

**Modulation of Voltage-Gated Calcium Channels by Group II
Metabotropic Glutamate Receptors in the Paraventricular
Nucleus of the Thalamus**

By

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Abstract

Compounds that interact with Group II metabotropic glutamate receptors (mGluRs) have antipsychotic effects in animal models. These drugs have also shown efficacy in the treatment of schizophrenia in humans. The mechanism of action is believed to arise from a reduction of glutamatergic transmission in limbic and forebrain regions commonly associated with this disorder. Previous anatomical tracer and lesion studies have revealed that neurons of the paraventricular nucleus of the thalamus (PVT) are an important source of the glutamatergic drive to these specific regions. However, the function of Group II mGluRs in the PVT remains to be determined. Whole-cell recordings from PVT neurons reveal that activation of these receptors has two interesting effects; it reduces calcium entry through voltage-gated calcium channels and it causes neurons to hyperpolarize. These two effects may contribute to affect the excitability of PVT neurons, an action that may underlie the effectiveness of Group II mGluR-activating compounds.

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Abbreviations

ACSF	artificial cerebrospinal fluid
ADP	after-depolarizing potential
AgTx	ω -agatoxin IVA
AMPA	α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor
BK channels	large conductance calcium-activated potassium channels
cAMP	cyclic adenosine monophosphate
CgTx	ω -conotoxin GVIA
CM	central medial nucleus of the thalamus
CNS	central nervous system
D3V	third ventricle
DCG-IV	(2 <i>S</i> , 19 <i>R</i> , 29 <i>R</i> , 39 <i>R</i>)-2-(29, 39-dicarboxycyclopropyl)-glycine
DHPG	(<i>RS</i>)-3, 5-dihydroxyphenylglycine
DHPs	dihydropyridines
fAHP	fast after-hyperpolarizing potential
GABA	γ -aminobutyric acid
GPCR	G-protein-coupled receptors
G$\beta\gamma$	G-protein $\beta\gamma$ subunits
HVA channels	high-voltage-activated calcium channels
I_{CAN}	calcium-dependent, non-selective cation current
LTS	low-threshold spike
LVA channels	low-voltage-activated calcium channels
MCPG	(<i>RS</i>)- α -methyl-4-carboxyphenylglycine

mGluR	metabotropic glutamate receptor
mPFC	medial prefrontal cortex
NMDAR	N-methyl-D-aspartate receptor
PLC	phospholipase C
PVT	the paraventricular nucleus of the thalamus
QX-314	lidocaine N-ethyl bromide
sADP	“sustained” after-depolarizing potential
sAHP	slow after-hyperpolarizing potential
SCN	hypothalamic suprachiasmatic nucleus
SK channels	small conductance calcium-activated potassium channels
TC	thalamocortical
TTX	tetrodotoxin
VGCC	voltage-gated calcium channel
VLPO	ventrolateral preoptic area

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Abstracts

1. **Jean-François Borduas**, A. Wong and R. Bergeron (2010) Modulation of Voltage-Gated Calcium Channels by Group II Metabotropic Glutamate Receptors in the Paraventricular Nucleus of the Thalamus. Canadian Association for Neuroscience. Ottawa.

2. **Jean-François Borduas**, A. Wong and R. Bergeron (2010) Modulation of Voltage-Gated Calcium Channels by Group II Metabotropic Glutamate Receptors in the Paraventricular Nucleus of the Thalamus. 2nd annual Brain Health Research Day. Roger-Guindon, Ottawa.

Chapter 1: INTRODUCTION

Metabotropic Glutamate Receptors

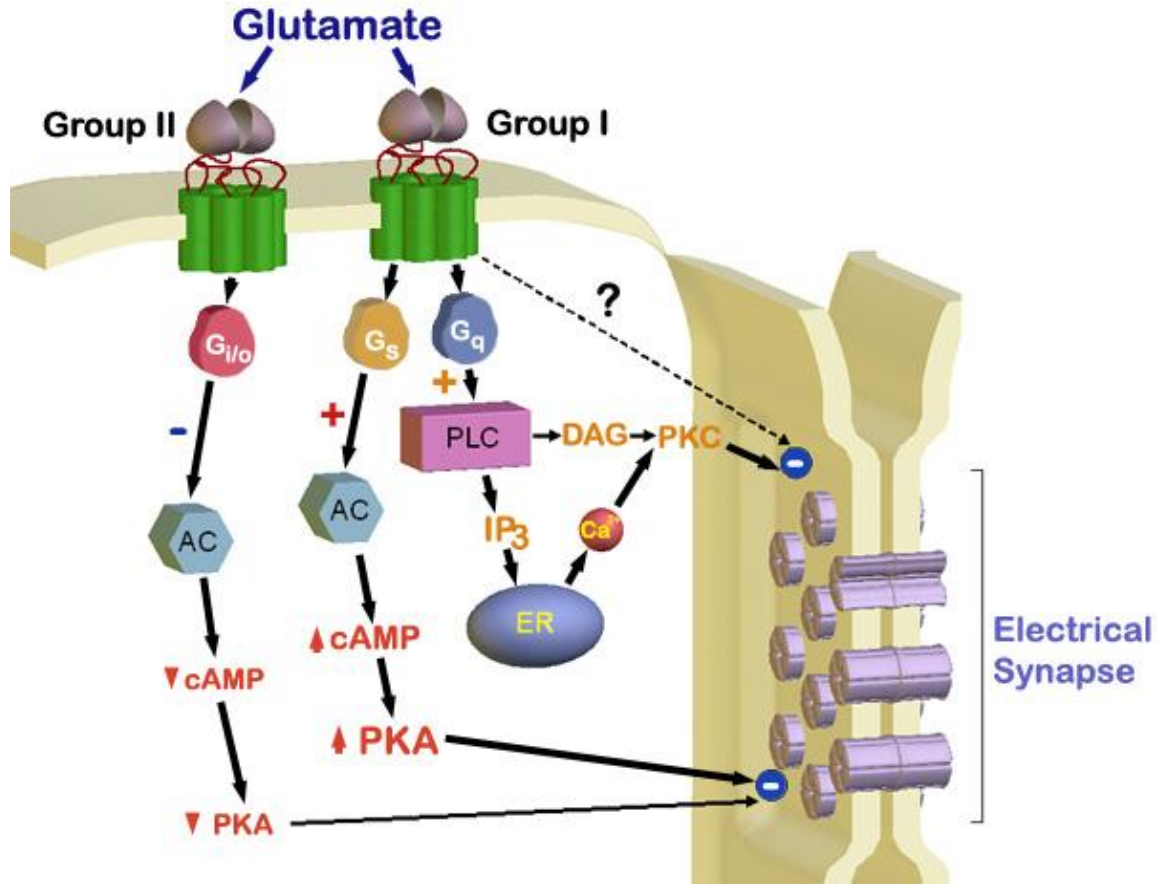
L-Glutamate acts as the major excitatory neurotransmitter in the mammalian CNS. Once released at synapses, glutamate not only binds to ionotropic glutamate receptors but also acts on metabotropic glutamate receptors (mGluRs). mGluRs are neuromodulatory receptors which provide the means by which glutamate can modulate cell excitability and synaptic transmission via second messenger signalling pathways (Conn & Pin, 1997, Nakanishi et al., 1998). These G-protein coupled receptors indirectly gate ion channels by activating a second messenger cascade which can exert not only an excitatory but also an inhibitory action. As many as eight mGluRs have been cloned (Nakanishi, 1992; Schoepp & Conn, 1993; Hollman & Heinemann, 1994). They are divided into three groups according to their amino acid sequence, pharmacological profile and putative transduction mechanism (**Table 1**). Group I mGluRs, which are comprised of mGluR1 and mGluR5, are positively linked to phospholipase C through coupling to the G_q class of G proteins. In contrast, Group II (mGluR2/3) and Group III (mGluR4,6-8) mGluRs inhibit cAMP formation through coupling to the G_i/G_o class of G proteins (**Fig. 1**). Although group II mGluR (particularly mGluR2) can be located on both sides of the synaptic cleft, group I and group III receptors are generally localized post and presynaptically, respectively (Reviewed in Cartmell & Schoepp, 2000; Niswender & Conn, 2010).

Group classification of metabotropic glutamate receptors			
	Receptor	Transduction mechanism	Agonists
Group I	mGluR1 mGluR5	activation of PLC	quisqualate 3,5-DHPG
Group II	mGluR2 mGluR3	inhibition of adenylate cyclase	DCG-IV 2R,4R-APDC LY354740 LY379268
Group III	mGluR4 mGluR6 mGluR7 mGluR8	inhibition of adenylate cyclase	L-AP4 (RS)PPG

Classification of metabotropic glutamate receptors

This classification was determined by the similarities in coupling mechanisms, molecular structure and homology of sequences, and the pharmacology of the receptors (Hermans & Challiss, 2001).

Table 1



Metabotropic glutamate receptor-mediated intracellular signalling

Activation of metabotropic glutamate receptors can indirectly regulate ion channel activity by acting on different intracellular pathways. Group I metabotropic glutamate receptors are typically found to be positively linked to phospholipase C (PLC) and therefore, direct activation of these receptors results in increased phosphoinositide turnover. On the other hand, Group II metabotropic glutamate receptors are negatively coupled to adenylyl cyclase (AC) in that upon activation, they inhibit forskolin-stimulated cyclic AMP formation (Hughes & Crunelli, 2006).

Figure 1

Interestingly, there is a wide diversity and heterogeneous distribution of mGluR subtypes in the CNS. This provides us with an opportunity for selectively targeting individual mGluR subtypes involved in only a limited number of neurological functions for the development of novel drug therapies for psychiatric and neurological disorders. Preclinical studies have revealed that compounds specific for a particular mGluR subtype have potential for the treatment of several CNS disorders, including depression (153), anxiety disorders (154), schizophrenia (71, 155), epilepsy (157), Parkinson's disease (159) and more. Moreover, evidence from clinical studies demonstrate promising clinical efficacy of some of these drugs in treatment of specific neurological diseases (Swanson et al., 2005; Conn et al., 2009; Niswender and Conn, 2010).

Group II mGluRs are excellent examples for the specific involvement of a particular mGluR subtype to particular CNS disorders. These glutamatergic receptors are express at high levels in limbic and forebrain regions (Ohishi et al., 1993, 1998; Gu et al., 2008) and thus have been targeted in possible therapies for stress, anxiety disorders and some symptoms of schizophrenia. In fact, compounds that interact with Group II metabotropic glutamate receptors (mGluRs) have anxiolytic and antipsychotic effects in animal models. These drugs have also shown efficacy in the treatment of both anxiety and schizophrenia in humans (Swanson et al., 2005; Conn et al., 2009; Niswender and Conn, 2010). The mechanism of action is believed to result from a reduction of glutamatergic transmission in relevant limbic and forebrain areas, including the amygdala and prefrontal cortex (Lin et al., 2000; Marek et al., 2000; Grueter and Winder, 2005; Muly et al., 2007).). Anatomical tracer and lesion studies have demonstrated that the

glutamatergic neurons of the paraventricular nucleus of the thalamus (PVT) play an important role in regulating excitability levels in the limbic system and forebrain by providing an important source of glutamatergic drive to these regions (Hur and Zaborsky, 2005; Huang et al., 2006; Hsu and Price, 2009). Interestingly, the PVT displays high expression levels of the Group II mGluRs (Ohishi et al., 1993, 1998; Gu et al., 2008). This raises the question that activation of Group II mGluRs in the PVT may be responsible for the effectiveness of Group II mGluR-activating compounds in anxiety and schizophrenia.

The Paraventricular Nucleus of the Thalamus

Located directly ventral to the third ventricle, the PVT is a unique member of the midline and intralaminar group of thalamic nuclei. Although very small in size, the PVT is a multisensorial structure as it is connected to a remarkably extensive set of limbic, striatal and midbrain structures as well as many hypothalamic cell groups. The PVT differs from other thalamocortical relay nuclei in terms of its putative role in stress, psychostimulants and reward-motivated behaviors (Bentivoglio et al., 1991; Groenewegen & Berendese, 1994; Hsu & Price, 2009) as well as its connection with the ventral aspects of medial prefrontal cortex (mPFC), a region associated with limbic function including motivation and attention (Cardinal et al., 2002; Christakou et al., 2004).

The PVT receives heavy monoamine inputs that include histamine, dopamine, noradrenaline, and serotonin fibers (Cornwall and Phillipson 1988; Otake and Ruggiero

1995; Panula et al. 1989; Rico and Cavada 1998), all of which have been implicated in the promotion and maintenance of wakefulness (Jones 2003; Siegel 2004). Additionally, the PVT shares a reciprocal connection with the hypothalamic suprachiasmatic nucleus (SCN), the primary circadian pacemaker (Klein et al., 1991; Moga et al., 1995; Moga & Moore, 1997). This suggests that the PVT might play a role in the regulation of many behavioral, neuroendocrine and autonomic circadian rhythms. This is further supported by evidence showing that expression of Fos (the product of the immediate-early gene *c-fos*) increases in the PVT during wakefulness and peaks while the animals engage in functions that are incompatible with sleep (Peng et al., 1995; Novak & Nunez, 1998). Additionally, this pattern of expression is 180° out of phase with that of the ventrolateral preoptic area (VLPO) (Novak & Nunez, 1998), a brain area involved in the onset and maintenance of sleep (Sherin et al., 1996).

The PVT efferents are unique among all other thalamic nuclei and project to medial prefrontal cortex, nucleus accumbens and amygdala, all of which are associated with limbic function including motivation and attention. Most of these projections are excitatory (Hur and Zaborsky, 2005; Huang et al., 2006; Hsu and Price, 2009). These axonal projections are of particular interest because they point to an important role of PVT neurons in regulating excitability levels in the limbic system and forebrain. Thus, the PVT might be the principal target of Group II mGluR-activating compounds which have been proven effective in anxiety and schizophrenia since their mechanism of action is believed to arise from a reduction in excitatory neurotransmission in brain areas which receive input from the PVT. However, the function of Group II mGluRs in the PVT has

not been determined. In most studies carried throughout the CNS, activation of Group II mGluRs is associated with inhibition of voltage-gated calcium channels (VGCCs) (Swartz and Bean, 1992; Sahara and Westbrook, 1993; Chavis et al., 1994; Rothe et al., 1994; Stefani et al., 1994; Ikeda et al., 1995; Lachica et al., 1995). This effect can greatly impact neuronal excitability since VGCCs are involved in many cellular functions, including repetitive firing behaviour and activation of calcium-dependent potassium conductances (Jones, 1998; Pape et al. 2004; Lacinová, 2005).

