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Elucidating the Subunit Identity of GABA_A Receptors
Using Subunit Selective Compounds

By

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A thesis submitted to the Faculty of Graduate Studies and Research, and the
Department of Psychology in partial fulfillment of the requirements for the degree of
Masters of Science Psychology (Neuroscience Specialization)

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Abstract

Cortical GABA\textsubscript{A} receptor mediated inhibitory postsynaptic currents (mIPSCs) occur as two behaviours. One decays with a mono-exponential rate while the other is bi-exponential. Using α subunit selective GABA\textsubscript{A} receptor modulators 1) zolpidem (α\textsubscript{1}), 2) SB-205384 (α\textsubscript{3}), 3) furosemide (α\textsubscript{4}), and 4) L-655,708 (α\textsubscript{5}), we tested the hypothesis that these differences are due to differential α subunit expression. Non-stationary fluctuation analysis showed the channel conductance of mono-exponential mIPSCs were smaller than those in bi-exponential mIPSCs. Zolpidem prolonged deactivation of mono-exponential mIPSCs but not the bi-exponentials. SB-205384 prolonged the bi-exponential mIPSCs but did not affect mono-exponential mIPSCs. Furosemide and L-655,708 reduced the amplitude of both populations but increased the deactivation rate of the bi-exponentials. Additionally, zolpidem and SB-205384 increased holding current while furosemide reduced it, providing evidence of extrasynaptic localization of α\textsubscript{1}, α\textsubscript{3}, and α\textsubscript{4} subunits. We found mono-exponential synapses contain α\textsubscript{4}, α\textsubscript{4} and α\textsubscript{5} subunits while bi-exponential ones contain α\textsubscript{3}, α\textsubscript{4} and α\textsubscript{5}.
Acknowledgements

This work is due in large part to many special people. From the Life Sciences Institute, I’d like to thank Jin Lu and Teresa Fortin, two great people whom I’ve had the pleasure of working with (and pestering with technical questions). Diane Trenouth has been great in helping me with any administrative tasks, big or small, that needed attention. The animal care staff of Anne Hogarth, Collinda Thivierge, and Andrew Mott have been great in helping me with inquiries and issues with the animals. And of course, I’d like to thank Dr. Mike Poulter, who has been a great mentor and supervisor. Outside the lab, I’d like to thank Cecilia Lam and her family and Mom and Kenneth for being supportive. There have been long days, but you all have been wonderful in helping me keep my focus and spirits up.
Table of Contents

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract .......................................................... ii</td>
</tr>
<tr>
<td>Acknowledgements .................................................. iii</td>
</tr>
<tr>
<td>Table of Contents .................................................. iv</td>
</tr>
<tr>
<td>Page .......................................................... iv</td>
</tr>
<tr>
<td>List of Tables .................................................. vi</td>
</tr>
<tr>
<td>List of Figures .................................................. vii</td>
</tr>
<tr>
<td>Overview .......................................................... 1</td>
</tr>
<tr>
<td>Background .......................................................... 2</td>
</tr>
<tr>
<td>Functional Properties of GABA_{A} Receptors Conferred by Specific α Subunits ................................................. 6</td>
</tr>
<tr>
<td>Kinetic Properties of GABA_{A} Receptors .......................................................... 13</td>
</tr>
<tr>
<td>Synaptic and Non-synaptic Transmission .......................................................... 16</td>
</tr>
<tr>
<td>Methods .......................................................... 25</td>
</tr>
<tr>
<td>Culture methods .................................................. 25</td>
</tr>
<tr>
<td>Solutions .......................................................... 25</td>
</tr>
<tr>
<td>Electrophysiology .................................................. 26</td>
</tr>
<tr>
<td>Data analysis .......................................................... 26</td>
</tr>
<tr>
<td>Statistical Analysis .................................................. 27</td>
</tr>
<tr>
<td>Results .......................................................... 29</td>
</tr>
<tr>
<td>Peak scaled non-stationary noise analysis reveals different unitary currents for mono- and biphasic synapses .......................................................... 29</td>
</tr>
</tbody>
</table>
Pharmacological studies reveal both similarities and differences in sensitivities to various α subunit selective compounds ................................................................. 30

Effects of zolpidem on mIPSCs at high and low concentrations ........................................ 30

The α1 subunit selective compound SB-205384 increased the slow decay time constant
of the bi-exponential mIPSCs ...................................................................................... 35

The α4/6 subunit selective compound furosemide effects on mIPSCs ......................... 37

The α5 subunit selective compound L-655,708 decreased both peak amplitude and
decay time constant in mono-exponential mIPSCs and fast decay time constant and
peak amplitude in bi-exponential mIPSCs .................................................................. 38

Pregnenolone sulphate (PS) reduced peak amplitude in both mono- and bi-exponential
mIPSCs ......................................................................................................................... 39

Tonic baseline experiments suggest an extrasynaptic localization of the α1, α5, and α4
subunits ....................................................................................................................... 40

Non-stationary noise analysis: .................................................................................. 46

Pharmacology: .......................................................................................................... 47

Effect of 0.5, 1.0, and 10 μM zolpidem ....................................................................... 47

Effect of 5 μM SB-205384 ....................................................................................... 48

Effect of 100 μM Furosemide .................................................................................... 49

Effect of 5 μM L-655,708 ......................................................................................... 50

Effect of 10 μM pregnenolone sulphate (PS) ............................................................ 50

Conclusions: .............................................................................................................. 51

References: .............................................................................................................. 52
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summary of pharmacological effects on mono-exponential mIPSCs</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>Summary of pharmacological effects on bi-exponential mIPSCs</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>Summary of effects on mIPSCs</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>Summary of synaptic and extrasynaptic α subunit localization</td>
<td>45</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1 - The unitary currents for mono- and bi-exponential mIPSCs differ. ........30

Figure 2 – The effects of 0.5 µM zolpidem on decay time constant and peak amplitude of averaged mono-exponential mIPSCs. ........32

Figure 3 – The effects of 0.5 µM zolpidem on decay time constants and peak amplitude of averaged bi-exponential mIPSCs ........33

Figure 4 - The effects of 1.0 µM zolpidem on decay time constant and peak amplitude of averaged mono-exponential mIPSCs. ........33

Figure 5 - The effects of 1 µM zolpidem on decay time constants and peak amplitude of averaged bi-exponentials mIPSCs. ........34

