159-1163.2001).
- Puia G, Vicini S, Seeburg PH, Costa E (Influence of recombinant γ -aminobutyric acid-A receptor subunit composition on the action of allosteric modulators of γ -aminobutyric acid-gated Cl⁻ currents. *Mol Pharmacol* 39:691-696.1991).
- Rabe H, Kronbach C, Rundfeldt C, Luddens H (The novel anxiolytic ELB139 displays selectivity to recombinant GABA(A) receptors different from diazepam. *Neuropharmacology* 52:796-801.2007).

- Racz A, Ponomarenko AA, Fuchs EC, Monyer H (Augmented hippocampal ripple oscillations in mice with reduced fast excitation onto parvalbumin-positive cells. *J Neurosci* 29:2563-2568.2009).
- Ramerstorfer J, Furtmuller R, Sarto-Jackson I, Varagic Z, Sieghart W, Ernst M (The GABA_A receptor α + β - interface: a novel target for subtype selective drugs. *J Neurosci* 31:870-877.2011).
- Ranna M, Sinkkonen ST, Moykkynen T, Uusi-Oukari M, Korpi ER (Impact of ϵ and τ subunits on pharmacological properties of α 3 β 1 GABA_A receptors expressed in *Xenopus* oocytes. *BMC Pharmacol* 6:1.2006).
- Ribak CE (Axon terminals of GABAergic chandelier cells are lost at epileptic foci. *Brain Res* 326:251-260.1985).
- Rossi S, Muzio L, De Chiara V, Grasselli G, Musella A, Musumeci G, Mandolesi G, De Ceglia R, Maida S, Biffi E, Pedrocchi A, Menegon A, Bernardi G, Furlan R, Martino G, Centonze D (Impaired striatal GABA transmission in experimental autoimmune encephalomyelitis. *Brain Behav Immun*.2010).
- Rudy B, Fishell G, Lee S, Hjerling-Leffler J (Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev Neurobiol* 71:45-61.2011).
- Saarelainen KS, Ranna M, Rabe H, Sinkkonen ST, Moykkynen T, Uusi-Oukari M, Linden AM, Luddens H, Korpi ER (Enhanced behavioral sensitivity to the competitive GABA agonist, gaboxadol, in transgenic mice over-expressing hippocampal extrasynaptic α 6 β GABA(A) receptors. *J Neurochem* 105:338-350.2008).
- Salichon N, Gaspar P, Upton AL, Picaud S, Hanoun N, Hamon M, De Maeyer E, Murphy DL, Mossner R, Lesch KP, Hen R, Seif I (Excessive activation of serotonin (5-HT)_{1B} receptors disrupts the formation of sensory maps in monoamine oxidase a and 5-ht transporter knock-out mice. *J Neurosci* 21:884-896.2001).
- Savic MM, Majumder S, Huang S, Edwankar RV, Furtmuller R, Joksimovic S, Clayton T, Sr., Ramerstorfer J, Milinkovic MM, Roth BL, Sieghart W, Cook JM (Novel positive allosteric modulators of GABA_A receptors: do subtle differences in activity at α 1 plus α 5 versus α 2 plus α 3 subunits account for dissimilarities in behavioral effects in rats? *Prog Neuropsychopharmacol Biol Psychiatry* 34:376-386.2010).
- Savic MM, Obradovic DI, Ugresic ND, Cook JM, Yin W, Van Linn M, Bokonjic DR (Benzodiazepine site inverse agonists and locomotor activity in rats: bimodal and biphasic influence. *Pharmacol Biochem Behav* 84:35-42.2006).
- Schmidt H, Arendt O, Brown EB, Schwaller B, Eilers J (Parvalbumin is freely mobile in axons, somata and nuclei of cerebellar Purkinje neurones. *J Neurochem* 100:727-735.2007).
- Schmiedt C, Brand A, Hildebrandt H, Basar-Eroglu C (Event-related theta oscillations during working memory tasks in patients with schizophrenia and healthy controls. *Brain Res Cogn Brain Res* 25:936-947.2005).
- Schofield CM, Kleiman-Weiner M, Rudolph U, Huguenard JR (A gain in GABA_A receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures. *Proc Natl Acad Sci U S A* 106:7630-7635.2009).