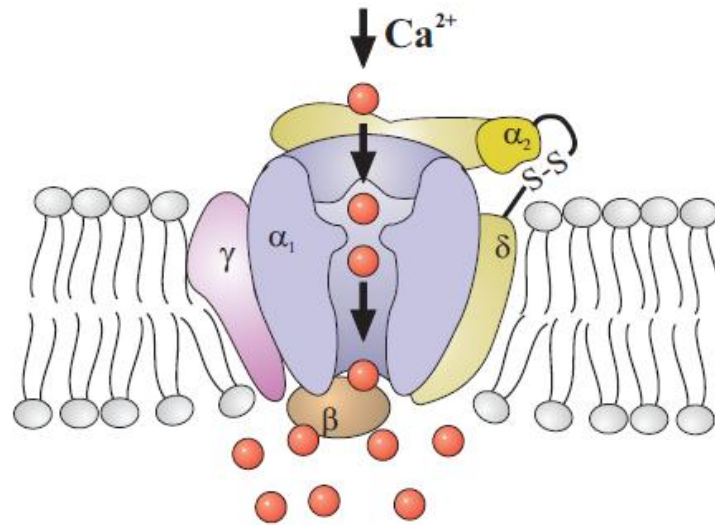
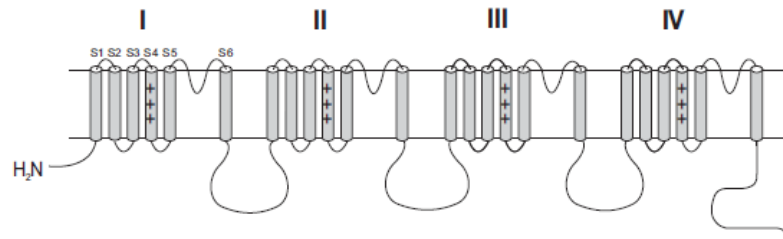
Voltage-gated Calcium Channels

Voltage-gated calcium channels (VGCCs) were first identified by Fatt and Katz (1953) in crustacean muscle fibres (Fatt & Katz, 1953). Subsequently, it became evident that some calcium channels need only a small depolarization to be activated, while others require a relatively high step in membrane voltage to open (Hagiwara, 1975; Llinás & Yarom, 1981). According to this criterion, calcium channels were classified into low-voltage activated (LVA) and high-voltage-activated (HVA). LVA channels are well distinguished by their low-voltage activation threshold potential (-60 mV), their rapid inactivation kinetics ($\tau \sim 15\text{-}30$ ms) and small single channel conductance (5-9 pS). For these reasons, they have been also named T-type, T for transient (fast inactivation) and tiny (small conductance). In contrast, HVA calcium channels required stronger depolarization to activate (threshold potential of -30 mV) and inactivation was much slower ($\tau \sim 2,000$ ms) (Fishman & Spector, 1981; Perez-Reyes, 2003; Lacinová, 2005). The first generally known representative of the HVA family was termed L-type calcium

channel due to its large-single channel conductance (~ 25 pS) and slow decay kinetics (L for large and long-lasting) (Fox & al., 1987b). L-type calcium channels were also characterized by their pharmacological sensitivity to dihydropyridines (DHPs). In the following years, recordings on neuronal cells revealed novel calcium currents, insensitive to DHPs with intermediate single channel conductances (Nowycky et al., 1985; Fox et al., 1987a). These calcium channels were therefore termed N-type (N for neuronal). However, these channels were later divided into two separate subtypes according to their sensitivity to different peptide toxins. Channels blocked by the cone snail toxin, ω -conotoxin GVIA (CgTx), kept the name N-type (Plummer et al., 1989). On the other hand, channels blocked by the funnel web spider toxin, ω -agatoxin IVA (AgTx), were termed P/Q type. P channels, originally characterized in Purkinje neurons of the cerebellum, are now defined by rapid block by AgTx at < 100 nM (Mintz et al., 1992). Q channels are also resistant to DHPs and CgTx but were found to be blocked less rapidly and/or potently by AgTx (Zhang et al., 1993). The distinction between P and Q channels has been difficult to establish in many neurons, so most studies refer to P/Q channels. The HVA channels resistant to DHPs, AgTx and CgTX were named R-type calcium channel (R for resistant) (Randall & Tsien, 1997). However, SNX-482, a peptide toxin isolated from a species of giant tarantula is now considered a potent and semi-selective inhibitor of these channels (Newcomb et al., 1998; Bourinet et al., 2001).

Cloning of cDNA encoding individual channel subtypes first began with the skeletal L-type calcium channel (Tanabe et al., 1987). These experiments revealed that VGCCs are composed of a pore-forming α_1 subunit as well as auxiliary subunits β , α_2 - δ ,

and γ (**Fig. 2**). These auxiliary subunits have different regulatory functions. For instance, the β and $\alpha_2\text{-}\delta$ subunits increase expression levels of HVA channels in heterologous systems such as *Xenopus* oocytes and mammalian cell lines, and also influence the kinetic and pharmacological properties of the channel (Singer et al., 1991). Additionally, genetic disorders of calcium channels can result from defects in either α_1 or β subunits (Fletcher et al., 1998). Further functional diversity arises from the observation that not all four modulatory proteins are necessarily present in each channel complex (Lacinová, 2005). The primary α_1 subunit is responsible for basic electrophysiological and pharmacological properties that formed the basis of previous channel classifications. Therefore, a second classification of VGCCs was developed based on their respective cloned α_1 subunit. The properties of α_1 (S, C, D, F) subunits matched those of L-type channels (Tanabe et al., 1987; Mikami et al., 1989; Seino et al., 1992; Bech-Hansen et al., 1998). On the other hand, the α_{1A} , α_{1B} and α_{1E} subunits were found to correspond to P/Q-type (Mori et al., 1991; Starr et al., 1991), N-type (Williams et al., 1992; Dubel et al., 1992) and R-type (Ellinor et al., 1993) channels, respectively. A short while after, three members of the LVA T-type subfamily were identified: α_{1G} (Perez-Reyes et al., 1998), α_{1H} (Cribbs et al., 1998), α_{1I} (Lee et al., 1999). In the year 2000, a more systemic nomenclature for calcium channels was proposed (Ertel et al., 2000). Since, VGCCs have been named according to the $\text{Ca}_v\text{x.y}$ scheme, where Ca_v stands for VGCC, x is a number indicating if the channel is part of the L-type (1), neuronal (2) or T-type (3) subfamilies, and y is a number designating individual members of subfamilies that are differentiated by their cloned α_1 subunit. **Table 2** shows an overview of VGCCs classification.

A**B**

Structure of voltage-gated calcium channels

A) Voltage-gated calcium channels are composed of a pore-forming α_1 subunit through which calcium can pass upon opening. The α_1 subunit can be further regulated by auxiliary subunits β , α_2 - δ , and γ . B) The α_1 subunit is composed of four homologous domain I-IV, each containing six transmembrane segments S1-S6. The fourth transmembrane segment S4 bears a net positive charge and is believed to act as the voltage sensor controlling VGCC gating (Lacinová, 2005).

Figure 2

Electrophysiological nomenclature		Molecular nomenclature		Main localization
		Old	New	
HVA (co-assembled with $\beta + \alpha_2\delta$)	L	$\alpha 1S$	Ca _v 1.1	Skeletal muscle
		$\alpha 1C$	Ca _v 1.2	Cardiac, smooth muscle, neuronal
		$\alpha 1D$	Ca _v 1.3	Sinoatrial node, cochlear hair cells, neuronal
		$\alpha 1F$	Ca _v 1.4	Retina
	P/Q	$\alpha 1A$	Ca _v 2.1	Neuronal (presynaptic)
	N	$\alpha 1B$	Ca _v 2.2	Neuronal (presynaptic)
	R	$\alpha 1E$	Ca _v 2.3	Neuronal
LVA	T	$\alpha 1G$	Ca _v 3.1	Neuronal, cardiac
		$\alpha 1H$	Ca _v 3.2	Neuronal (+ many other tissues)
		$\alpha 1I$	Ca _v 3.3	Neuronal

Classification of voltage-gated calcium channels

This table summarizes how calcium channels are classified according to their electrophysiological and molecular (old and new) nomenclature (Dolphin, 2009).

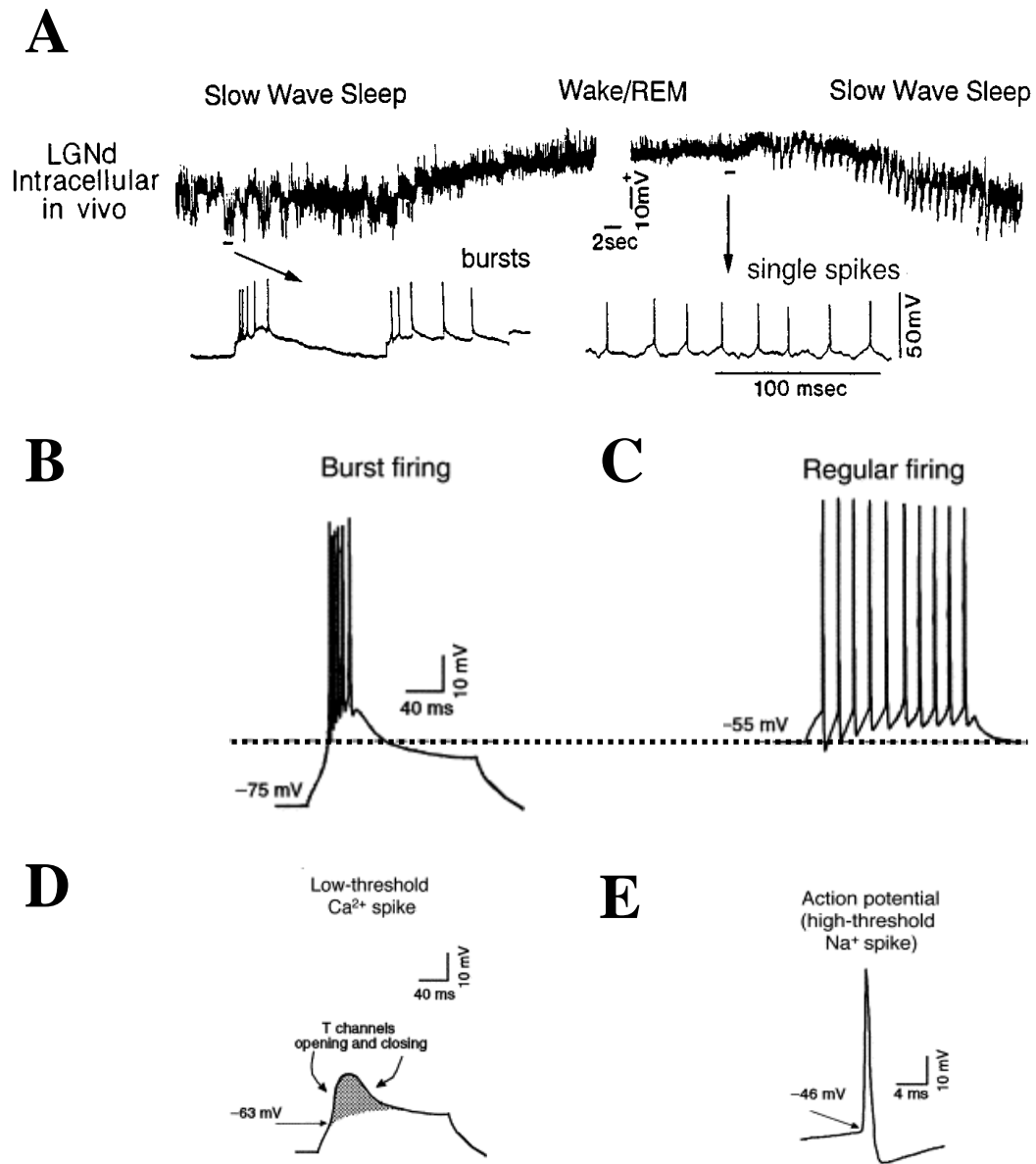
Table 2

The Roles of LVA and HVA Calcium Channels in the Thalamus

Although many functional studies on the PVT have been made, VGCCs have not been fully characterized in this region. Moreover, their influence on neuronal excitability of PVT neurons remains unexplored. Most of the information on the role of VGCCs in the thalamus comes from the lateral geniculate nucleus (LGN), the primary processing center for visual information received from the retina.

The LGN and other thalamic nuclei play a pivotal role in integrating and relaying information from other brain regions to the cerebral cortex. A great diversity of signals are first transformed into a thalamocortical (TC) neuron firing rate code and then transmitted to forebrain circuits (cortex, amygdala, striatum). Therefore, much attention has been given to the ionic basis of the various conductances modulating these complex firing patterns (McCormick & von Krosigk, 1992; Steriade et al., 1993; Sherman and Guillery, 1996; Llinás et al., 1998; Jones, 2000; Le Masson et al., 2002). Studies indicate that mutations of these ion channels may be responsible for aberrant thalamic function in some neurological diseases. For instance, channelopathies in the thalamus are thought to be responsible for the low threshold for sensory arousal that occurs in certain forms of insomnia (Anderson et al. 2005), the sensory auras that occur in certain forms of epilepsy (Kalachikov et al., 2002) and the sensory hallucinations that occur in schizophrenia (Sim et al., 2006).