Figure 6 - The effects of 10 µM zolpidem on decay time constant and peak amplitude of averaged mono-exponential mIPSCs. ........35

Figure 7 - The effects of 10 µM zolpidem on decay time constants and peak amplitude of averaged bi-exponential mIPSCs. ........36

Figure 8 - The effects of 5 µM SB-205384 on averaged mono- and bi-exponential mIPSCs. ........37

Figure 9 - The effects of 100 µM furosemide on averaged mono- and bi-exponential mIPSCs. ........38

Figure 10 - The effects of 5 µM L-655,708 on averaged mono- and bi-exponential mIPSCs. ........39

Figure 11 - The effects of 10 µM PS on averaged mono- and bi-exponential mIPSCs. ........40
**Figure 12** - The effects of 1 μM zolpidem, 5 μM SB-205384, and 100 μM furosemide on holding current.
Overview:

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the central nervous system (CNS) and its importance is reflected by the widespread distribution of its receptors throughout the brain (Mortensen et al., 2004). An estimated 20-50% of all central synapses are GABAergic (Sieghart, 1995). Prior to 1987 when the first GABA_A receptor subunits cDNAs were sequenced and cloned, the prevailing notion was that there were two receptor subtypes in the brain based on benzodiazepine binding studies (Whiting, 1999). Today, there appears to be enormous heterogeneity that results from the various building blocks since many GABA_A receptors can form from subunits belonging to any of the α, β, γ, δ, θ, π, ε, and ρ families.

Pharmacological modulation of GABA_A receptors causes a wide range of behavioural effects including changes in motor activity, muscle tone, seizure genesis, sedation, hypnosis, and anxiety (Collins et al., 2002). Clinically relevant compounds such as benzodiazepines (BZDs), barbiturates, steroids, anaesthetics, and anti-convulsants all modulate GABA to varying extents (Sieghart, 1995). Disturbances in GABAergic transmission have been implicated in various neurological and psychiatric disturbances (Kristiansen and Lambert, 1996). It has been shown that impaired GABAergic function contributes at least in part to epilepsy (Brooks-Kayal et al., 1998).

Previously, miniature inhibitory postsynaptic currents (mIPSCs) were classified into either mono- or bi-exponential groups (Hutcheon et al., 2000). The existence of two kinetically different mIPSCs suggests different receptor subtypes mediate these differences. Using subunit selective compounds, we attempt to determine whether specific subunits are included/excluded from these potentially different receptors. The
following subunit selective compounds were used: zolpidem ($\alpha_1$), SB-205384 ($\alpha_3$), furosemide ($\alpha_4/6$), and L-655,708 ($\alpha_5$). Additionally, the pharmacology of the neuroactive steroid pregnenolone sulphate (PS) was examined to determine if it selectively modulates synaptic activity. Using these compounds, I wish to explore the hypothesis that these kinetically distinct synaptic behaviours arise from differing GABA$_A$ receptors constructed from various $\alpha$ subunits.

**Background:**

There are 3 types of GABA receptors: GABA$_A$, GABA$_B$, and GABA$_C$. GABA$_A$ and GABA$_C$ receptors belong to a superfamily of ligand-gated ion channels and respond to the presence of GABA by opening an intrinsic anion channel (Luddens et al., 1995). The majority of GABA physiological actions are produced through GABA$_A$ receptors (Sieg hart et al., 1999).

GABA$_A$ receptors are the most extensively studied as they are the target of many clinically relevant drugs (Chou, 2004). Its main function in the mature CNS is to inhibit neuronal firing by increasing chloride (Cl$^-$) conductance (Cherubini et al., 1991). The benzodiazepines (BZDs) are anxiolytics whose efficacy impinges on modulation of GABA$_A$ receptor kinetics (Jones & Westbrook, 1995). Conversely, GABA$_A$ receptors antagonists such as bicuculline give rise to epileptic activity (Cherubini et al., 1991).

GABA$_B$ receptors are G-protein coupled receptors that have not been studied as thoroughly, perhaps due to their BZD/barbiturate/bicuculline insensitivity (Zorumski & Isenberg, 1991). They inhibit neuronal activity primarily by inhibiting transmitter release and inducing a hyperpolarising potassium current.
GABA<sub>C</sub> receptors are similar to GABA<sub>A</sub> in that they are also ligand-gated chloride channels but they are bicuculline- and baclofen-insensitive and unaffected by BDZs/pentobarbitol and steroids (Sieghart, 1995; Martinez-Torres et al., 2000). GABA<sub>C</sub> receptors are formed from <i>p</i> subunits and they display little desensitization (Martinez-Torres et al., 2000). While the function of these receptors is unknown, the apparent evolutionary conservation of these receptors across different animals implies an important role (Martinez-Torres et al., 2000). Such receptors are found primarily in the retina (Martinez-Torres et al., 2000), spinal cord, and dorsal root ganglia (Zheng et al., 2003).

GABA<sub>A</sub> receptors are heteropentameric and involve at least 15 protein subunits (Sieghart, 1995). The subunits are divided into several classes: α, β, γ, δ, θ, π, and ε. The various subunits share a common structural motif consisting of four putative transmembrane segments (M1-M4), a large NH<sub>2</sub>-term signal peptide featuring a disulfide-bonded β structural loop and a long variable intracellular domain between M3 and M4 (Saxena and Macdonald, 1994). This allows for great diversity in structural composition with the number of subunit permutations in the thousands (Sieghart, 1995). However, the actual number of combinations is much lower due to regional separation imposed by the brain as well as the failure of subunits to aggregate and form functional receptors (Davies et al., 2001). αβγ and αβδ combinations are believed to be the prominent isoforms (McKernan and Whiting, 1996). The relevance of this heterogeneity can be seen on several levels, affecting maximum single-channel conductance, BZD pharmacology, and desensitization kinetics. The role of component subunits in GABA receptor function emerged from functional studies of recombinant receptors. Expression
systems provided evidence subunits assembled into various combinations, producing ion channels with distinct electrophysiological and pharmacological profiles (Angelotti et al., 1993).

*In situ* hybridization studies have localized subunit distributions of mRNA transcripts *in vivo*, allowing investigation of the structure and function relationships of native receptor channels (Serafini et al., 1995).