- Scott-Stevens P, Atack JR, Sohal B, Worboys P (Rodent pharmacokinetics and receptor occupancy of the GABA_A receptor subtype selective benzodiazepine site ligand L-838417. *Biopharm Drug Dispos* 26:13-20.2005).
- Senior TJ, Huxter JR, Allen K, O'Neill J, Csicsvari J (Gamma oscillatory firing reveals distinct populations of pyramidal cells in the CA1 region of the hippocampus. *J Neurosci* 28:2274-2286.2008).
- Serafini R, Bracamontes J, Steinbach JH (Structural domains of the human GABA_A receptor β 3 subunit involved in the actions of pentobarbital. *J Physiol* 524:649-676.2000).

- Sidman RL, Lane PW, Dickie MM (Staggerer, a new mutation in the mouse affecting the cerebellum. *Science* 137:610-612.1962).
- Sieghart W, Eichinger A, Richards JG, Mohler H (Photoaffinity labeling of benzodiazepine receptor proteins with the partial inverse agonist [³H]Ro 15-4513: a biochemical and autoradiographic study. *J Neurochem* 48:46-52.1987).
- Sieve AN, King TE, Ferguson AR, Grau JW, Meagher MW (Pain and negative affect: evidence the inverse benzodiazepine agonist DMCM inhibits pain and learning in rats. *Psychopharmacology (Berl)* 153:180-190.2001).
- Sigel E, Luscher BP (A closer look at the high affinity benzodiazepine binding site on GABA_A receptors. *Curr Top Med Chem* 11:241-246.2011).
- Simon AP, Poindessous-Jazat F, Dutar P, Epelbaum J, Bassant MH (Firing properties of anatomically identified neurons in the medial septum of anesthetized and unanesthetized restrained rats. *J Neurosci* 26:9038-9046.2006).
- Sinkkonen ST, Hanna MC, Kirkness EF, Korpi ER (GABA_A receptor ϵ and τ subunits display unusual structural variation between species and are enriched in the rat locus ceruleus [In Process Citation]. *J Neurosci* 20:3588-3595.2000).
- Sinkkonen ST, Luscher B, Luddens H, Korpi ER (Autoradiographic imaging of altered synaptic $\alpha\beta\gamma 2$ and extrasynaptic $\alpha\beta$ GABA_A receptors in a genetic mouse model of anxiety. *Neurochem Int* 44:539-547.2004).
- Slimko EM, McKinney S, Anderson DJ, Davidson N, Lester HA (Selective electrical silencing of mammalian neurons in vitro by the use of invertebrate ligand-gated chloride channels. *J Neurosci* 22:7373-7379.2002).
- Spampanato J, Polepalli J, Sah P (Interneurons in the basolateral amygdala. *Neuropharmacology* 60:765-773.2011).
- Steiner JP, Hamilton GS, Ross DT, Valentine HL, Guo H, Connolly MA, Liang S, Ramsey C, Li JH, Huang W, Howorth P, Soni R, Fuller M, Sauer H, Nowotnik AC, Suzdak PD (Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. *Proc Natl Acad Sci U S A* 94:2019-2024.1997).
- Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing GABA_A receptors. *Proc Natl Acad Sci U S A* 100:14439-14444.2003).
- Stephens DN, Schneider HH, Kehr W, Jensen LH, Petersen E, Honore T (Modulation of anxiety by β -carbolines and other benzodiazepine receptor ligands: relationship of pharmacological to biochemical measures of efficacy. *Brain Res Bull* 19:309-318.1987).
- Stevenson A, Wingrove PB, Whiting PJ, Wafford KA (β -Carboline γ -aminobutyric acid_A receptor inverse agonists modulate γ -aminobutyric acid via the loreclezole binding site as well as the benzodiazepine site. *Mol Pharmacol* 48:965-969.1995).
- Stork O, Ji FY, Kaneko K, Stork S, Yoshinobu Y, Moriya T, Shibata S, Obata K (Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65. *Brain Res* 865:45-58.2000).
- Taft WC, DeLorenzo RJ (Micromolar-affinity benzodiazepine receptors regulate voltage-sensitive calcium channels in nerve terminal preparations. *Proc Natl Acad Sci U S A* 81:3118-3122.1984).
- Takahashi H, Yoshimoto M, Higuchi H, Shimizu T, Hishikawa Y (Different effects of L-type and T-type calcium channel blockers on the hypnotic potency of triazolam and zolpidem in rats. *Eur Neuropsychopharmacol* 9:317-321.1999).