Intracellular recordings of TC neurons *in vivo* revealed that these neurons display two distinct modes of action potential generation in relation to the state of consciousness of the animal. During deep sleep, these cells generate repetitive bursts of action potentials that ride on top of a slower depolarizing potential. In contrast, arousal occurs with the progressive depolarization of TC neurons, which halts the rhythmic activity and switches the neurons to the tonic, or single spike, mode of action potential generation (Hirsch et al., 1983; McCarley et al., 1983) (**Fig. 3A**). Intracellular recordings in slices revealed a similar pattern of activity (McCormick & Pape, 1990; Leresche et al., 1991; Soltesz et al., 1991; Huguenard, 1998). When these neurons are activated from a relatively depolarized state (greater than or equal to approximately -60 mV), TC neurons respond with a regular, non-adapting, train of action potentials, which is maintained throughout the duration of the applied stimulus (tonic mode) (**Fig. 3C**). However, when these neurons are hyperpolarized (less than or equal to approximately -65 mV), weak depolarization reveals a rebound potential crowned by a group of high-frequency spikes (burst mode) (**Fig. 3B**). While tonic firing is thought to enable the faithful transfer of sensory signals to the cortex during wakefulness, the functional consequences of burst firing are a little less obvious. Some authors have proposed that this mode of firing provides a means to “uncouple” sensory thalamocortical signaling during sleep (Steriade et al., 1993). The rhythmic bursts of action potentials observed during sleep may also maintain the forebrain neurons in a state of biochemical readiness for a quick transition to an aroused state (Steriade, 1989). Others believe that burst firing is essential for learning, as information is edited and reorganized during sleep (Crick & Mitchison, 1983). Interestingly, such firing has been linked to spike-wave discharges reported during



Burst and tonic firing modes

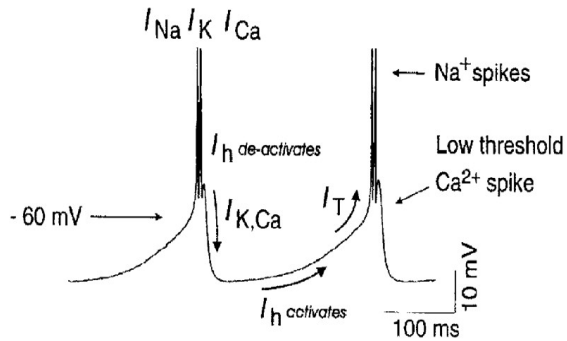
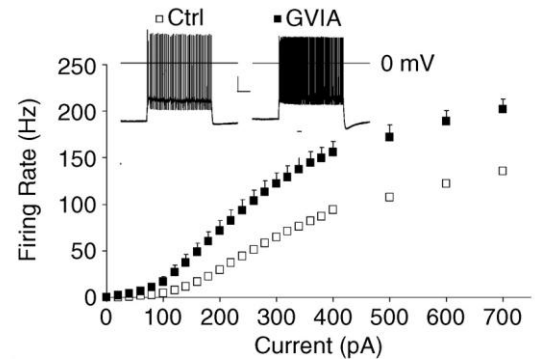
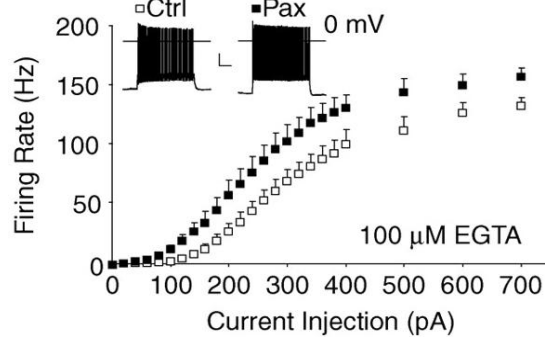
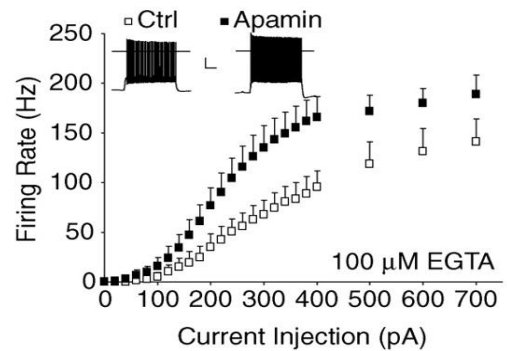
A) *In vivo* intracellular recording of LGN neurons during the transition between burst (slow wave sleep) and tonic mode (wake) of action potential generation indicate that it is accomplished by depolarization of the membrane (McCormick & Bal, 1997). B) *In vitro* recording revealed that these neurons fire in burst mode when depolarized from a hyperpolarized state C) and fire in tonic mode if held at a more depolarized level (≥ -60 mV). D) Block of voltage-gated sodium channels by TTX reveals the LTS underlying burst firing reflecting opening and closing of LVA calcium channels. E) Note the difference in voltage threshold for activation for Na^+ -mediated action potentials (-63 mV vs -46 mV) (Huguenard, 1998).

Figure 3

absence seizures (Huguenard, 1999). In fact, magnetoencephalography has revealed similar patterns of brain activity in many neurological disorders. These patterns have been termed thalamocortical dysrhythmias (Llinás et al., 2001).

Jahnsen & Llinás (1984) were the first to demonstrate that activation of a specialized calcium current is responsible for the rebound potential observed following weak depolarization of hyperpolarized TC neurons. This rebound potential was therefore named low-threshold spike (LTS) and was thought to be carried by LVA calcium channels (**Fig. 3D**). Definitive proof that thalamic LTS are mediated by LVA channels was later provided by studies on transgenic mice, where knockout of the Cav3.1 (LVA) gene abolishes these spikes and burst firing (Kim et al., 2001). Initial voltage-clamp analysis of thalamic LVA currents revealed that activation occurs at membrane potential positive to approximately -65 mV, while inactivation becomes complete, at steady-state, at membrane potentials positive to approximately -65 mV (Coulter et al. 1989; Crunelli et al., 1989; Hernández-Cruz & Pape, 1989). Therefore, most LVA channels are completely inactivated near resting membrane potential (typically -60 mV). However, hyperpolarization (by intracellular injection of current or by the natural occurrence of an inhibitory postsynaptic potential) causes the channels to switch from the inactivated state to a closed state, a process called de-inactivation (Perez-Reyes, 2003). Consequently, if these channels are subsequently activated, the resulting influx of calcium directly depolarizes the membrane of TC neurons, generating the LTS. These calcium spikes in turn bring the membrane potential positive to threshold (approximately -55 mV) for the generation of a burst of tetrodotoxin (TTX)-sensitive sodium spikes (Jahnsen & Llinás,

1984) (**Fig. 3E**). HVA calcium channels will also begin to open at potentials more positive than -40 mV, leading to more influx of calcium (Sundgren-Andersson & Johansson, 1998). Calcium entry through LVA and HVA channel activates different calcium-dependent potassium channels leading to repolarization of the membrane and burst termination (Steriade & Llinás, 1988; Avanzini et al., 1989; Bal et al., 1995) (**Fig. 4A**). Activation of calcium-dependent potassium channels is often associated with an afterhyperpolarization (AHP) of the membrane. AHPs have a great impact on neuronal excitability and discharge patterns over variable time periods. Typically, they can be divided into a fast (fAHP) and a slow component (sAHP). Immediately after an action potential, there is a fast hyperpolarizing potential, the fAHP, which typically lasts 1-10 ms and is due to the activation of large conductance calcium-activated potassium channels (BK channels) (Adams et al., 1982, Lancaster and Nicoll, 1987; Sah, 1992). Therefore, BK channels have a primordial role in spike repolarization. Following the fAHP, there may be a prolonged hyperpolarization, the sAHP, lasting between several hundreds of milliseconds and several seconds. The slower component is thought to be mediated by the small conductance calcium-activated potassium channels (SK channels) (Sah, 1996; Bowden et al., 2001). In contrast to the fAHP, the sAHP does not contribute to action potential repolarization (Lancaster and Nicoll, 1987; Sah, 1992), but simply slows the maximal firing frequency (Engel et al., 1999). Hence, SK channels are major contributors to setting the firing frequency of neurons.

A**B****C****D**

Ionic conductances underlying burst and tonic firing

A) Representative example of currents that generate burst firing. Depolarization of the membrane following hyperpolarizing leads to activation of LVA currents (I_T) which yields the LTS. Apart from activating voltage-gated sodium channels (I_{Na}), the LTS can also activate HVA calcium channels (I_{Ca}). The resulting calcium entry leads to activation of calcium-activated potassium channels ($I_{K,Ca}$) which, in combination with voltage-gated potassium channels (I_K), repolarize the membrane (Bal & McCormick, 1997). B) Tonic firing rates in TC neurons are increased by block of N-type calcium channels, C) BK channels and D) SK channels (Kasten et al., 2007).

Figure 4

During wakefulness, thalamic neurons are depolarized and fire tonic sequences of Na^+/K^+ -mediated action potentials (Jahnsen & Llinás, 1984). This switch in fire behavior results mainly due to inactivation of LVA calcium channels (McCormick & Bal, 1997; Steriade, 1991). Therefore, only HVA calcium channel contribute to calcium influx during tonic firing. As with burst firing, calcium entry through HVA channels can activate SK and/or BK channels, triggering a repolarizing mechanism during tonic firing (Adams et al. 1982; Fagni et al., 1991). Extensive investigations on the specific ionic conductances underlying TC tonic firing are quite limited. A variety of K^+ currents that could potentially regulate firing have been defined in thalamic relay neurons (Huguenard et al. 1991; Huguenard & Prince, 1991). However, such studies predated the common use of high affinity peptide toxins that can link specific calcium channel subtypes to the regulation of thalamic relay neuron firing. Interestingly, a recent study did provide evidence that block of N-type calcium channel results in an increase firing rate of TC neurons (Kasten et al., 2007) (**Fig. 4B**). This study also reported a similar effect by blocking either BK (**Fig. 4C**) or SK channels (**Fig. 4D**). However, inhibiting L-type or P/Q-type calcium channels had small or no effects.

It is important to mention that although PVT neurons are considered TC neurons, their unique projections to the limbic system and their high expression of Group II mGluRs functionally separate them from other thalamic nuclei such as the LGN. Thus, the role of VGCCs in the PVT might differ from what has been described above.

Modulation of VGCCs by Metabotropic Glutamate Receptors

Activity of VGCCs must be tightly regulated to ensure proper control on calcium-dependent processes inside the cell. A classical route of inhibition of certain VGCC subtypes (notably N-, L- and P/Q-types) involves modulation by activation of heterotrimeric G proteins by seven transmembrane G-protein-coupled receptors (GPCRs) (Reviewed in Dolphin, 2003). Although the GPCRs typically involved in this type of modulation are α 2-adrenoreceptors, μ and δ opioid receptors, GABA-B receptors and adenosine A1 receptors (Dunlap & Fischbach, 1978; Dolphin & al., 1986; Scott & Dolphin, 1986), metabotropic glutamate receptors (mGluRs) have also been shown to play a crucial role in regulation of calcium channel activity in different regions of the CNS (Lester & Jahr, 1990; Nawy & Jahr, 1990; Sayer et al., 1992; Swartz & Bean, 1992; Trombley & Westbrook, 1992; Sahara & Westbrook; 1993; Swartz et al., 1993; Chavis et al., 1994, 1995; Hay & Kunze, 1994; Rothe et al., 1994; Stefani et al. 1994, 1996; Ikeda et al., 1995; Choi & Lovinger, 1996).

The hypothesis that glutamate might affect calcium conductances via G-protein-activated mechanisms was first investigated by Lester and Jahr in 1990. Subsequently, activation of all three subfamilies of mGluRs has been linked to inhibition of different subtypes of VGCCs (reviewed in **Table 3**). Although this table suggests that mGluR-mediated modulation of VGCCs is restricted to L- and N-type HVA calcium channels, more recent investigations have revealed that other VGCCs are implicated.

mGluR-Mediated Modulation of HVA Calcium Currents

Channel	Structure	Effect	mGluR	Reference
L	Hippocampal neurons	Reduction	G-I (high sens. to Quis)	Lester and Jahr, 1990
N	Hippocampal neurons	Reduction	G-II?	Swartz and Bean, 1992
N/L	Hippocampal neurons	Reduction	G-II? G-III (?/1-AP4 as agonist)	Sahara and Westbrook, 1993
N	Cortical neurons	Reduction	G-II?	Swartz et al., 1993
N	Cortical neurons	Reduction	G-I and G-II (Quis- and DCG-IV as agonists)	Choi and Lovinger 1996
N/L	Cortical neurons	Reduction	G-III (1-AP4 as agonist)	Stefani et al., 1996
L/N	Cortical neurons	Reduction	G-I (high sen. to Quis)	Sayer et al., 1992
N/L	Olfactory bulb neurons	Reduction	G-III (1-AP4 as agonist)	Trombley and Westbrook, 1992
N	Striatal neurons	Reduction	G-II?	Stefani et al., 1994
N	Visceral neurons	Reduction	G-II	Hay and Kunze, 1994
L	Cerebellar granule	Reduction	G-II (DCG-IV as agonist)	Chavis et al., 1994
L	Cerebellar granule	Increase	G-I	Chavis et al., 1994
L	Cochlear neurons	Reduction	G-II?	Lachica et al., 1994
L	Retinal cells	Reduction/ increase	G-III/G-II	Rothe et al., 1994
N	Transfected DRG cells	Reduction	G-II	Ikeda et al., 1996

Modulation of high-voltage-activated calcium channels by metabotropic glutamate receptors in the CNS

This table summarizes the reported effects of metabotropic glutamate receptor activation on different subtypes of high-voltage-activated calcium channels (Stefani et al. 1996).

Table 3

For instance, these reports indicate that mGluR activation also inhibits P/Q-type calcium channels in cultured cerebellar cells and in synaptosomes from hippocampus and striatum (Perroy et al., 2000; Mela et al. 2006; Martín et al., 2007). Moreover, some studies even indicate that LVA channels are also regulated by mGluRs in the cerebellum and retina (Robbins et al., 2003; Hildebrand et al., 2009). Modulation of VGCCs is of great importance since they are involved in many cellular functions, including repetitive firing behaviour, activation of calcium-dependent potassium conductances, regulation of intracellular calcium stores and gene expression (Jones, 1998; Pape et al. 2004; Lacinová, 2005). Interestingly, calcium and potassium channel proteins, as well as the mGluRs, have been reported to be closely associated with the cell membrane in cultured cerebellar granule cells (Chavis et al. 1995). This lead to the speculation that a sort of functional triplet, constituted by an mGluR in the proximity of depolarizing VGCCs and hyperpolarizing calcium-activated potassium channels (SK or BK channels), might play crucial roles in regulating cell firing.

Modulation of VGCCs by mGluRs has often been linked to a G-protein-mediated, membrane-delimited and voltage-dependent inhibition. This particular inhibition has been shown to slow down the activation kinetics of the inhibited channel and shift the voltage threshold for activation to a more depolarized potential. Interestingly, this inhibitory effect can be removed by a strong conditioning depolarizing prepulse (reviewed in Hille, 1994; Dolphin, 2003; Tedford & Zamponi, 2006). Physiologically, this voltage-dependent feature has been proposed to contribute to short-term plasticity by virtue of relieving G-protein inhibition during high-frequency action potential firing

(Brody et al., 1997; Williams et al., 1997; Bertram et al., 2003). Mechanistically, such channel inhibition has been shown to reflect direct binding of G protein $\beta\gamma$ subunits ($G\beta\gamma$) to VGCCs (Herlitze et al., 1996; Ikeda, 1996; Zamponi & Snutch, 1998). Once attached, the $G\beta\gamma$ -bound channels enter a reluctant gating mode, requiring stronger depolarizations to open (Bean, 1989; Elmslie et al., 1990). However, activation of these channels results in the dissociation of $G\beta\gamma$ from the channel (Boland & Bean, 1993). Thus, the voltage-dependence of mGluRs-mediated inhibition of VGCCs stems mainly from the voltage-dependent unbinding of $G\beta\gamma$.