The use of *Xenopus* oocytes allowed investigators to observe the GABA<sub>A</sub> receptor subunit stoichiometry and subunit functions. Injections of single subunits gave rise to homomeric receptors; in only a few cases only high GABA concentrations activate these BZD insensitive receptors (Sieghart, 1995). Furthermore, GABA<sub>A</sub> homomeric receptors comprised of α, β, γ, δ, π, or ε have not been identified natively (Sieghart, 1999). Only ρ has been shown to form homomeric receptors (Sieghart, 1999). Paired subunit combinations formed receptors more efficiently and required lower activating GABA concentrations (Sieghart, 1995). Injections of three subunits gave receptors that exhibited large GABA-gated Cl<sup>−</sup> currents that were inhibited by bicuculline and picrotoxin (Sieghart, 1995). These triple subunit receptors closely resembled the GABA<sub>A</sub> receptors in the brain (Sieghart, 1995). Current knowledge posits native GABA<sub>A</sub> receptors contain 2α, 2β, and a γ subunit although 2α, β, 2γ and α, 2β, 2γ are also possible (McKernan and Whiting, 1996). The amino acid homology among different subunit classes is 30-40% while intragroup homology is 60-80% (Sieghart, 1995).

Early pharmacological and biochemical data suggested the existence of GABA<sub>A</sub> receptor subtypes, but the full diversity was not fully appreciated until the advent of molecular cloning (Luddens et al., 1995).
Embryonic and early postnatal cortex express α₂, 3, 5 and β₃ subunits mRNA as early as E15 (Laurie et al., 1992; Poulter et al., 1992). In particular, the α₅ subunit expression is high at E19, especially in the hippocampus and thalamus (Poulter et al., 1992). The expression profile of α₁, α₄, β₂, and δ subunits increases with age (Laurie et al., 1992). α₂, 5, β₃, and γ₂ subunits appear in the hippocampus (Laurie et al., 1992; Poulter et al., 1993). The expression of γ₁ and γ₃ subunits peaks high early and decreases throughout development (Laurie et al., 1992). In contrast, γ₂ expression is widespread and remains constant with age (Laurie et al., 1992; Poulter et al., 1993). These changes in GABAₐ subunit mRNA expression suggest a rationale for receptor combinations at each age (Laurie et al., 1992). In particular, the change from perinatal subunits (α₂, α₃, α₅, β₃) to adult (α₁, α₄, β₂, δ) may have a temporal profile synchronized with the change of GABA function from an excitatory to inhibitory neurotransmitter. Mature synapse formation occurs in the 3rd and 4th postnatal weeks in rats (Laurie et al., 1992).

The regulation of functional properties by GABA and its analogues differ dramatically among subunits (Luddens et al., 1995). The 53 kDa α subunit (Olsen & Tobin, 1990) appears involved in channel activation by influencing the affinity of GABA activation sites and reportedly affecting the affinity for GABA gating (Levitan et al., 1988). The α subunit is also the determinant of the BZD pharmacology, with α₁ containing receptors of Type I nature and α₂, 3, 5 being Type II. BZD Type I receptors are abundant in the cerebellum while BZD Type II receptors are strongly expressed in hippocampus; both are expressed to the same degree in the cortical layers (Luddens et al., 1995). While the presence of at least one α subunit is required for induction of large
GABA-gated ion currents, it is not required to observe GABA activated currents (Sigel et al., 1990).

Functional Properties of GABA<sub>A</sub> Receptors Conferred by Specific α Subunits:

The α<sub>1</sub> subunit is the most widely expressed α subunit, with expression increasing during development (McKernan and Whiting, 1996; Laurie et al., 1992; Poulter et al., 1992). It is abundant in the cerebral cortex, thalamus, and cerebellum of adult rat (Fritschy and Mohler, 1995). Various functional assays have assessed the contribution of α<sub>1</sub>. Co-expression of mutated α<sub>1</sub> with β<sub>3</sub>γ<sub>2L</sub> in HEK-293 cells revealed a number of kinetic changes that were α<sub>1</sub> dependent (Fisher, 2004). GABA sensitivity was significantly reduced (EC<sub>50</sub> 419 μM mutant vs. 14.1 μM control) and the peak current was significantly smaller (90 pA mutant vs. 818 pA control, Fisher 2004). Desensitization was also affected as α<sub>1</sub> mutants displayed a slower mono-exponential time course of decay as opposed to the bi-exponential time course seen in controls (Fisher, 2004). Single-channel conductance was not affected in the mutants (Fisher, 2004). Behavioural assays using α<sub>1</sub> KOs have shown the sedative and motor effects of diazepam are mediated by the α<sub>1</sub> subunit (McKernan et al., 2000). The α<sub>1</sub> subunit may be sensitive to epilepsy induced changes. In a pilocarpine-induced model of epilepsy, the α<sub>1</sub> subunit significantly decreased in seizure prone rats and this effect precluded manifestation of spontaneous seizures by up to two weeks (Brooks-Kayal et al., 1998). Expectedly, zolpidem, an α<sub>1</sub> subunit selective compound, failed to potentiate GABA currents (Brooks-Kayal et al., 1998). The importance of the α<sub>1</sub> subunit is underscored by the fact its mRNA encodes
the most prevalent and ubiquitous GABA_A receptor subunit in rat brain (Luddens et al., 1995).

α_2 containing receptors display slower deactivation kinetics and faster channel openings compared to α_1 containing receptors (McClellan and Twyman, 1999). Nusser et al. (1996) demonstrated hippocampal expression of α_2 was abundant at the axon initial segment, but much less common in somatic and dendritic synapses (Nusser et al., 1996), suggesting α_2 containing receptors may mediate on/off signalling (Rudolph and Mohler, 2004).

The α_3 subunit appears to reduce the activating site affinity in experiments replacing α_1 with α_3 in α_1β_2γ_2 combination (Gingrich et al., 1995). In adult rats, α_3 is abundant in the amygdala (Fritschy and Mohler, 1995).

α_4 subunit containing receptors are diazepam-insensitive and abundantly expressed in hippocampus, striatum, cerebral cortex, thalamus, and olfactory bulb. Following pilocarpine-induced seizures, α_4 subunit expression increases (Brooks-Kayal et al., 1998). A relationship appears to exist between the steroid progesterone and the α_4 subunit (Smith et al., 1998). In a progesterone withdrawal paradigm, progesterone withdrawal was followed by insensitivity to BZD sedatives (Smith et al., 1998). As expected, α_4 transcription increased due to reduced levels of the progesterone derivative 3α, 5α-THP (Smith et al., 1998).