- Tamas G, Somogyi P, Buhl EH (Differentially interconnected networks of GABAergic interneurons in the visual cortex of the cat. *J Neurosci* 18:4255-4270.1998).

- Tan EM, Yamaguchi Y, Horwitz GD, Gosgnach S, Lein ES, Goulding M, Albright TD, Callaway EM (Selective and quickly reversible inactivation of mammalian neurons in vivo using the *Drosophila* allatostatin receptor. *Neuron* 51:157-170.2006).
- Thomet U, Baur R, Scholze P, Sieghart W, Sigel E (Dual mode of stimulation by the β -carboline ZK 91085 of recombinant GABA(A) receptor currents: molecular determinants affecting its action. *Br J Pharmacol* 127:1231-1239.1999).
- Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, Thompson KR, Gradinaru V, Ramakrishnan C, Deisseroth K (Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature* 471:358-362.2011).
- Uematsu M, Hirai Y, Karube F, Ebihara S, Kato M, Abe K, Obata K, Yoshida S, Hirabayashi M, Yanagawa Y, Kawaguchi Y (Quantitative chemical composition of cortical GABAergic neurons revealed in transgenic venus-expressing rats. *Cereb Cortex* 18:315-330.2008).
- Uhlhaas PJ, Haenschel C, Nikolic D, Singer W (The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophr Bull* 34:927-943.2008).
- Uhlhaas PJ, Singer W (Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* 11:100-113.2010).
- Uusi-Oukari M (Characterization of two cerebellar binding sites of [³H]Ro 15-4513. *J Neurochem* 59:568-574.1992).
- Uusisaari M, De Schutter E (The mysterious microcircuitry of the cerebellar nuclei. *J Physiol*.2011).
- Wafford KA, Bain CJ, Quirk K, McKernan RM, Wingrove PB, Whiting PJ, Kemp JA (A novel allosteric modulatory site on the GABA_A receptor β subunit. *Neuron* 12:775-782.1994).
- Walters RJ, Hadley SH, Morris KD, Amin J (Benzodiazepines act on GABA_A receptors via two distinct and separable mechanisms. *Nat Neurosci* 3:1274-1281.2000).
- Wang DD, Kriegstein AR (Defining the role of GABA in cortical development. *J Physiol* 587:1873-1879.2009).
- Vekovischeva OY, Aitta-Aho T, Echenko O, Kankaanpaa A, Seppala T, Honkanen A, Sprengel R, Korpi ER (Reduced aggression in AMPA-type glutamate receptor GluR-A subunit-deficient mice. *Genes Brain Behav* 3:253-265.2004).
- Welsh JP, Yamaguchi H, Zeng XH, Kojo M, Nakada Y, Takagi A, Sugimori M, Llinas RR (Normal motor learning during pharmacological prevention of Purkinje cell long-term depression. *Proc Natl Acad Sci U S A* 102:17166-17171.2005).
- Werner DF, Swihart A, Rau V, Jia F, Borghese CM, McCracken ML, Iyer S, Fanselow MS, Oh I, Sonner JM, Eger EI, 2nd, Harrison NL, Harris RA, Homanics GE (Inhaled anesthetic responses of recombinant receptors and knockin mice harboring $\alpha 2$ (S270H/L277A) GABA(A) receptor subunits that are resistant to isoflurane. *J Pharmacol Exp Ther* 336:134-144.2011).
- Whittington MA, Traub RD, Jefferys JG (Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* 373:612-615.1995).
- Wick MJ, Radcliffe RA, Bowers BJ, Mascia MP, Luscher B, Harris RA, Wehner JM (Behavioural changes produced by transgenic overexpression of $\gamma 2L$ and $\gamma 2S$ subunits of the GABA_A receptor. *Eur J Neurosci* 12:2634-2638.2000).
- Wieland HA, Luddens H, Seeburg PH (A single histidine in GABA_A receptors is essential for benzodiazepine agonist binding. *J Biol Chem* 267:1426-1429.1992).
- Wilson RI, Nicoll RA (Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410:588-592.2001).

- Wingrove PB, Thompson SA, Wafford KA, Whiting PJ (Key amino acids in the γ subunit of the γ -aminobutyric acid_A receptor that determine ligand binding and modulation at the benzodiazepine site. *Mol Pharmacol* 52:874-881.1997).