Aim of Study

Drugs that interact with Group II mGluRs have anxiolytic and antipsychotic effects in animal models. These drugs have also shown efficacy in the treatment of both anxiety and schizophrenia in humans (Swanson et al., 2005; Conn et al., 2009; Niswender and Conn, 2010). The mechanism of action is believed to result from a reduction of glutamatergic transmission in relevant limbic and forebrain areas, including the amygdala and prefrontal cortex (Lin et al., 2000; Marek et al., 2000; Grueter and Winder, 2005; Muly et al., 2007). Anatomical tracer and lesion studies have demonstrated that the PVT plays an important role in regulating excitability levels in the limbic system and forebrain by providing an important source of glutamatergic drive to these regions (Hur and Zaborsky, 2005; Huang et al., 2006; Hsu and Price, 2009). Interestingly, the PVT displays high expression levels of the Group II mGluRs (Ohishi et al., 1993, 1998; Gu et al., 2008). This raises the question that activation of Group II mGluRs in the PVT may be

responsible for the effectiveness of Group II mGluR-activating compounds in anxiety and schizophrenia. However, their function in this location has not been determined. Therefore, the main objective of this study was to elucidate the effect of Group II mGluR activation on PVT neurons.

In most studies carried throughout the CNS, activation of Group II mGluRs is associated with inhibition of VGCCs (Swartz and Bean, 1992; Sahara and Westbrook, 1993; Chavis et al., 1994; Rothe et al., 1994; Stefani et al., 1994; Ikeda et al., 1995; Lachica et al., 1995). However, the existence of such a coupling mechanism between Group II mGluRs and VGCCs in the PVT has not yet been identified. Based on the literature, we can hypothesize that activation of Group II mGluRs in the PVT will reduce calcium currents carried by VGCCs. However, in order to test this hypothesis, it was first necessary to properly characterize and separate both main types of calcium currents (LVA and HVA calcium currents) using the whole-cell patch-clamp technique.

Although prior investigations have demonstrated evidence that both types of VGCCs (LVA and HVA) are present in the PVT (Richter et al., 2005, 2006; Zhang et al., 2009), the identity of the different HVA calcium channel subtypes (P/Q-, L-, R- and N-type) and their contribution on total HVA current remains unexplored. This information is critical in order to eventually decipher the implication of each calcium channel subtype on PVT neuronal excitability and to elucidate a possible coupling mechanism between these channels and Group II mGluRs. Therefore, the second aim of this study consisted in applying different HVA calcium channel blockers to assess the above question. Once the

calcium currents in the PVT were well identified and characterized, the third aim was to examine if Group II mGluR activation could actually reduce calcium currents in the PVT. Results from this study will allow us to elucidate the role of Group II mGluRs in PVT, a role that might explain the effectiveness of Group II mGluR-activating compounds in anxiety and schizophrenia.

Chapter 2: MATERIALS AND METHODS

Preparation of PVT Slices

Coronal brain slices containing the PVT were obtained from wild-type Sprague Dawley rats (3 to 4-weeks old). Animals were housed in a temperature-controlled environment under 12-h light/dark conditions and were decapitated in the morning (subjective quiet period). Prior to decapitation, the animals were anaesthetized using an isoflurane vaporizer (Stoelting, Wood Dale, IL, USA), in agreement with the guidelines of the Canadian Council of Animal Care. The brain was quickly removed and placed in an oxygenated (95% O₂ – 5% CO₂) and cooled (<4°C) artificial cerebrospinal fluid (ACSF) solution containing (mM): 126 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃ and 10 glucose (pH 7.3, osmolarity 300 mOsm). PVT-containing slices 300 µm in thickness were cut in the coronal plane with a vibrating microtome (Leica VT 1000S, Germany) and incubated for more than an hour in an oxygenated chamber at room temperature before they were used for experiments. Cells were subsequently transferred to a submerged recording chamber and superfused (2-4 ml/min) with oxygenated ACSF at room temperature.

Data Recording and Analysis

In voltage-clamp experiments, whole-cell electrophysiological recordings were obtained with borosilicate micropipettes filled with either a K-gluconate-based or a Cs-

methanesulfonate-based intracellular solution (see below for specification of use and composition). The internal solution's pH and osmolarity were adjusted to 7.3 and 300 mOsm, respectively. Pipette resistance ranged from 4-7 M Ω and access resistance < 25 M Ω was considered acceptable.

Initially, recordings were obtained while slices were perfused with ACSF and electrodes were filled with a K-gluconate-based intracellular solution of the following composition (in mM): 130 K-gluconate, 10 KCl, 2 MgCl₂, 10 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES), 2 Mg-ATP, 0.2 GTP-tris. The use of this internal solution proved to be however problematic since strong outward currents mask the existence of a HVA calcium inward current when cells were depolarized to high voltages (>-40 mV).

To isolate LVA and HVA calcium currents, outward potassium currents had to be blocked. Consequently, electrodes were filled with a Cs-methanesulfonate-based internal solution composed of (in mM): 130 Cs-methanesulfonate, 10 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES), 10 CsCl, 2 MgCl₂, 2 Mg-ATP, 0.2 GTP-tris. Additionally, sodium currents were blocked by adding 0.5 mM tetrodotoxin (TTX) to the ACSF. After recording control calcium currents for several experiments, it became evident that there was a current run-down over the time-course of the experiment (approximately 20 min.), especially HVA calcium currents (see results). To remedy this problem, external calcium was replaced by 5 mM barium. Substitution of calcium by barium is a procedure that is often used to enhance currents carried through calcium

channels, because barium permeates better than calcium in most calcium channels, and calcium-dependent inactivation is less pronounced (Fishman & Spector, 1981; Hagiwara & Byerly, 1981).

In current-clamp experiments, slices were perfused with normal ACSF and recordings were obtained with electrodes filled with a K-gluconate-based internal solution (same composition as previously mentioned) in order to record PVT action potential firing.

Voltage-clamp recordings were obtained with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA, USA) under visual control using differential interference contrast and infrared video microscopy (IR DIC; Leica DMLFSA, Germany). The recordings were performed at room temperature from individual PVT neurons voltage-clamped at -60 mV. Patched cells were photographed shortly after the experiments with the microscope's camera to confirm their localization in the PVT. For some cells, Lucifer Yellow was added to the internal solution and, subsequently, stained neurons were photographed by confocal microscopy.

Data were collected using pCLAMP 9 software (Axon Instrument, Foster City, CA, USA). Analysis was performed *off-line* with the software Clampfit 9.0 (Axon Instrument, Foster City, CA, USA). Statistical significance of the results was determined with a one-way Analysis of Variance (Dunnett's test). A $P < 0.05$ was considered statistically significant. All values are expressed as means \pm SEM.

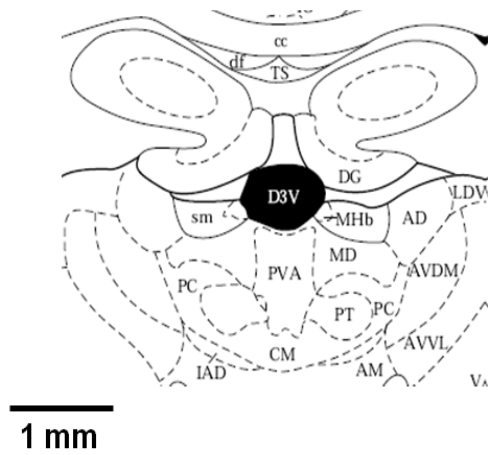
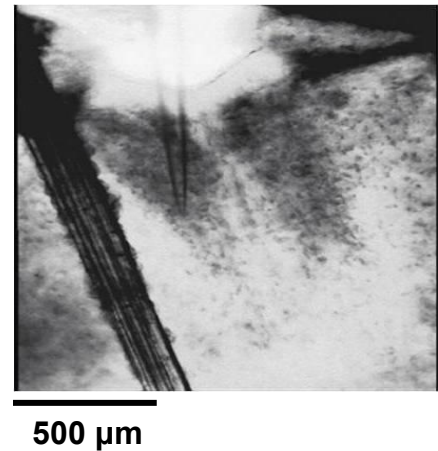
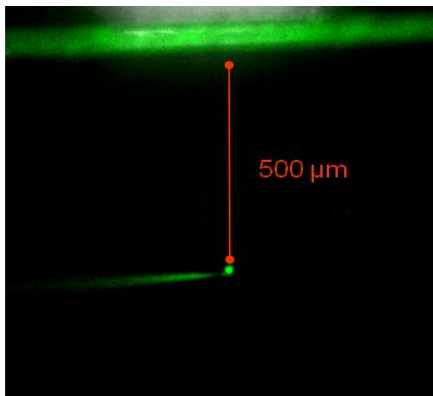
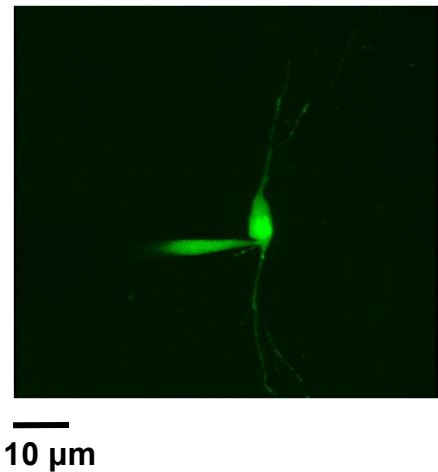
Drugs: Tetrodotoxin (TTX), (2*S*, 19*R*, 29*R*, 39*R*)-2-(29, 39-dicarboxycyclopropyl) glycine (DCG-IV), LY341495, (*RS*)-3, 5-dihydroxyphenylglycine (DHPG) and (*RS*)- α -methyl-4-carboxyphenylglycine (MCPG) were obtained from Sigma-Aldrich (MO, USA). Lidocaine N-ethyl bromide (QX-314), ω -agatoxin IVA, nifedipine, ω -conotoxin GVIA and Lucifer Yellow were purchased from Tocris (Bristol, UK).

Chapter 3: RESULTS

PVT Neurons: Localization, Morphology and Firing Modes

The PVT is located medially in the rat thalamus, spanning the entire anteroposterior extent of the midline-intralaminar complex (approximately 3 mm) (Paxinos & Watson, 1998). For the purpose of this study, the vast majority of recorded cells were localized in slices from the anterior part of the PVT. In these sections, the PVT lies directly ventral to the third ventricle (D3V) and dorsal to the central medial nucleus of the thalamus (CM) (**Fig. 5A**). In order to confirm that the whole-cell recordings were performed within the nucleus, cells were intracellularly stained with Lucifer Yellow and their distance from the D3V was measured (**Fig. 5B**). Localization of the patched cells within the PVT could also be confirmed by photograph of the slice by the digital camera of the microscope in 50X magnification (**Fig. 5C**). PVT neurons filled with Lucifer Yellow displayed ovoid somata and two main dendrites which extended to branch into two or three secondary dendrites (**Fig. 5D**).

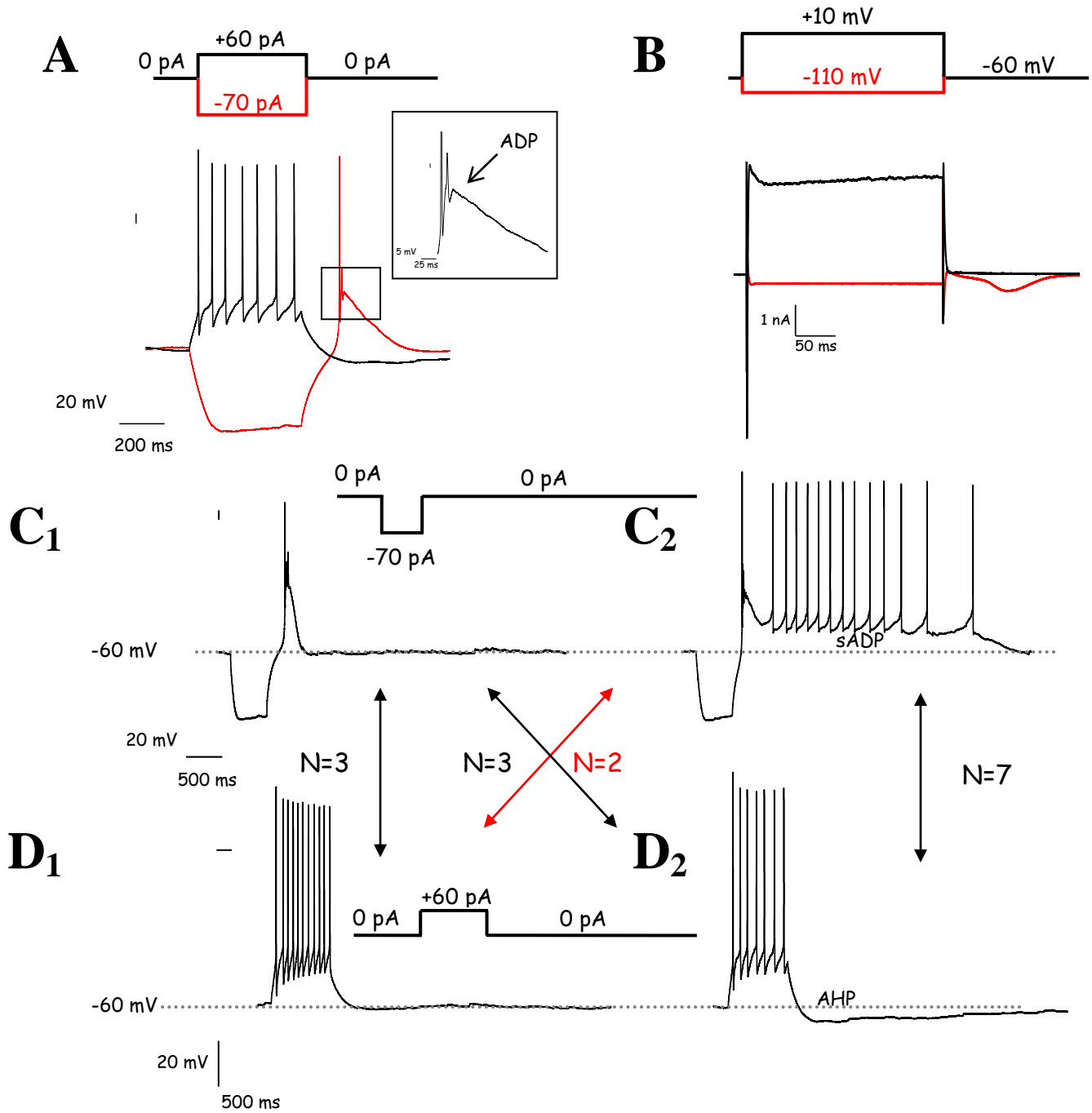
Consistent with recordings from other thalamocortical relay neurons (McCormick & Pape, 1990; Leresche et al., 1991; Soltesz et al. 1991; Huguenard, 1998), PVT neurons display state-dependent firing patterns. Under current-clamp conditions, depolarizing the neurons (+60 pA current injection) from a resting membrane potential of -60 mV (0 pA current injection) results in tonic firing. In other words, the neurons fire a train of action potentials of similar amplitude that lasts the length of the depolarizing step (**Fig. 6A**;

A**C****B****D**

Localization and morphology of neurons from the paraventricular nucleus of the thalamus

A) The anterior PVT (PVA) lies directly ventral to the third ventricle (D3V) and dorsal to the central medial nucleus of the thalamus (CM). B) In order to confirm whole-cell recordings were performed within the nucleus, cells were intracellularly stained with Lucifer Yellow and their distance from the D3V was measured. C) Localization of the patched cells within the PVT could also be confirmed by photograph of the slice. D) PVT neurons filled with Lucifer Yellow displayed ovoid somata and two main dendrites which extended to branch into two or three secondary dendrites.