The α_5 subunit appears to be involved in mediating tonic inhibition (Caraiscos et al., 2004). While it comprises <5% of total GABA_A receptors globally, its expression is high in the hippocampus, accounting for >20% of GABA_A receptors (Caraiscos et al., 2004). α_5 has low expression levels in the cerebral cortex. It has been shown to localize
at extrasynaptic sites on pyramidal neurons in CA1 and CA3 regions of hippocampus (Caraiscos et al., 2004). The \( \alpha_5 \) subunit appears to play a role in cognition as \( \alpha_5 \) KO mice demonstrate improved performance in the water maze (Caraiscos et al., 2004; Whiting, 2003).

\( \alpha_6 \) subunit expression is restricted to cerebellar granule cells (Luddens et al., 1995; Mody, 2001). \( \mathrm{GABA}_A \) receptors containing \( \alpha_6 \) have a higher affinity for GABA than receptors containing the more ubiquitous \( \alpha_1 \) subunit. \( \alpha_{4,6} \) subunit containing receptors are insensitive to classical BZDs. However, \( \alpha_{4,6} \) containing receptors bind other BZD ligands with high affinity including the inverse agonist Ro15-4513 and the partial agonist bretazenil (Benke et al., 1996). The BZD insensitivity in these \( \alpha_{4,6} \) containing receptors lies in an arginine residue that is a histidine in other \( \alpha \) family members (Wieland et al., 1992).

The 56 kDa \( \beta \) subunit (Olsen & Tobin, 1990) binds GABA agonists at the receptor sites that trigger channel activation (Gingrich et al., 1995). \( \beta_1 \) subunits can form homomeric channels that are open in the absence of GABA (Sigel et al., 1990). The \( \beta_1 \) subunit (but not \( \beta_2 \)) is required for picrotoxin sensitivity (Sigel et al., 1990). During synaptogenesis and differentiation, \( \beta_2,3 \) subunits are abundantly expressed, but expression becomes region specific upon CNS maturation (Poulter et al., 1993). \( \beta_2,3 \) expression in adult rat is abundant in olfactory bulb, cerebral cortex, hippocampus, amygdala, basal ganglia, thalamus, and cerebellum (Fritschy and Mohler, 1995). While, the \( \beta \) subunit is not involved in BZD pharmacology (Collins et al., 2002), it appears to affect BZD efficacy as replacement of \( \beta_2 \) with \( \beta_1 \) led to diminished diazepam potentiation (Sigel et al., 1990).
γ₂ is essential for modulation of GABA channels by drugs acting at the BZD binding site (Sigel et al., 1990). When co-expressed with α and β subunits, the resulting GABAₐ channels consistently respond to BZD ligands (Luddens et al., 1995). Expression of α₁β₁γ₂ combination in HEK-293 cells led to full sensitivity to BZD receptor ligands (Luddens et al., 1995). γ₂ is not required for steroid sensitivity as demonstrated by 3α, 5α-THPROG, which is more effective at α₁β₁ than α₁β₁γ₂ (Lambert et al., 2003). The γ₂ subunit is the dominant γ subtype (Collins et al., 2002). γ₁ is localized to glia and does not support BZD pharmacology (DeFazio and Hablitz, 1998). Indeed, γ₁ containing receptors are of comparatively low abundance compared to γ₂ (Benke et al., 1996). γ₃ containing receptors show high affinity for the BZD antagonist Ro15-1788 and Ro15-4513 and reduced affinity for BZDs (Sieghart, 1995); they also have high affinity for Cl-218,872 (DeFazio and Hablitz, 1998). Recent studies in epilepsy have identified genetic mutations in the γ₂ subunit as having distinct roles in pathogenesis/maintenance (Macdonald et al., 2003; Wallace et al., 2001; Baulac et al., 2001). Xenopus oocytes were injected with one of two γ₂ mutations, R43Q or K289M. The R43Q mutation abolished GABAergic current enhancement with diazepam (Wallace et al., 2001) while K289M mutants had generally smaller GABAergic peak current amplitude (Wallace et al., 2001; Baulac et al., 2001). Wallace et al. suggested the lack of diazepam effect in R43Q mutants may be explained by an endogenous BZD ligand, but this effect has not been corroborated by other groups (Macdonald et al., 2003; Bianchi et al., 2002). Interestingly, single-channel conductance was not affected by either mutation (Bianchi et al., 2002). Lorez et al. (2000) demonstrated γ₂ KO mice had lower single-channel conductance states compared to wildtype (12 pS vs. 28 pS respectively). In addition,
these KOs were highly sensitive to zinc (Lorez et al., 2000). Indeed, recombinant studies have shown zinc potently inhibits $\alpha\beta$ subunits more than $\alpha\beta\gamma$ (DeFazio and Hablitz, 1998).

The $\delta$ subunit expression is restricted to specific regions, including thalamic relay neurons, cerebellar granule neurons (Fritschy and Mohler, 1995) and the dentate gyrus (Haas and Macdonald, 1999). It appears to play a role in steroid sensitivity (Lambert et al., 2003). The $\delta$ subunit colocalizes with $\alpha_1$, $\alpha_6$, $\beta_1$ and $\gamma_2$ subunits in the cerebellum and $\alpha_1$, $\beta_1$, $\gamma_{2L}$ in hippocampal dentate gyrus cells (Saxena and Macdonald, 1994). When the $\gamma$ subunit is replaced with $\delta$, steroid sensitivity increases (Lambert et al., 2003) as does zinc sensitivity (Brooks-Kayal, 1998). That $\delta$ subunit containing receptors are sensitive to zinc, but insensitive to BZDs, suggests an exclusion of the $\gamma_2$ subunit that confers BZD sensitivity (Mody, 2001). Saxena and Macdonald (1994) showed the $\delta$ subunit could incorporate into an established tertiary subunit combination. Zinc sensitivity was still observed despite the presence of the $\gamma$ subunit (Saxena and Macdonald, 1994). The $\alpha_1\beta_1\delta$ combination was blocked by zinc; $\alpha_1\beta_1\gamma_{2L}\delta$ displayed zinc sensitivity despite the presence of $\gamma$ subunit (Saxena and Macdonald, 1994). Brooks-Kayal et al. (1998) demonstrated zinc sensitivity increased in seizure prone rats. Appropriately, increases in $\delta$ subunit expression were found (Brooks-Kayal et al., 1998). In addition, in vivo studies using $\delta$ knockouts show blunted effects to anti-convulsant and anaesthetic properties of pregnane steroids as well as the appearance of spontaneous seizures (Lambert et al., 2003; Haas and Macdonald, 1999). The $\delta$ subunit may be involved in the receptor subtype that mediates the tonic inhibition (Yeung et al., 2003). The incorporation of the $\delta$ subunit into $\alpha_1\beta_1$ combinations significantly reduces desensitization of GABA-induced
responses (Saxena and Macdonald, 1994). The $\alpha_1\beta_1\delta$ combination was not enhanced by diazepam whereas $\alpha_1\beta_1\gamma_2\lambda$ and $\alpha_1\beta_1\gamma_2\delta$ showed diazepam enhancement (Saxena and Macdonald, 1994).