- Wingrove PB, Wafford KA, Bain C, Whiting PJ (The modulatory action of loreclezole at the γ -aminobutyric acid type A receptor is determined by a single amino acid in the β 2 and β 3 subunit. *Proc Natl Acad Sci U S A* 91:4569-4573.1994).
- Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (The EEG effects of THIP (Gaboxadol) on sleep and waking are mediated by the GABA(A)delta-subunit-containing receptors. *Eur J Neurosci* 25:1893-1899.2007).
- Wisden W, Cope D, Klausberger T, Hauer B, Sinkkonen ST, Tretter V, Lujan R, Jones A, Korpi ER, Mody I, Sieghart W, Somogyi P (Ectopic expression of the GABA(A) receptor α 6 subunit in hippocampal pyramidal neurons produces extrasynaptic receptors and an increased tonic inhibition. *Neuropharmacology* 43:530-549.2002).
- Wisden W, Korpi ER, Bahn S (The cerebellum: a model system for studying GABA_A receptor diversity. *Neuropharmacology* 35:1139-1160.1996).
- Wisden W, Laurie DJ, Monyer H, Seeburg PH (The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* 12:1040-1062.1992).
- Wittner L, Eross L, Czirjak S, Halasz P, Freund TF, Magloczky Z (Surviving CA1 pyramidal cells receive intact perisomatic inhibitory input in the human epileptic hippocampus. *Brain* 128:138-152.2005).
- Woo TU, Spencer K, McCarley RW (Gamma oscillation deficits and the onset and early progression of schizophrenia. *Harv Rev Psychiatry* 18:173-189.2010).
- Woo TU, Whitehead RE, Melchitzky DS, Lewis DA (A subclass of prefrontal γ -aminobutyric acid axon terminals are selectively altered in schizophrenia. *Proc Natl Acad Sci U S A* 95:5341-5346.1998).
- Woodruff A, Xu Q, Anderson SA, Yuste R (Depolarizing effect of neocortical chandelier neurons. *Front Neural Circuits* 3:15.2009).
- Woodruff AR, Anderson SA, Yuste R (The enigmatic function of chandelier cells. *Front Neurosci* 4:201.2010).
- Wouterlood FG, Hartig W, Bruckner G, Witter MP (Parvalbumin-immunoreactive neurons in the entorhinal cortex of the rat: localization, morphology, connectivity and ultrastructure. *J Neurocytol* 24:135-153.1995).
- Wulff P, Ponomarenko AA, Bartos M, Korotkova TM, Fuchs EC, Bahner F, Both M, Tort AB, Kopell NJ, Wisden W, Monyer H (Hippocampal theta rhythm and its coupling with gamma oscillations require fast inhibition onto parvalbumin-positive interneurons. *Proc Natl Acad Sci U S A* 106:3561-3566.2009a).
- Wulff P, Schonewille M, Renzi M, Viltono L, Sassoe-Pognetto M, Badura A, Gao Z, Hoebeek FE, van Dorp S, Wisden W, Farrant M, De Zeeuw CI (Synaptic inhibition of Purkinje cells mediates consolidation of vestibulo-cerebellar motor learning. *Nat Neurosci* 12:1042-1049.2009b).
- Yagle MA, Martin MW, de Fiebre CM, de Fiebre NC, Drewe JA, Dillon GH ([³H]Ethynylbicycloorthobenzoate ([³H]EBOB) binding in recombinant GABA_A receptors. *Neurotoxicology* 24:817-824.2003).
- Zezula J, Fuchs K, Sieghart W (Separation of α 1, α 2 and α 3 subunits of the GABA_A-benzodiazepine receptor complex by immunoaffinity chromatography. *Brain Res* 563:325-328.1991).
- Zhang ZJ, Reynolds GP (A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophr Res* 55:1-10.2002).

- Zhou FW, Chen HX, Roper SN (Balance of inhibitory and excitatory synaptic activity is altered in fast-spiking interneurons in experimental cortical dysplasia. *J Neurophysiol* 102:2514-2525.2009).
- Zhu XO, McNaughton N (Minimal changes with long-term administration of anxiolytics on septal driving of hippocampal rhythmical slow activity. *Psychopharmacology (Berl)* 118:93-100.1995).

10 ORIGINAL PUBLICATIONS