Figure 5



Burst and tonic firing in the PVT

A) In current-clamp mode, return to resting membrane potential following hyperpolarization elicits burst firing. Inset: note the after-depolarizing potential (ADP) following the bursts. In contrast, depolarization triggers tonic firing. B) Voltage-clamp recording demonstrating the different currents underlying these two firing modes. C & D) PVT neurons displayed heterogeneity in the events following burst and tonic firing. All recordings were conducted by using a K^+ -gluconate-based internal solution and perfusing the slices with ACSF (containing Ca^{2+}).

Figure 6

black trace and Fig. D₁ & D₂). However, if these neurons are given a 500 ms hyperpolarizing step (-70 pA current injection) from a holding potential of -60 mV (0 pA current injection), returning to -60 mV (0 pA current injection) triggers a low threshold spike (LTS) crowned by a burst of a single action potential followed by one or two smaller spikes (**Fig. 6A; red trace and Fig. C₁ & C₂**). These action potentials were followed by an after-depolarizing potential (ADP) which has been shown to be calcium-dependent in LGN thalamic neurons (Jahnsen & Llinàs, 1984; Hernández-Cruz & Pape, 1989) (**Fig. 6A inset**).

In order to understand the different ionic conductances underlying these firing patterns, I performed similar protocols in voltage-clamp (**Fig. 6B**). In voltage-clamp mode, depolarization to +10 mV from -60 mV revealed a fast inward current followed by a transient outward current and a sustained and delayed outward current (**Fig. 6B; black trace**). The fast inward current was TTX-sensitive suggesting that this current is carried by voltage-gated sodium channels (data not shown). The outward currents are likely to be carried by voltage-gated potassium channels since they were blocked by substituting potassium for cesium as the main cation present in the internal solution (data not shown). This will become evident in Figure 7B where depolarizing voltage steps up to +10 mV only elicit inward currents in neurons recorded with a cesium-based internal solution.

On the other hand, when neurons were given a 500 ms hyperpolarizing pulse to -110 mV from -60 mV, returning to -60 mV revealed an inward current with much slower

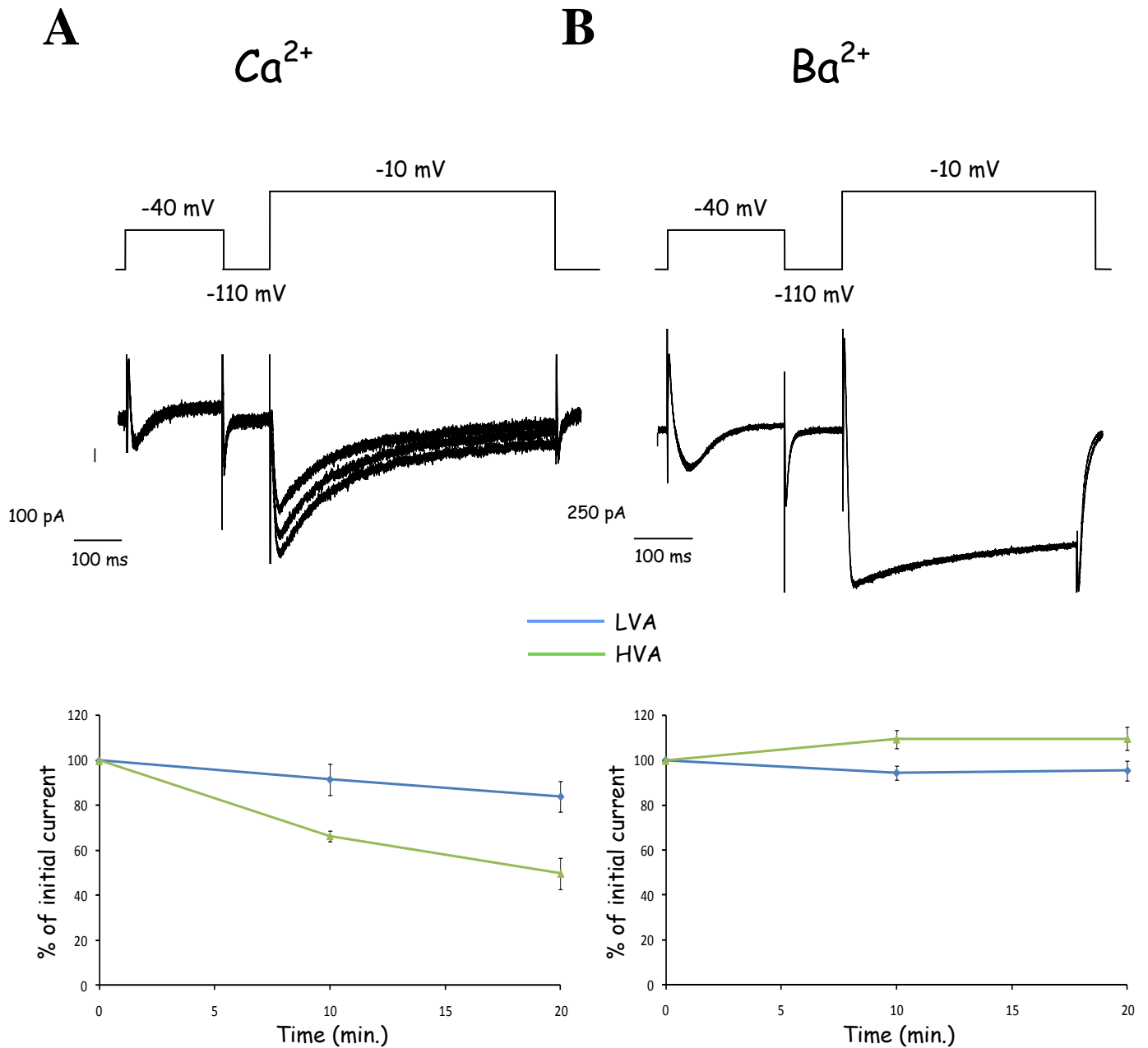
activation and inactivation kinetics then the fast inward current recorded during depolarizing voltage steps from -60 mV (**Fig. 6B; red trace**). This current is likely to be carried by LVA calcium channels since these channels require hyperpolarization to remove inactivation (de-inactivation) before they can activate at relatively low voltages (i.e. -60 mV) (Perez-Reyes, 2003). Moreover, this current is completely abolished in the presence of 500 μ M nickel (data not shown), a well-known inorganic blocker of LVA calcium channels (Perez-Reyes, 2003). This suggests that these channels are responsible for the rebound depolarizing potential (i.e. LTS), observed in current-clamp conditions (**Fig. 6A; red trace**) since proof that the thalamic LTS is mediated by LVA channels was previously provided by studies on transgenic mice, where knockout of the Cav3.1 (LVA) gene abolishes these spikes and burst firing (Kim et al., 2001).

Interestingly, PVT neurons displayed heterogeneity in the events following burst and tonic firing. In a sample of fifteen recorded cells, four different firing behaviours were observed. The majority of neurons (n=7) displayed a prominent and sustained after-depolarizing potential (sADP) following the LTS-induced burst. These neurons also displayed a slow after-hyperpolarization potential (sAHP) following the train of action potentials elicited during tonic firing (6.10 ± 0.51 mV in amplitude; 1.89 ± 0.19 s in duration) (**Fig 6C₂ & D₂**). However, three neurons lacked a discernable sADP but showed a sAHP (**Fig 6C₁ & D₂**). Conversely, two neurons displayed a sADP but lacked a sAHP (**Fig 6C₂ & D₁**), and three neurons showed no sADP following the LTS-induced burst and no sAHP following the train of action potentials elicited during tonic firing (**Fig 6C₁ & D₁**).

LVA and HVA Calcium Currents in the PVT

The results from Figure 6B suggest the presence of LVA calcium channels in PVT neurons. The low-threshold inward calcium current can be observed in voltage-clamp when neurons are depolarized to -60 mV after the channels have been deactivated by hyperpolarization (**Fig. 6B**). However, the outward potassium currents, elicited during stronger depolarization (+30 mV), mask the existence of the inward HVA calcium current in these neurons. Consequently, the HVA calcium current was isolated by blocking both sodium and potassium channels by external addition of TTX and internal substitution of potassium by cesium, respectively. Therefore, the following results (**Fig. 7-10**) were generated by recording with a cesium-based internal solution with TTX included in the external solution.

The LVA and HVA calcium current could be separated by using a two-pulse steady-state protocol (**Fig. 7**). In this protocol, the LVA current was defined as the current that decayed completely within a 200 ms step at -40 mV following a 500 ms hyperpolarizing step to -110 mV. In contrast, the HVA current amplitude was defined as the current flowing at the end of a 200 ms step at -10 mV. The use of this protocol will be justified in the following sections.



Calcium-dependent “rundown” of current in the PVT

A) HVA calcium currents were subject to significant rundown when calcium was used as the charge carrier (ACSF containing 2.5 mM calcium). On the other hand, LVA currents were much less affected. B) Replacement of external calcium by barium prevented rundown of current as the current remained fairly constant over the twenty minutes recording period (ACSF containing 5 mM barium). All recordings were conducted by using a Cs^+ -methanesulfonate-based internal solution (to block voltage-gated potassium currents) and by adding TTX to the ACSF (to block voltage-gated sodium channels).

Figure 7

After recording HVA calcium currents for several experiments, it became evident that there was a significant rundown of current when calcium was used as the charge carrier (49.7 ± 6.9 %; $n=3$, after twenty minutes of initial recording). This rundown of current is likely a result of calcium-dependent inactivation of HVA channels, a phenomenon also present in TC neurons (Meuth et al., 2001). In contrast, the LVA currents were much less affected (84.0 ± 6.9 %; $n=3$) (**Fig. 7A**). This likely reflects the fact that HVA calcium channels are much more sensitive to calcium-dependent inactivation than LVA calcium channels (Pape et al., 2004). When calcium was substituted by barium, both currents remained fairly constant after twenty minutes of initial recorded current (LVA: 95.4 ± 4.5 %; HVA: 109.7 ± 5.3 %; $n=5$) (**Fig. 7B**). Substitution of calcium by barium is a procedure that is often used to enhance currents carried through calcium channels, because barium permeates better than calcium, and calcium-dependent inactivation is less pronounced (Fishman & Spector, 1981; Hagiwara & Byerly, 1981). Therefore, the following voltage-clamp experiments (**Fig. 8-10**) were all conducted by using barium as the charge carrier in order to allow a sufficient recorded period to properly study LVA and HVA calcium currents.

In these conditions, LVA and HVA currents can be easily separated on the basis of their voltage dependency of activation by application of a depolarizing voltage ramp (from -90 to +30 mV) after a 100 ms hyperpolarizing step to -110 mV (to de-inactivate channels). This ramp protocol evokes a twin peaked inward current envelope demonstrating the presence of a low-voltage- and a high-voltage-activated component

(**Fig. 8A**). LVA and HVA calcium currents can also be differentiated on the basis of their steady-state inactivation kinetics. Application of 250 ms depolarizing steps (from -100 to +10 mV) of 10 mV increments, following a 1s hyperpolarizing step to -110 mV, displays transient and non-inactivating currents (**Fig. 8B**). Initially, no current is observed during the -100 to -70 mV range of test potentials. Transient and inactivating inward calcium currents were first observed at -60 mV. These currents decayed completely within the 250 ms step. At -30 mV, an increase in amplitude was observed, and the current decay became less pronounced. With further depolarization, steady-state inactivation of the current was almost nonexistent, highlighting the electrophysiological properties of HVA calcium channels. Note the shoulder at approximately -25 mV in the I/V curve which further supports the presence of two current components with different activation ranges. Although hard to distinguish, the peak current-voltage (I/V) curve (**Fig. 8C**) seems to indicate that the LVA current peaks at approximately -40 mV whereas the HVA current peaks closer to -10 mV.