$\varepsilon$ subunits are most related to the $\gamma$ subunit with 38-43% identical residues between them (Davies et al., 1997). $\varepsilon$ subunit expression is abundant in the amygdala, hypothalamic and hippocampal regions (Davies et al., 1997). $\varepsilon$ subunits do not appear to form homomeric GABAergic channels (Davies et al., 1997). Receptors incorporating the $\varepsilon$ subunit are reportedly constitutively active in addition to being BZD insensitive (Lambert et al., 2003). Neelands et al. (1999) demonstrated $\alpha_1\beta_1$ combined with $\varepsilon$ to form spontaneously active GABA gated channels. These recordings displayed a large holding current and noisy baseline (Neelands et al., 1999). Davies et al. (2001) found transfections using stably expressed $\alpha_1\beta_3\gamma_2$ with $\varepsilon$ displayed leak currents. In addition, reductions in peak amplitude were observed but no concomitant changes occurred in EC$_{50}$ (Davies et al., 2001). The potentiating effects of propofol and flunitrazepam are reduced in $\varepsilon$ carrying receptors (Davies et al., 2001). While it is possible to have quarternary GABA subunit receptors, another scenario is the $\varepsilon$ subunit competing with the $\gamma$ subunit for receptor occupancy (Davies et al., 2001). $\varepsilon$ subunit containing receptors can be enhanced by 5$\alpha$-pregnan-3$\alpha$-ol-20-one (Neelands et al., 1999). Davies et al. (1997) showed $\varepsilon$ containing receptors were insensitive to anaesthetic potentiation, but retained sensitivity to direct activation. This suggests the anaesthetic activation site differs from the potentiating site (Davies et al., 1997).

The $\rho$ subunits are expressed in the retina where they form bicuculline-insensitive GABA$_C$ receptors. The $\rho_1$ and $\rho_2$ subunits are expressed at similar levels. While able to
form homomeric receptors, ρ does not assemble with α₁, β₁, γ₂ subunits (Enz and Cutting, 1998).

The use of recombinant receptors, while invaluable, must be interpreted with care. These expression systems may not fully predict the properties of native GABA<sub>A</sub> receptors as the latter express multiple subunits simultaneously (Brooks-Kayal et al., 1999). Information regarding coordinated subunit expression is comparatively sparse. Brooks-Kayal et al. (1999) identified the coordinated expression of α₄β₂δ in epileptic human hippocampal dentate gyrus cells and this was consistent with data collected in control and epileptic rats. As well, α₃, β₂, and ε mRNA also displayed coordinated expression (Brooks-Kayal et al., 1999). That these effects occurred in both epileptic and control rats eliminates epilepsy as a causative factor (Brooks-Kayal et al., 1999).

In the adult CNS, it is believed the majority of GABA<sub>A</sub> receptors contain the α₁ subunit while α₂–6 subunits are expressed in a more region-specific manner (Poulter et al., 1997). The most prevalent subunit combination in rat brain is α₁β₂γ₂ with a relative abundance of 43% in rat brain (McKernan and Whiting, 1996). α₂β₂γ₂ and α₃β₃γ₂/γ₃ have a relative abundance of approximately 18% (McKernan and Whiting, 1996). α₅β₃γ₂/γ₃ makes up <5% of GABA<sub>A</sub> receptors and is localized to hippocampal pyramidal neurons (McKernan and Whiting, 1996). α₆βγ₂ and α₆βδ are expressed at <2% and are present in the cerebellar granule cells (McKernan and Whiting, 1996) while α₄βδ is present in thalamus and hippocampal dentate gyrus (McKernan and Whiting, 1996).

Alterations in channel properties can lead to developmental changes in response to neurotransmitters and in some cases permit regulation of Ca²⁺ influx (Spitzer, 1991). This may be mediated by altered subunit composition (Spitzer, 1991).
Kinetic Properties of GABA<sub>A</sub> Receptors:

Based on the studies of recombinant and native receptors, it is now generally accepted that the time course of inhibitory transmission is largely governed by the biophysical properties of the receptor. During synaptic transmission, GABA appears only briefly in the synaptic cleft. It rises to a concentration of 0.5-3.0 mM in 20-50 μs (Maconochie <i>et al.</i>, 1994) and then re-distributes and diffuses away with a time constant of 2 ms. The synaptic cleft is approximately 50 nm, which ensures the transmitter reaches its postsynaptic targets at a high concentration (Isaacson, 2000). The lifetime of the transmitter in the cleft is dependent on the combination of diffusion of the molecules away from the postsynaptic receptor and the reuptake mechanisms (Isaacson, 2000).

GABA<sub>A</sub> receptor mediated mIPSCs usually activate and deactivate with time constants of 50-100 μs and 5-60 ms, respectively. While the fast rising and saturating wave of GABA guarantees activation, the decay of transmitter from the cleft does not account for the much slower deactivation of mIPSCs (Jones and Westbrook, 1995). GABA<sub>A</sub> mediated mIPSCs have been shown to decay with either a mono- or bi-exponential time course (Galarreta & Hestrin, 1997). Hutcheon <i>et al.</i> (2000) quantified this relationship and found 63% of mIPSCs were fit by a mono-exponential time course.

The role of decay is important for determining the temporal profile of hyperpolarization in response to excitation (Jones & Westbrook, 1995). Receptor occupancy and desensitization effects are influential on the amplitude and time course of the postsynaptic response (Clements, 1996). In turn, the binding, activation, and extent of desensitization are determined by the peak concentration and timing of transmitter
clearance from the synaptic cleft in response to synaptic activation (Clements, 1996). It has also been consistently shown mIPSCs recorded from various central neurons show large variations in amplitude due to differences in vesicle size (Frerking et al., 1995). The lengthening of the deactivating time course is central to the therapeutic and sedative effects of BZDs and general anaesthetics (Jones & Westbrook, 1995).