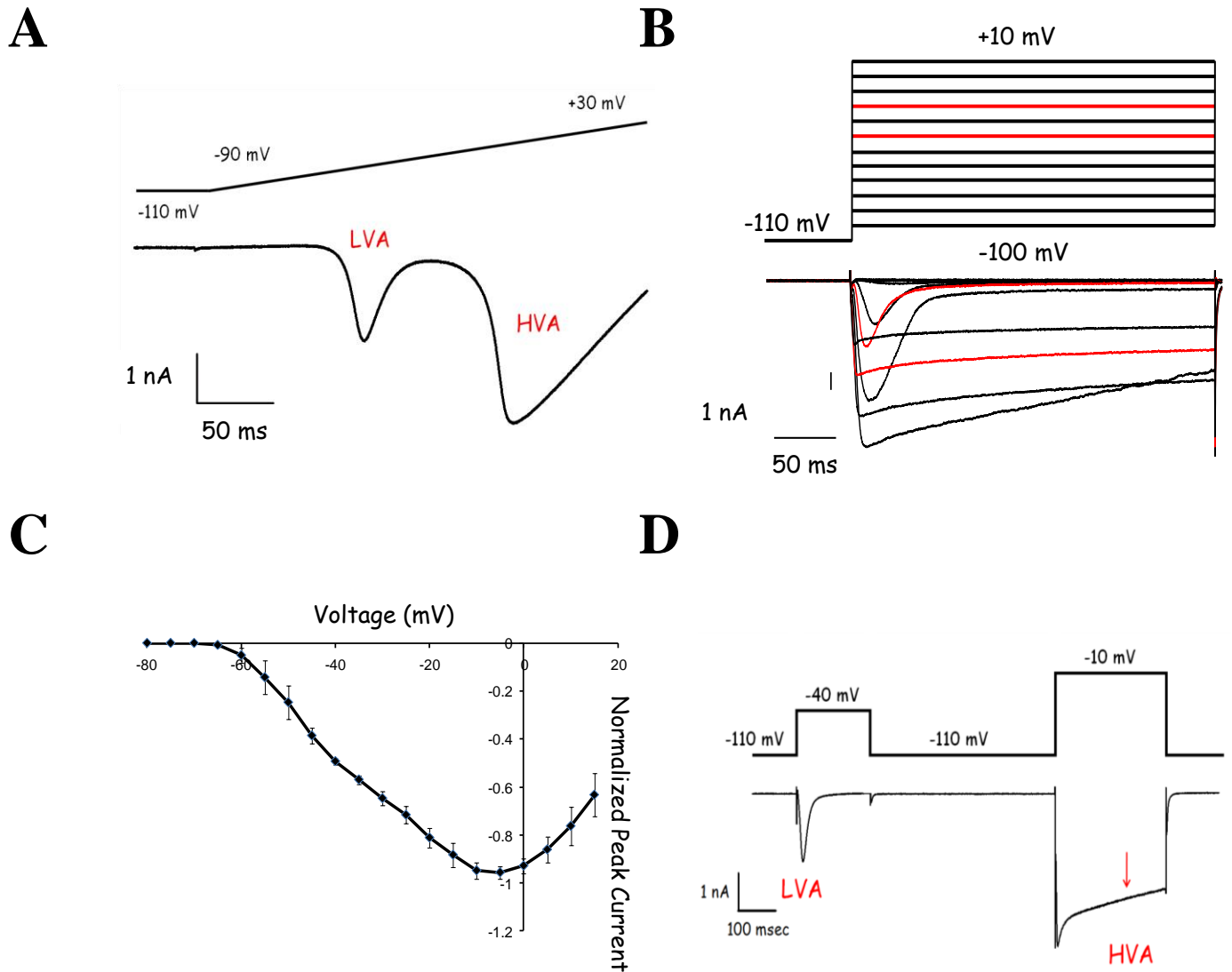
The above results indicate that the LVA current peaks close to -40 mV and decays completely within approximately 100 ms. In contrast, the HVA current peaks at -10 mV and shows little inactivation within the 250 ms step. These differences in voltage dependency of activation and steady-state inactivation kinetics allow us to properly separate and study these currents for quantitative analysis. In order to achieve this, a specific two-pulse steady-state protocol was designed (**Fig. 8D**). In this protocol, the LVA current was defined as the current that decayed completely within a 200 ms step at -40 mV following a 500 ms step to -110 mV. The LVA current reached an average

amplitude of -2.76 ± 0.53 nA (n=6) and decayed with a time course well fitted by a single exponential ($\tau = 17.6 \pm 1.13$ ms; n=6). In contrast, the HVA current amplitude was defined as the current flowing at the end of a 200 ms step at -10 mV (**Fig. 8D; red arrow**). The HVA current measured on average -2.95 ± 0.27 nA (n=10). Decay kinetics of the total current generated by the -10 mV test pulse could be fitted with a double exponential with a fast time constant of 17.11 ± 1.53 ms and a slow time constant of 242.75 ± 24.67 ms (n=6). Note that this protocol might underestimate the total HVA current as some inactivation might have occurred within 200 ms of activation. However, it provides us with an easy way to completely separate both LVA and HVA calcium currents based on their electrophysiological properties.

N-, P/Q-, L-type Calcium Channel Contribution to Total HVA Current

To date, no prior investigations have focused on the identity of the different subtypes of HVA channels comprising the whole-cell recorded current. Moreover, no study has focused on either their additive or their exclusive effects on PVT neuronal firing patterns. This information is absolutely essential to understand Group II mGluR modulation of HVA channels since these receptors have been shown to modulate specific subtypes of HVA channels in the CNS (Chavis et al., 1994; Lester & Jahr, 1990; Sayer et al., 1992; Pin & Duvoisin, 1995).

Since the definition of each subtype depends primarily on pharmacology, different selective HVA calcium channel blockers were applied to assess their effect on

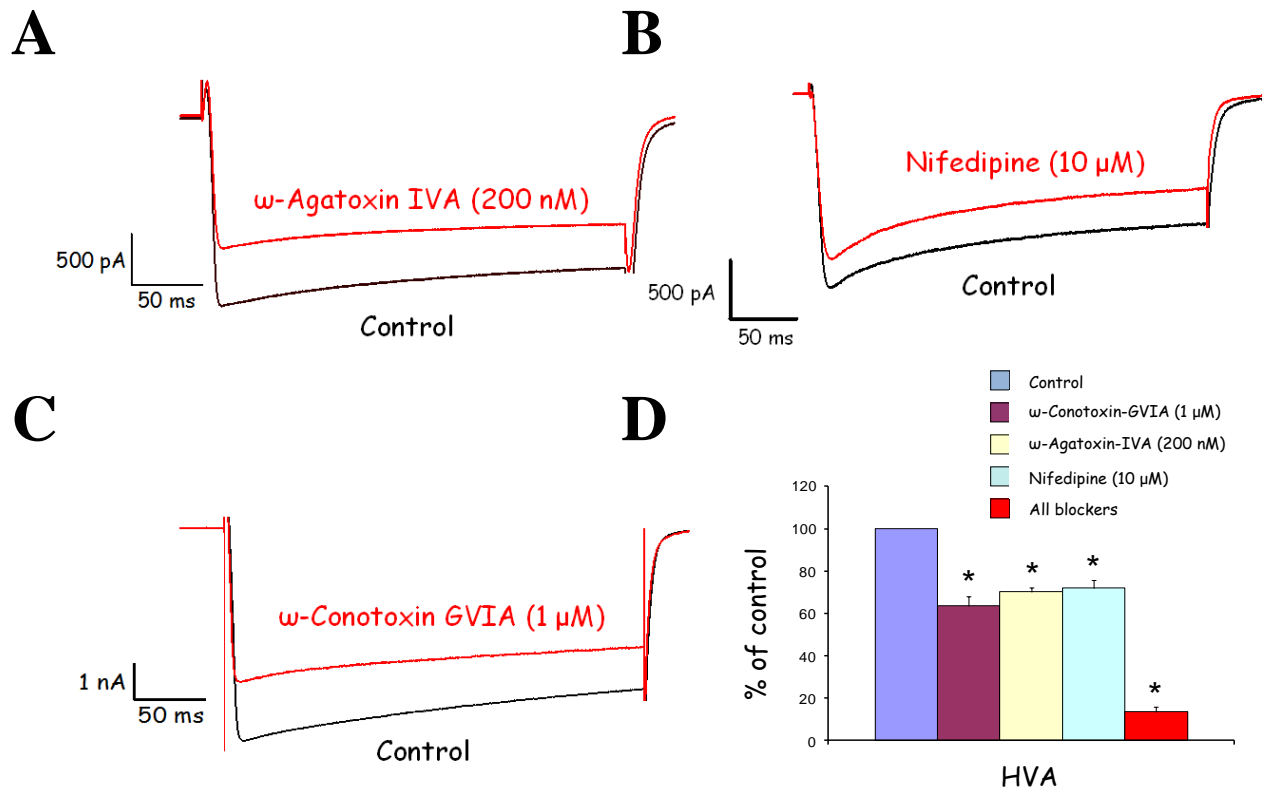


Low-voltage-activated and high-voltage-activated calcium currents in the paraventricular nucleus of the thalamus

A) Application of a depolarizing voltage ramp (from -90 to +30 mV) after a 100 ms hyperpolarizing step to -110 mV demonstrates the presence of a low-voltage-activated and a high-voltage-activated inward current in the PVT. B) Application of 250 ms depolarizing steps (from -100 to +10 mV) of 10 mV increments, following a 1 s hyperpolarizing step to -110 mV, displays transient and non-inactivating currents. C) Peak current-voltage (I/V) curve indicates two peaks at -40 mV and -10 mV (n=3). D) Separation of LVA and HVA current could be achieved by using a two-pulse steady-state protocol. All recordings were conducted by using a Cs⁺-methanesulfonate-based internal solution (to block voltage-gated potassium currents) and by perfusing slices with ACSF containing barium (to limit current rundown) and TTX (to block voltage-gated sodium channels).

Figure 8

the current flowing at the end of a 200 ms step to -10 mV. P/Q-type calcium channels are blocked by the toxin AgTx of the funnel web spider *Agelenopsis aperta* (Mintz et al., 1992) whereas N-type calcium channels are blocked by CgTx, a peptide isolated from the venom of the marine cone snail, genus *Conus* (Plummer et al., 1989). On the other hand, L-type calcium channels are highly sensitive to DHPs, such as nifedipine (Tsien & Ellinor, 1991). Many neurons also have a component of HVA current that is resistant (R-type) to all of the above blockers (Randall & Tsien, 1997). Bath application of AgTx (200 nM) significantly reduced the total control HVA current by 29.79 ± 1.98 % (n=7; p<0.05; Student's paired t-test). Similarly, application of nifedipine (10 μ M) or CgTx (1 μ M), significantly reduced the HVA control current by 28.82 ± 2.90 % (n=5; p<0.05) and 36.54 ± 4.57 % (n=4; p<0.05), respectively. Simultaneous application of all three blockers blocked the total HVA current by 13.51 ± 2.21 % (n=4; p<0.05) (**Fig. 9**). These results suggest that the R-type contribution would be very little (~ 5-10%) and that each of the other subtypes of calcium channel contribute about 30% of the total HVA current in the PVT. According to this data, L-, P/Q- and N-type calcium channels have similar contributions on HVA current. However, they might differentially modulate PVT neuronal firing patterns depending on their respective cellular localization. Additionally, they might be coupled to different regulatory pathways (i.e. mGluR-regulated). Hence, our knowledge of the identity of the different subtypes of calcium channels in the PVT will lead to future investigations that will focus on understanding the role of these different calcium currents in this thalamic nucleus.



Contribution of individual HVA calcium channel subtypes to total whole-cell HVA calcium current

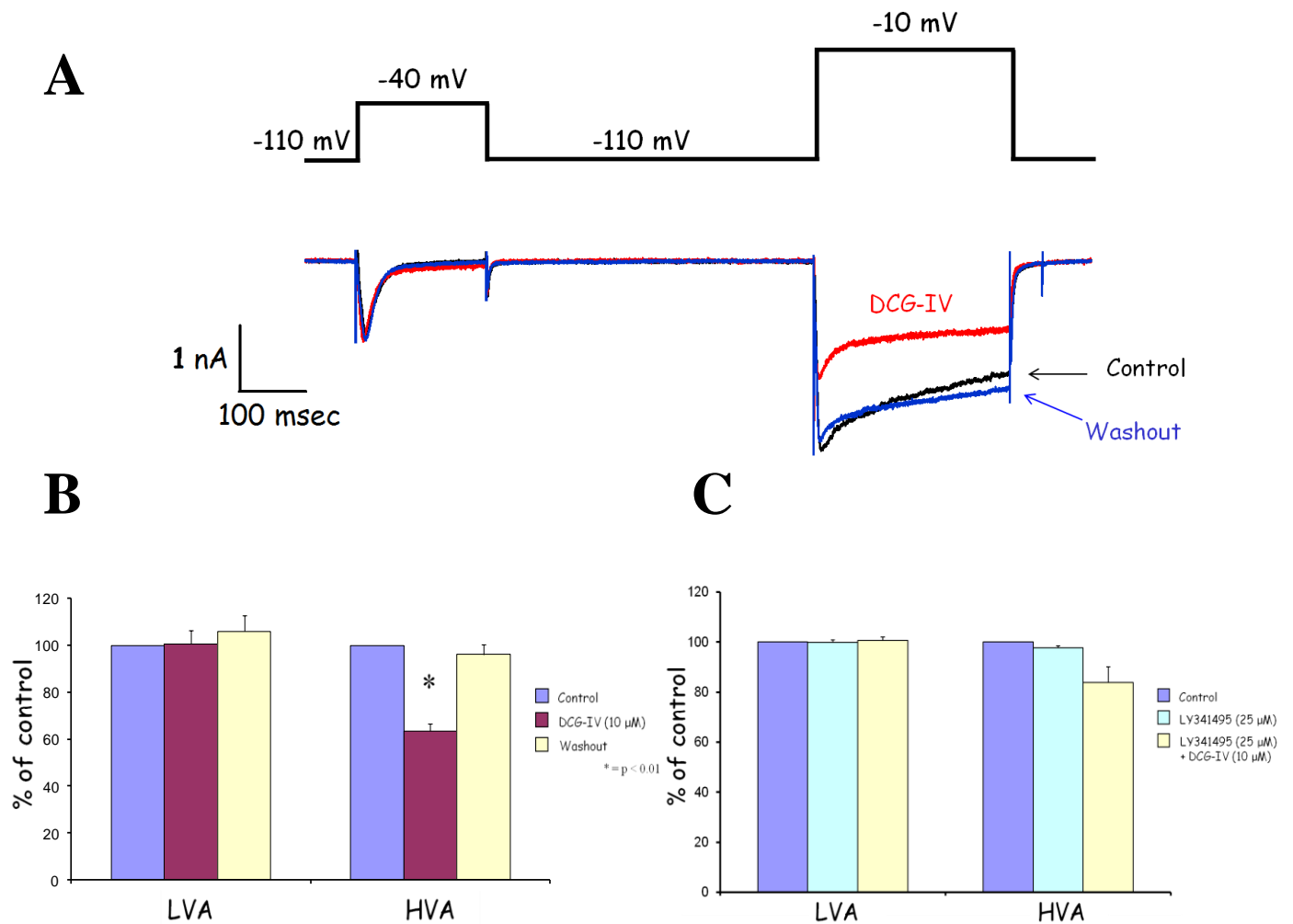
A-C) Representative traces demonstrating that application of AgTx (A), Nifedipine (B) and CgTx (C) reduce the total whole-cell HVA calcium current. D) AgTx, Nifedipine and CgTx significantly reduced the control current by 29.79 ± 1.98 % (n=7), 28.82 ± 2.90 % (n=5) and 36.54 ± 4.57 % (n=4), respectively. Additionally, combination of all blockers (AgTx, CgTx and Nifedipine) significantly reduced the total whole-cell HVA current to 13.51 ± 2.21 % (n=4; p<0.05). All recordings were conducted by using a Cs⁺-methanesulfonate-based internal solution (to block voltage-gated potassium currents) and by perfusing slices with ACSF containing barium (to limit current rundown) and TTX (to block voltage-gated sodium channels).