The outside-out patch technique has been used to compare decay kinetics currents in excised membrane patches with mIPSCs (Jones & Westbrook, 1995; Galarreta & Hestrin, 1997). These studies have shown the bi-exponential decay of IPSCs is likely due to the rapid entry and exit of GABA_A receptors through desensitized states (Jones and Westbrook, 1995; Galarreta and Hestrin, 1997).

Desensitization is an important mechanism in maintaining a physiological balance of excitation and inhibition (Frosch et al., 1992). Arguably, desensitization may have an important role in developing neurons at a time when various changes occur. While in adults, GABA is the main inhibitory neurotransmitter, in immature neurons, it acts as an excitatory neurotransmitter (Cherubini et al., 1991). It is during the early neonatal period GABA provides the excitatory drive while glutamatergic synapses are quiescent (Cherubini et al., 1991). The importance of this role is underscored by the fact GABAergic synaptic connections form in advance of glutamatergic counterparts (Ben-Ari et al., 1997). Intracellular experiments confirm the predominant form of synaptic activity is excitatory in immature neurons (Cherubini et al., 1991). Neonatal neurons have two defining characteristics that differentiate them from adultsː first, GABAergic currents in neonatal cells display little desensitization (Cherubini et al., 1991). Second, immature GABAergic mediated currents are potentiated by barbiturates but are
insensitive to BZDs (Cherubini et al., 1991). In assessing the role of desensitization in developing neurons, GABA_A receptor development was classed into 3 periods: 1-4 days \textit{in vitro} (DIV), where few GABA_A-ergic synapses are present; 7-10 DIV, where synaptogenesis begins; 14-17 DIV, where rapid synaptogenesis occurs (Hutcheon et al., 2000). In outside-out patches, older neurons displayed a fast component of desensitization not seen in younger cultures (Hutcheon et al., 2000). This was mirrored in patches where the fast component was responsible for shaping the initial phase of deactivation (Hutcheon et al., 2000). The late onset of this fast component may result from a specific \textit{\alpha} subunit combination being upregulated (Hutcheon et al., 2000).

There are clear developmental changes in mIPSC properties over time. mIPSC frequency increases notably with development, which may represent an increase in innervation by inhibitory interneurons (Cohen et al., 2000). mIPSC responses from postnatal day 1-14 showed larger amplitude, longer decay, and slower rise times when compared with adults (Cohen et al., 2000). Responses taken from postnatal day 15-21 more closely resembled adults (Cohen et al., 2000).

In addition to synaptic transmission, GABA may exert effects on distant targets by way of extrasynaptic receptors (Isaacson, 2000). The notion of spillover is not novel as ambient levels of glutamate have been shown to act on extrasynaptic NMDA receptors (Mody, 2001). Spillover can occur at highly active synapses (Dittman and Regehr, 1997). Neurotransmitters can also engage in cross talk at the extrasynaptic level. Dittman and Regehr (1997) found tetanic stimulation of granule cells preceded an extracellular GABA increase; this manifested as a brief depression of the Purkinje EPSC. GABA_B receptors may be involved in extrasynaptic transmission (Dittman and Regehr,
1997; Scanziani, 2000). When the GABA_A response was blocked by bicuculline and GABA reuptake transporters were selectively blocked, a slow IPSC was observed and was blocked by a GABA_B antagonist (Scanziani, 2000). GABA_B receptors also have a higher sensitivity for GABA than GABA_A receptors, making them suitable for detecting low concentrations of GABA (Isaacson, 2000). Separation of extrasynaptic from synaptic receptors revealed the former possessed three conductance states: 28, 17, and 12 pS (Brickley et al., 1999). These three conductance states may be evidence for three different tonic receptors (Brickley et al., 1999). Synaptic receptors had a uniform conductance of 28 pS, which is in close agreement with experimental data (Brickley et al., 1999).

*Synaptic and Non-synaptic Transmission:*

Recent efforts have focused on distinguishing tonic from phasic inhibition (Yeung et al., 2003; Caraiscos et al., 2004). Many pharmacological studies place the emphasis of drug effect on changes occurring at the synaptic level (Mody, 2001). However, it has been shown by Nusser et al. (1995) that in some cases, the density of extrasynaptic receptors may be larger than synaptic. Thus, it is possible these extrasynaptic receptors may play a larger role in drug effects than previously appreciated (Mody, 2001). Phasic inhibition is believed to be the result of vesicular GABA release while tonic results from low ambient GABA concentrations (Yeung et al., 2003). Tonic conductances have been identified in the thalamus, cerebellum, CA1 of hippocampus, and cortex (Yeung et al., 2003; Bai et al., 2001). Evidence from the cerebellum and hippocampus indicate phasic receptors found in synapses are primarily GABA_Aergic (Stell and Mody, 2002). As the
functions of phasic and tonic receptors differ, it follows the subunit identities may differ (Stell and Mody, 2002). While its functions are unclear, tonic mediating receptors may be possible targets for clinical therapeutics including anaesthetics and sedatives (Yeung et al., 2003). Nusser et al. (1995) determined the subunit distribution in synapses differed from extrasynaptic receptors using immunogold and electron microscopy. GABAergic Golgi synapses were enriched with $\alpha_{1,6}$, $\beta_{2/3}$, and $\gamma_2$; these same subunits are also expressed extrasynaptically at a lower density. The $\delta$ subunit was found exclusively in extrasynaptic locales (Nusser et al., 1998). Subunit candidates for tonic inhibition include $\alpha_{4,5,6}$ and the $\delta$ subunit as they are localized extrasynaptically (Mody, 2001); potential subunit combinations for these tonic receptors include $\alpha_4\beta_3\delta$, $\alpha_5\beta_3\gamma_{2/3}$, and $\alpha_6\beta_{2/3}\delta$ (McKernan and Whiting, 1996). In mouse CA1 neurons, the $\alpha_3$ subunit was identified in tonic mediating receptors (Caraiscos et al., 2004). The tonic current was not affected by zolpidem, but use of L-655,708, an $\alpha_3$ selective inverse agonist, partially inhibited the tonic current (Caraiscos et al., 2004). An interplay appears to exist between $\alpha_6$ and $\delta$ subunits, where null-mutant mice for $\alpha_6$ are also devoid of $\delta$ in the cell membrane (Jones, 1997). However, $\delta$ subunit expression remained at wildtype levels, suggesting a post-translational loss (Jones et al., 1997). $\delta$ containing receptors have 50x higher affinity for GABA than other GABA_A receptors; they do not desensitize in prolonged presence of agonist (Mody, 2001). These features make them amenable as tonic current mediating receptors. GABA_C receptors may also be candidates for mediating tonic inhibition as they exhibit low desensitization rates. The competitive GABA_A antagonist GABAZINE has been shown to selectively inhibit phasic inhibition in hippocampal dentate gyrus granule cells without affecting tonic conductance at low
concentrations (200 nM) while higher concentrations inhibited both types of current (Stell and Mody, 2002). However, Yeung et al. (2003) found 10 μM GABAZINE was sufficient to selectively block phasic inhibition while >100 μM GABAZINE blocked phasic currents and induced changes in holding current. Similar results were obtained in pyramidal cell recordings from CA1 (Stell and Mody, 2002). In addition to GABAZINE, penicillin-G was shown to selectively inhibit phasic inhibition without affecting tonic inhibition using doses as high as 20 mM (Yeung et al., 2003). Hence, GABAZINE and penicillin-G are two pharmacological compounds that may be used to delineate the phasic from tonic currents.