Figure 9

Effect of Group II mGluR Activation on LVA and HVA Currents

The main objective of this study was to elucidate the effect of Group II mGluR activation in PVT neurons. In most cases throughout the CNS, activation of Group II mGluRs have been linked to inhibition of VGCCs (Swartz and Bean, 1992; Sahara and Westbrook, 1993; Chavis et al., 1994; Rothe et al., 1994; Stefani et al., 1994; Ikeda et al., 1995; Lachica et al., 1995). After properly characterizing both LVA and HVA calcium currents in PVT, the effect of Group II mGluR activation on these ion channels was assessed.

In order to accomplish this analysis, we used that same two-pulse steady-state protocol as described previously. Interestingly, bath application of DCG-IV (10 μ M), a potent Group II mGluR agonist, significantly reduced the HVA current by $36.55 \pm 3.06\%$ of recorded control current (n=8; $p < 0.01$). This inhibition of HVA current was almost completely reversible upon washout of the drug. In contrast, activation of Group II mGluRs had no significant effect on LVA current (n=5) (**Fig. 10A, B**). Subsequently, we attempted to confirm that this inhibition of HVA currents was truly a Group II mGluR-mediated process by pre-incubating the Group II mGluRs antagonist, LY341495, prior to application of the agonist, DCG-IV. Bath application of LY341495 (25 μ M) blocked the DCG-IV effect as the reduction in HVA current was found to be statistically non-significant ($16.26 \pm 6.28\%$ of control current; n=3; $p > 0.05$) (**Fig. 10C**).



Effect of Group II metabotropic glutamate receptor activation on voltage-gated calcium currents in the PVT

A) Representative trace demonstrating that activation of Group II mGluRs by DCG-IV (10 μ M) reversibly inhibits HVA calcium channels without affecting LVA calcium channels. B) Application of DCG-IV significantly reduced the HVA current by $36.55 \pm 3.06\%$ of recorded control current (n=8; $p < 0.01$) without significant effect on LVA current (n=5). C) Pre-incubation of the Group II mGluRs antagonist, LY341495 (25 μ M) blocked the DCG-IV effect as the reduction in HVA current was found to be statistically non-significant ($16.26 \pm 6.28\%$ of control current; n=3; $p > 0.05$). All recordings were conducted by using a Cs^+ -methanesulfonate-based internal solution (to block voltage-gated potassium currents) and by perfusing slices with ACSF containing barium (to limit current rundown) and TTX (to block voltage-gated sodium channels).

Figure 10

The role of HVA calcium channels in tonic firing of PVT neurons

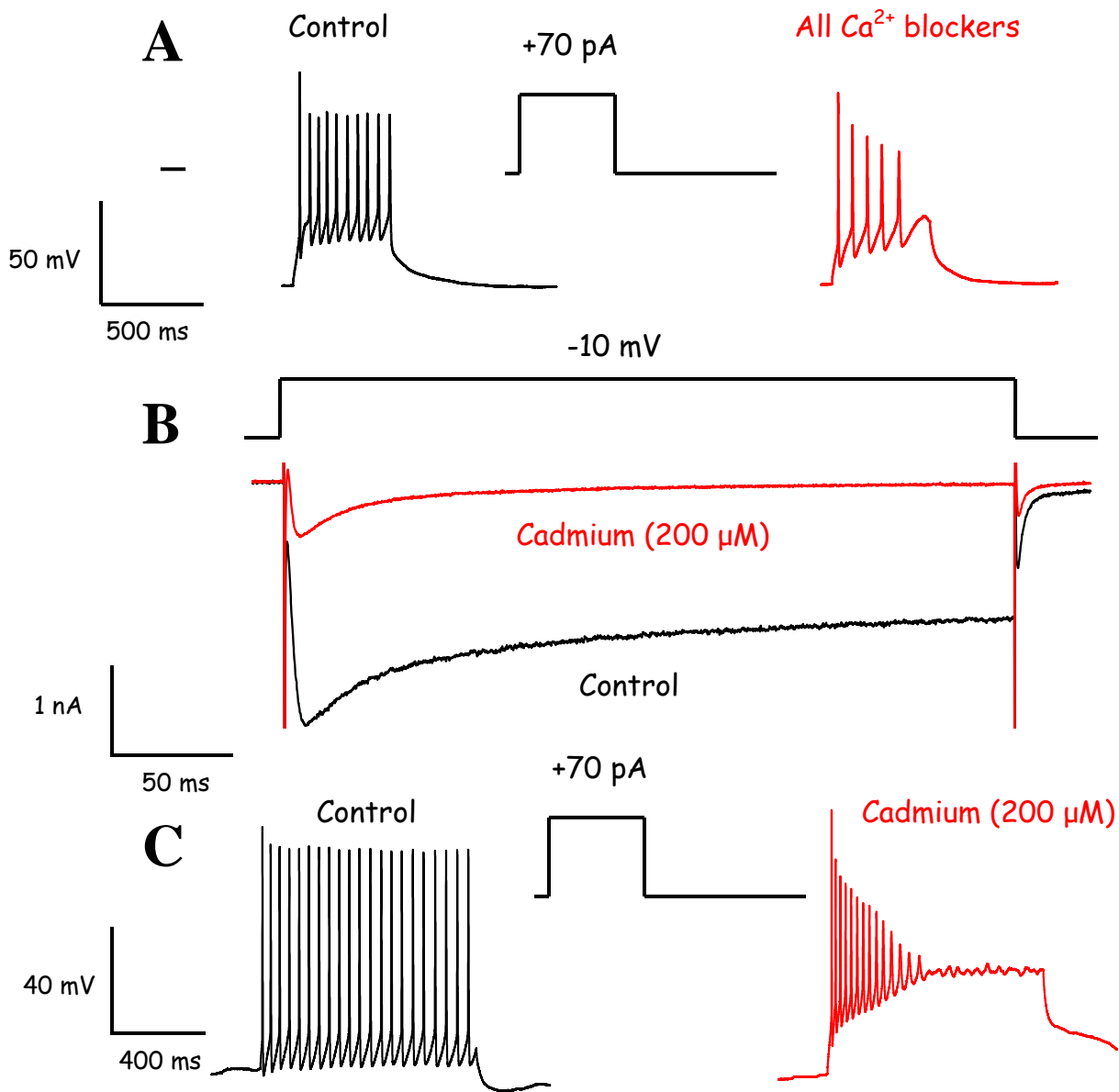
The above results demonstrate that activation of Group II mGluRs results in a reduction of calcium entry through HVA calcium channels. However, the function of HVA calcium channels in PVT neuronal firing is unknown. Theoretically, if calcium influx is reduced upon Group II mGluR activation, a smaller number of calcium-activated potassium channels (SK and BK channels) will be activated which would affect repolarization during tonic firing. Therefore to address this question, the effect of simultaneous blockade of all HVA calcium channel subtypes (P/Q-, N- and L-type) on PVT tonic firing was investigated. Interestingly, application of CgTx (1 μ M), AgTx (200 nM) and nifedipine (10 μ M) disrupted tonic firing elicited by a +70 pA current step. (**Fig. 11A**). Note that the after-spike repolarization level does not remain constant, as observed in control conditions. This might occur as a result of a reduction in the activity of the big conductance calcium-activated potassium channels (BK channels), a major contributor to the repolarizing phase immediately following an action potential (Adams et al., 1982; Lancaster & Nicoll 1987; Shao et al., 1999) (see discussion).

In order to further support the observation that HVA calcium channels are essential to prevent spike failure during tonic firing, the general and non-specific HVA calcium channel blocker cadmium was applied to the external solution. In voltage-clamp mode, application of cadmium (200 μ M) almost completely abolished the HVA current elicited by a depolarizing voltage step to -10 mV (4.20 ± 0.38 % of initial recorded current; n=3). A representative trace is illustrated in figure 11B. Therefore, cadmium was

applied in current-clamp mode to confirm that the results observed with the combination of all specific HVA calcium channel blockers (CgTx, AgTx and nifedipine) was reproducible (**Fig. 11A**). Consistent with those results, application of cadmium (200 μ M) disrupted tonic firing in PVT neurons (**Fig. 11C**). The same effect was observed in all 6 PVT neurons tested with cadmium. In contrast, there was no evident effect on burst firing (data not shown).

Effect of Group II mGluR Activation on resting membrane potential of PVT neurons

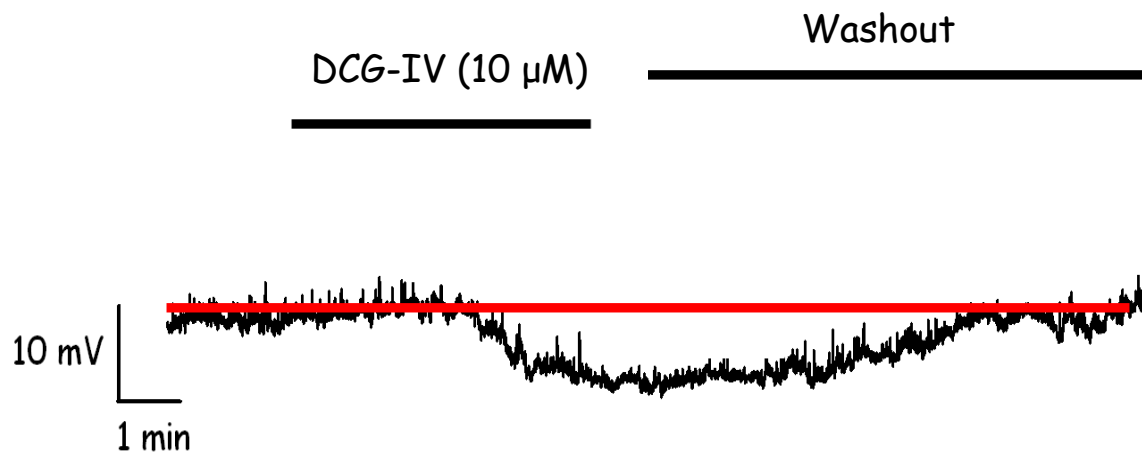
Current-clamp recordings of PVT neurons revealed that activation of Group II mGluRs did not only affect HVA calcium currents but also influences the resting membrane potential of these neurons. Bath application of DCG-IV (10 μ M) caused PVT neurons to hyperpolarize (from -57.39 ± 1.43 to -67.59 ± 1.27 mV; n=8) within 2-3 minutes after application of the agonist. This effect on resting membrane potential was almost completely reversible upon washout (from -67.59 ± 1.27 to -59.38 ± 2.05 mV; n=8) (**Fig. 12**). Although similar responses following activation of Group II mGluRs have been described in several other areas of the mammalian brain, including amygdala (Muly et al., 2007), cerebellum (Knoflach and Kemp, 1998; Watanabe and Nakanishi, 2003), and reticular thalamic nucleus (Cox and Sherman, 1999), this is the first report of this effect in the PVT. This is presumably in part attributable to the high density of Group II mGluRs in this location (Ohishi et al., 1993, 1998; Gu et al., 2008).



Effect of HVA calcium channel block on tonic firing

A) Current-clamp recording demonstrating that application of all three HVA blockers (CgTx, Nifedipine and AgTx) results in disruption of tonic firing in PVT. B) Voltage-clamp recording illustrating that addition of the non-specific HVA channel blocker cadmium (200 μM) to the ACSF inhibits most of the whole-cell HVA current. Current-clamp recording demonstrating the application of cadmium (200 μM) produces a similar effect on tonic firing as observed in A. Current-clamp recordings were conducted by using a K^+ -gluconate-based internal solution and perfusing the slices with normal ACSF (containing Ca^{2+}). Voltage-clamp recordings were conducted by using a Cs^+ -methanesulfonate-based internal solution and by perfusing slices with ACSF containing barium and TTX.

Figure 11



Effect of Group II mGluR activation on burst and tonic firing in the PVT

A) Representative current-clamp trace demonstrating that bath application of DCG-IV (10 μM) caused PVT neurons to hyperpolarize in a reversible manner. All current-clamp recordings using the Group II mGluR agonist, DCG-IV, were conducted by using a K^+ -gluconate-based internal solution and perfusing the slices with normal ACSF (containing Ca^{2+}).

Figure 12

Chapter 4: DISCUSSION

The main objective of this study was to assess the effect of Group II mGluR activation in the PVT. Results from this present study indicate that activation of these receptors reduces calcium entry through HVA calcium channels (**Fig. 10, p. 43**). They have also shown that various subtypes of HVA calcium channels (P/Q-, N-, L-type) comprise the total whole-cell recorded HVA current (**Fig. 9, p. 41**), a current that has been demonstrated to be crucial for maintenance of tonic firing in PVT (**Fig. 11C, p. 46**). Moreover, activation of Group II mGluRs induces PVT neurons to hyperpolarize (**Fig. 12, p. 47**).

Firing Properties of PVT Neurons

Initially, firing properties of PVT neurons were analyzed. Consistent with recordings from other thalamocortical relay neurons (McCormick & Pape, 1990; Leresche et al., 1991; Soltesz et al. 1991; Huguenard, 1998), PVT neurons have two distinct modes of action potential firing: burst and tonic (**Fig. 6A, p. 34**). However, these neurons displayed heterogeneity in the events following burst and tonic firing (**Fig. 6C & D, p. 34**). Immunocytochemistry and tracer studies have revealed that a subpopulation (approximately 60%) of PVT neurons are glutamatergic, whereas other PVT neurons use unidentified transmitters (Hur and Zaborsky, 2005; Huang et al., 2006; Hsu and Price, 2009). Results from this study suggest that, albeit presenting similar morphology, there are important differences in the electrophysiological properties of neurons within the

nucleus. Further investigations will need to be completed in order to elucidate the different types of neurons within PVT.

HVA Calcium Channels in the PVT

Although the identity of the individual subtypes of HVA channels and their relative contributions to the total recorded HVA current have been studied in the thalamus (**Table 4, p. 50**), no such investigation has been conducted in the PVT. Data from this study indicate that the HVA current in the PVT is composed of at least three main components: a nifedipine-sensitive L-current (29%), a CgTx-sensitive N-current (37%) and a AgTx-sensitive P/Q current (30%). The small amount of current remaining in the presence of the all the above blockers ($\approx 5-10\%$) could possibly be attributed to an R current. The effects of CgTx and nifedipine on HVA currents were generally comparable with previous reports on thalamic neurons. However, reported effects of AgTx are more variable (**Table 4, p. 50**). In fact, Guyon and Leresche (1995) found that AgTx (100 nM) had no effect on HVA current in thalamic dorsal lateral geniculate. On the other hand, Kammermeier and Jones (1997) reported a small block of the current in the ventrobasal thalamic nucleus. Nevertheless, the assumption that PVT neurons functionally express that same relative amount of specific calcium channels as other thalamic neurons may not be correct.