In addition, channels can also open in the absence of ligand (Birnir et al., 2000). Such channels are indeed GABAergic as confirmed by chloride sensitivity, bicuculline block, and BZD modulation by diazepam (Birnir et al., 2000). Application of GABA to these channels resulted in rapidly synchronized activity (Birnir et al., 2000). These spontaneously opening GABA channels have been observed in rat hippocampal CA1 neurons (Birnir et al., 2000). Spontaneous GABA_A channel openings can be seen in β1 (Birnir et al., 2000) and β3 homomeric receptors as well as the α4β1 combination (Neelands et al., 1999). Although the functional consequence of these spontaneously active channels is unknown, it is possible the additional conductance offers a means to adjust baseline excitability (Neelands et al., 1999) or these channels may have a role in control of tonic inhibition (Birnir et al., 2000). The combination of α1β3ε produced spontaneously opening GABA channels with a single-channel conductance of 24 pS (Neelands et al., 1999).
Single-Channel Conductance Estimates:

Non-stationary noise analysis (NSNA) is a necessary tool in determining single-channel properties of GABA_A receptors in the intact synapse as size and structural limits make access impossible. It was initially used to evaluate whether transmembrane currents were carried by pores, each being capable of passing the same unit current when open (Katz and Miledi, 1972). From these experiments, it was deduced such membrane currents were the summation of many smaller currents (Katz and Miledi, 1972). Sigworth pioneered the adaptation of noise analysis to time-varying currents arising from stochastic properties of Na^+ activated currents (Traynelis and Jaramillo, 1998). The goal of NSNA is to obtain estimates of these unitary currents and this is accomplished by exploiting the probabilistic nature of channel function (Traynelis and Jaramillo, 1998). Single-channel conductance values have been obtained (28 pS at 35°C vs. 20 pS at 22°C in rat hippocampal neurons, DeKoninck and Mody, 1994), 26.7 pS in HEK-293 cells (Mortensen et al., 2004), 28 pS in recombinants (Angelotti and Macdonald, 1993), and 22-28 pS in dentate gyrus (Nusser et al., 1998).

The conductance of GABA_A receptors at room temperature ranges from 22 pS (De Koninck and Mody, 1994) to upwards of 85 pS (Birnir et al., 2000). Typical estimates are reported <30 pS for GABA_A receptors at room temperature. Recombinant receptors do not display conductances >40 pS (Everitt et al., 2004). Diazepam and pentobarbital have been shown to significantly increase single-channel conductance in cultured rat hippocampal neurons as high as 80 pS (Eghbali et al., 1997; Eghbali et al., 2000; Birnir et al., 2000). The high conductance state may be explained by the presence of GABARAP, a protein involved with clustering (Everitt et al., 2004). In recombinants,
the co-expression of GABARAP with $\alpha_1 \beta_1 \gamma_{2S}$ displayed single-channel conductances of $>40$ pS as well as diazepam-enhancement of single-channel conductance (Everitt et al., 2004). In the absence of GABARAP, the single-channel conductance was never higher than 40 pS and was not enhanced by diazepam (Everitt et al., 2004). One explanation for this phenomena may be the clustering of receptors leading to changed single-channel characteristics (Everitt et al., 2004).

**Benzodiazepine Pharmacology:**

BZDs act through GABA$_A$ receptors and facilitate GABA-evoked Cl$^-$ currents; these GABAergic currents increase inhibitory tone throughout the CNS. Generally safe and efficacious, the effects of central BZD receptor ligands on animal behaviour ranges from positive modulatory effects (anxiolysis, sedation, hypnosis) to negative modulatory effects (anxiety, alertness, and convulsions; Luddens et al., 1995). The ongoing dilemma with full agonists is the balance between the narrowly defined therapeutic window and the unacceptable side effects (Kristiansen and Lambert, 1996). As such, interest in partial agonists has surfaced. Such compounds have the potential to mediate minor GABAergic perturbations with a reduced side effects profile (Kristiansen and Lambert, 1996). Diazepam is a well-known BZD that while widely used, possesses a range of side effects including sedation, alcohol potentiation, amnesic properties, and tolerance/dependence issues (Whiting, 2003). Thus, diazepam is a full agonist at the BZD site and cannot be used to discriminate GABA$_A$ receptor subtypes (Collins et al., 2002). The different locations of GABA subunits would suggest different subtypes are related to specific physiological processes (Collins et al., 2002). Using $\alpha_1$ mouse KOs, researchers
determined the sedative/motor effects of diazepam were mediated by the $\alpha_1$ subunit (McKernan et al., 2000). Hence, the need to dissect GABA<sub>A</sub> receptors into derivative subunits has led to the search for subunit specific compounds (Whiting, 2003).