To date, no prior investigations have focused on the function of HVA channels in firing of PVT neurons. Calcium entry through HVA calcium channels can activate SK

Localization	Percentage Inhibition of HVA current			Reference
	DHP antagonist	CgTx (conc.)	AgTx (Conc.)	
Whole Thalamus	16 ± 1 (Nif. 50 µM)	32 ± 1 (2.5 µM)		1
Dorsal Lateral Geniculate	25 ± 1 (Nif. 10 µM)	23 ± 1 (20 µM)	No effect (100 nM)	2
Ventrobasal Thalamic Nucleus	15 ± 7 (Nim. 1 µM)	18 ± 2 (0.5 µM)		3
Ventrobasal Thalamic Nucleus	33 ± 1 (Nim. 5 µM)	25 ± 5 (1 µM)	8 ± 3 (100 nM)	4
Paraventricular Thalamic Nucleus	29 ± 3 (Nif. 10 µM)	37 ± 1 (1 µM)	30 ± 2 (200 nM)	This study

Effects of HVA calcium channel blockers on thalamic neurons

Summary of the inhibition values reported in different areas of the thalamus after application of different specific HVA calcium channel blockers. Values are means ± SE. Concentrations are in parentheses. Nif. and Nim. stand for nifedipine and nimodipine, respectively. References 1-4 correspond to (Pfrieger et al., 1992), (Guyon & Leresche, 1995), (Suzuki and Rogawski, 1989) and (Huguenard and Prince, 1992), respectively.

Table 4

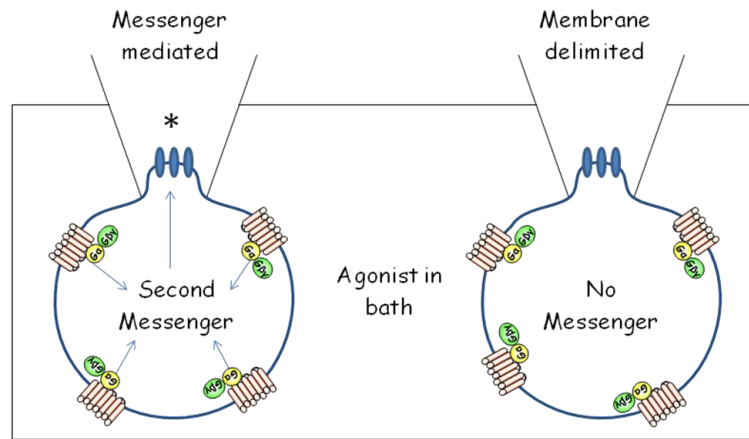
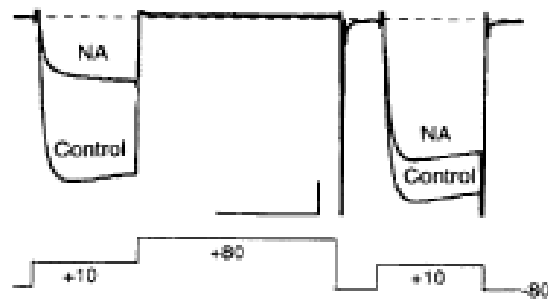
and/or BK channels, triggering a repolarizing mechanism during tonic firing (Adams et al. 1982; Fagni et al., 1991). Therefore, blocking these channels should interfere with the maintenance of tonic firing in PVT neurons. Indeed, complete HVA calcium channel blockade with the broad-spectrum calcium channel blocker cadmium (200 μM) disrupted tonic firing in these neurons. Figure 11C demonstrates that blocking HVA channels affects the repolarization immediately after each spike. This suggests that BK channels might be affected since these calcium-activated potassium channels are known to be responsible for the fAHP which contributes to spike repolarization (Adams et al., 1982, Lancaster and Nicoll, 1987; Sah, 1992). This data does not correspond with what was previously known about the function of HVA calcium channel in TC neurons. In LGN neurons, application of cadmium (200 μM) greatly enhanced neuronal firing. Additionally, application of the BK channel blocker, paxilline (2 μM), produced the same effect (Kasten et al., 2007). Therefore, the role of HVA calcium channels in the tonic firing of PVT neurons seems to differ from other TC neurons. Differences between TC neurons and PVT neurons are not surprising considering the latter's specific role in controlling excitability levels of the limbic system.

Modulation of HVA Calcium Channels by Group II mGluRs in the PVT

In most studies carried throughout the CNS, activation of Group II mGluRs is associated with inhibition of VGCCs (Swartz and Bean, 1992; Sahara and Westbrook, 1993; Chavis et al., 1994; Rothe et al., 1994; Stefani et al., 1994; Ikeda et al., 1995; Lachica et al., 1995). However, the existence of such a coupling mechanism between

Group II mGluRs and VGCCs in the PVT has not yet been identified. Results from this study demonstrate that activating these G-protein receptors inhibits approximately 40% of the total recorded HVA current (**Fig. 10, p. 43**). In most cases throughout the mammalian brain, this effect results from a direct interaction between $G\beta\gamma$ (coupled to GPCRs) and the α_1 subunit of specific HVA calcium channels (reviewed in Hille, 1994; Dolphin, 2003; Tedford & Zamponi, 2006). However, it is still uncertain if this G-protein-mediated, membrane-delimited and voltage-dependent inhibition underlies the observed response in the PVT. One way to answer this issue is to record PVT neurons in the cell-attached patch mode (**Fig. 13A, p. 53**) (Hille, 1994). In this experimental condition, agonist applied in the bath to the entire cell surface is unable to depress the current of the calcium channels isolated in the pipette. On the other hand, inhibition is observed if the receptor agonist is present in the pipette. This indicates that the inhibitory process is very localized and that a soluble second messenger is not involved. On the other hand, the voltage-dependency of this type of modulation can be easily verified by comparing calcium currents elicited by two identical test pulses (+10 mV) separated by a large conditioning depolarization (+80 mV) (**Fig. 13B, p. 53**) (Ikeda, 1996). If this Group II mGluR-mediated effect involves voltage-dependent binding of $G\beta\gamma$ to calcium channels, the large conditioning depolarization should relieve inhibition upon the following test pulse, reflecting $G\beta\gamma$ unbinding.

Consistent with these results, in most studies, it is found that this direct linkage only applies to the HVA family of calcium channels. This differential modulation could possibly be explained by structural differences between channel subfamilies. As expected

A**B**

Experimental procedures for determining if the Group II metabotropic glutamate receptor-mediated effect on high-voltage-activated calcium channels is A) membrane-delimited and B) voltage-dependent

A) With a messenger-mediated mechanism, bath application of the agonist acts on numerous receptors to make a messenger that diffuses to the channels in the patch and modulates their function (asterisk). In contrast, in a membrane-delimited mechanism, there is no diffusible cytoplasmic second messenger, and channels in the patch are unaffected by the agonist in the bath (modified from Hille, 1994). B) G-protein-mediated inhibition of HVA current is greatly relieved after application of a large conditional depolarization (+80 mV) demonstrating voltage-dependent binding of $G\beta\gamma$ (Ikeda, 1996).

Figure 13

from the functional differences, the α_1 subunit of LVA channels are only distantly related to the α_1 subunit of HVA channels (Perez-Reyes, 1998; Perez-Reyes et al., 1998). However, it is important to mention that additional non-voltage-dependent pathways, which may be direct or via down-stream soluble intracellular messengers, also occur in certain cell types and may apply to LVA channels (Robbins et al., 2003; Wolfe et al., 2003; Hildebrand et al., 2009).

This type of G-protein-mediated modulation of HVA channels can be interpreted as an endogenous neuronal protective mechanism. It is absolutely essential for neurons to be endowed with regulatory mechanisms designed to carefully buffer intracellular calcium, especially since it is well known that excessive calcium entry produces deleterious effects and may result in cell death. Excitotoxicity, which usually refers to the death of neurons arising from prolonged exposure to glutamate or from hyperfunction of calcium-permeable NMDA receptors, is a great example. The resulting calcium overload is particularly neurotoxic, leading to activation of enzymes that degrade proteins, membranes and nucleic acid (reviewed in Berliocchi et al., 2005). In this regard, the Group II mGluR-mediated inhibition of HVA channels described in this present study might be viewed as a mechanism by which endogenous glutamate limits damaging levels of intracellular calcium. Noticeably, calcium blockers, as well as agonist at group II mGluRs, strongly attenuated NMDA-related toxicity in cultured cortical and cerebellar granule cells, suggesting that the block of HVA calcium channels may be a target for limiting excitotoxicity (Copani et al., 1995).

Effect of Group II mGluR Activation on Resting Membrane Potential

In current-clamp mode, activating Group II mGluRs caused PVT neurons to hyperpolarize approximately 10 mV within 2-3 minutes of bath application of the Group II mGluR agonist (**Fig. 12, p. 47**). In most cases throughout the CNS, activation of mGluRs by the non-specific mGluR agonist l-amino-cyclopentane-1,3-dicarboxylic acid (ACPD) results in a postsynaptic excitation (Charpak et al., 1990; Mercuri et al., 1993; Eaton & Salt, 1996; Lee & McCormick, 1997). However, several studies have indicated that mGluR activation may also produce a postsynaptic inhibitory response (Shirasaki et al., 1994; Holmes et al., 1996; Fiorillo & Williams, 1998). Moreover, in basolateral amygdala, application of ACPD produced several different responses: either; 1) a membrane hyperpolarization followed by a depolarization; 2) a hyperpolarization; 3) a depolarization; or 4) no response (Holmes et al., 1996). This issue was finally resolved by evidence demonstrating that the polarity of the postsynaptic response (depolarization or hyperpolarization) was dependent on the mGluR subtype (Cox & Sherman, 1999). Activation of Group I mGluRs produced a long-lasting depolarization that usually resulted in action potential discharge, whereas activation of Group II mGluRs induced membrane hyperpolarizations. In both cases, this effect on resting membrane potential appeared to result from modification of a potassium conductance mediated by G protein-regulated inwardly rectifying potassium channels ($K_{ir3.X}$) (Lee & Sherman, 2009).

The observation that the general agonist ACPD produces mixed effects on resting membrane potential throughout the CNS could possibly be explained by differential

distribution of Group I versus Group II mGluRs in different regions of the brain. For instance, the predominant effect of application of ACPD to TC neurons is generally associated with membrane depolarization (McCormick & Krosigk, 1992; Godwin et al., 1996). This might be explained by the fact that TC neurons express high levels of mGluR1 (Shigemoto et al., 1992) and mGluR activity is especially linked to PLC activity in these neurons (Miyata et al., 2003). However, perhaps Group II mGluRs are also activated, but the level of activity is insufficient to be detected by somatic recordings. Interestingly, a recent study has revealed that Group II mGluRs are highly and discretely expressed in cell bodies in almost all of the key regions of the limbic system in the forebrain, including the PVT (Gu et al., 2008), suggesting that Group II mGluRs might play important roles in mood disorders. Concordantly, the first observation that ACPD produced mixed effects on resting membrane potential was obtained from basolateral amygdala, a key component of the limbic system (Holmes et al., 1996). An obvious question is whether endogenous activation of these different mGluR subtypes is specific to particular glutamatergic afferents. An excellent example for such specificity exists in the thalamus. Activation of Group I mGluRs on TC neurons, which induces depolarization of the membrane, appears specific to the corticothalamic pathway and not the retinogeniculate, both of which are glutamatergic (McCormick & Krosigk, 1992; Godwin et al., 1996). However, many questions remained unanswered. For instance, whether individual glutamatergic afferents can activate both Group I and Group II mGluRs or whether these afferents are confined to one subtype is still unclear.

Functional Implications

Compounds that interact with Group II mGluRs have been shown to have anxiolytic and antipsychotic effects in animal models (Swanson et al., 2005; Conn et al., 2009; Niswender and Conn, 2010). The mechanism of action is believed to result from a reduction of excitatory transmission in relevant limbic and forebrain areas, including the amygdala and prefrontal cortex (Lin et al., 2000; Marek et al., 2000; Grueter and Winder, 2005; Muly et al., 2007). Previous studies have demonstrated that the PVT projects to these areas and plays an important role in regulating their excitability levels by providing an important source of glutamatergic drive (Hur and Zaborsky, 2005; Huang et al., 2006; Hsu and Price, 2009). Interestingly, the PVT displays high expression levels of the Group II mGluRs (Ohishi et al., 1993, 1998; Gu et al., 2008). This raises the question that activation of Group II mGluRs in the PVT may be responsible for the effectiveness of Group II mGluR-activating compounds in anxiety and schizophrenia.

Results from this study demonstrate that Group II mGluR activation in the PVT leads to a reduction of calcium entry through HVA calcium channels. They have also shown that these calcium channels are crucial for the maintenance of tonic firing of these neurons. Therefore, we can hypothesize that the inhibitory effect on HVA calcium channels and the resulting disruption of tonic firing will have a great impact on the excitatory drive of PVT neurons to their respective targets in the limbic and forebrain regions. Moreover, activation of these G-protein receptors leads to direct hyperpolarization of PVT neurons. Thus group II mGluR agonists may inhibit PVT

neurons both by diminishing their excitatory drive and directly hyperpolarizing them, an action that may be responsible for the anxiolytic actions of group II mGluR agonists.

Future Developments

The results presented in this study are innovative because they show for the first time a regulatory coupling mechanism between Group II mGluRs and HVA calcium channels in the PVT. However, it is still uncertain if this coupling mechanism is membrane-delimited and voltage-dependent, reflecting direct binding of $G\beta\gamma$ to the calcium channel (**Fig. 13**). Moreover, we still need to investigate if Group II mGluRs act on only one specific subtype of HVA channels or more than one, and how these VGCC subtypes contribute to PVT firing patterns.

Group II mGluR activation also leads to hyperpolarization of PVT neurons. However, it is still unclear how this effect will impact excitability levels of these neurons. Therefore, future current-clamp recordings using the Group II mGluR agonist will help us understand how this effect on resting membrane potential will impact neuronal firing.

Chapter 5: CONCLUSION

Many functional studies have demonstrated that the PVT is possibly involved in many neurological disorders, especially anxiety and stress. Therefore, it is absolutely essential to understand how the PVT receives and integrates different inputs and then transmits the information to its postsynaptic targets. My results demonstrate that Group II mGluRs might play a crucial role in shaping PVT firing patterns by inhibiting calcium entry through HVA channels and/or by hyperpolarizing the membrane. Hence, Group II mGluRs may act as potential targets to treat action potential firing anomalies in the PVT, which possibly underlie some of the neurological disorders mentioned above.

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