Type I BZD receptors contain $\alpha_1\beta_1\gamma_2$ subunits (Whiting, 2003) while Type II BZD receptors are believed to contain either of the $\alpha_{2,3,5}$ subunits and have low affinity for the BZD analogue Cl-218, 872 (Pritchett et al., 1989). The Type I BZD receptors are found in the cerebellum while the Type II BZD receptors are strongly expressed in the hippocampus (Luddens et al., 1995). $\alpha_{4/6}$ subunit containing receptors are BZD insensitive (Collins et al., 2002). The $\beta$ subunit is not involved in BZD pharmacology (Collins et al., 2002). While most BZDs bind to GABA/BZD receptors with similar affinities, the binding properties of specific compounds Cl-218, 872 and 2-oxo-quazepam illustrate the heterogeneity of GABA<sub>A</sub>/BZD receptors (Luddens et al., 1995).

BZD type I receptors (high affinity) have greater affinity for Cl-218, 872 and 2-oxo-quazepam than Type II receptors (low affinity); they also exist as the predominant GABA<sub>A</sub> receptor class in the CNS (Luddens et al., 1995). Type II receptors are found in the hippocampus, striatum, and spinal cord.

In addition to the BZDs, the anaesthetic family is known to mediate its effects at the GABA<sub>A</sub> receptor. While propofol and other intravenous anaesthetics demonstrate no significant subunit selectivity, etomidate has $\beta_{2,3}$ selectivity over $\beta_1$ (Whiting, 2003). GABAergic currents are enhanced by etomidate (Beelute et al., 1997). Loreclezole, the anti-epileptic, also demonstrates similar preference for $\beta_{2,3}$ over $\beta_1$, suggesting it acts at the same site.
Neurosteroid Modulators:

Neurosteroids are endogenous in the CNS and are synthesized by glial cells and neurons (Poisbeau et al., 1997). They circulate at concentrations that may modulate GABAergic synaptic communication (Mennerick et al., 2001). They play a role in several physiological states, including the menstrual cycle, pregnancy, and parturition (Reddy, 2003). In 1941, Selye established progesterone metabolites had potent sedative and anaesthetic properties. 40 years later, Schofield demonstrated a close analogue of these steroids, alphaxalone, prolonged inhibition in guinea-pig olfactory slices, implicating a GABAergic target. 5α-pregnan-3α-ol-20-one, 5β-pregnan-3α-ol-20-one, and 5α-pregnan-3α, 21-diol-20-one are steroid metabolites even more potent than alphaxalone (Lambert et al., 1995). Neuroactive steroids appear to possess properties synonymous with positive allosteric modulators, including allosteric enhancement of agonist, BZD binding, and displacement of non-competitive antagonists (Lambert et al., 1995). At concentrations higher than required for potentiation, neurosteroids can activate GABA_A receptors in the absence of GABA (Lambert et al., 1995). The anaesthetic profile 5β-pregnan-3α-ol-20-one is well documented, with notable features including rapid induction and greater potency than propofol (Lambert et al., 1995). Hence, there is renewed interest in developing steroid-derived anaesthetics (Lambert et al., 1995). 5α- and 5β-pregnan-3α-ol-20-one are also effective anti-convulsants, protecting against various GABA_A receptor antagonist induced seizures (Lambert et al., 1995). Thus, there is a possibility neurosteroids are endogenous anti-convulsants (Lambert et al., 1995). Disturbances in steroid circulation may manifest at a pathological level. For example, altered progesterone levels are associated with anxiety and seizures, features also
associated with the premenstrual cycle (Smith et al., 1998). The use of subunit selective compounds can help discriminate between the various types of behaviours seen (Collins et al., 2002). Our work has centred on the pharmacology of miniature inhibitory postsynaptic currents (mIPSCs). mIPSCs are the unit of choice as they persist in TTX and are insensitive to Ca\textsuperscript{2+} blockade. More importantly, mIPSCs are the result of single synapse activation and this is preferential to the tens of thousands of synapses activated in evoked/stimulated conditions.

**Rationale for Experiments:**

As previously described by Hutcheon et al. (2000), mIPSCs can be classified into mono- and bi-exponential classes. The existence of these distinct distributions form our premise: are separate receptors responsible for these distinct phenomena and if so, what are their subunit identities? We have used the α subunit selective compounds zolpidem, SB-205384, L-655,708 and furosemide in cultured rat embryonic neurons to address this question. In addition, we have also employed the neurosteroid pregnenolone sulphate to test for subunit selectivity.

Zolpidem is an imidazopyridine and is α\textsubscript{1} subunit selective. It positively potentiates α\textsubscript{1} containing receptors.

SB-205384 is an α\textsubscript{3} subunit selective compound that positively potentiates α\textsubscript{3} containing receptors.

While there is general agreement that the non-competitive antagonist furosemide is a negative modulator, there appears to be some disagreement in its subunit selectivity.
Bosman et al. (2002) used 100 μM furosemide as an α₄ selective antagonist; Cohen et al. (2000) used furosemide at 600 μM. Tia et al. (1996) used 100 μM as an α₅ antagonist.

L-655,708 is an α₅ subunit selective compound with inverse agonist properties and a 50x greater affinity for the BZD site than any other α subunit when combined with β₃γ₂ (Quirk et al., 1996).

Pregnenolone sulphate (5-pregnen-3α-ol-20-one sulphate) is a neurosteroid with demonstrated negative modulatory effects. It is presently unknown whether pregnenalone sulphate is produced in the neurointermediate lobe of the pituitary or whether it exists at sufficient endogenous quantities to negatively modulate GABAergic activity (Poisebeau et al., 1997).

The aim of this thesis was to demonstrate the presence/absence of specific α subunits in kinetically distinct GABAergic synapses and the extrasynaptic membrane. Our approach to this was to employ different positive and negative modulators with putative α subunit selectivity.
Methods:

Culture methods:

Pregnant Sprague-Dawley rats (Charles River) were euthanized on embryonic day 18 (E18) using CO₂. Pups were removed and transferred to ice cold PBS where they were decapitated. Cortices were surgically removed, suspended in 2 mL plating media comprised of Eagle’s MEM (GIBCO), 10% horse serum, 10% fetal bovine serum, 2 mM glutamine, and 20 mM glucose, and mechanically dissociated. The cell suspension was then centrifuged at 1100 RPM for 6 minutes at 20°C. After removal of the supernatant, the resulting pellet was resuspended in 8-10 mL plating media. A live cell count was obtained by trypan blue exclusion. Cells were plated at a density of 2 million cells/mL on Corning 35 mm dishes previously treated with poly-D-lysine. After week 1, media is exchanged with Neurobasal supplemented with B-27 (GIBCO). Further media changes occurred bi-weekly.

Solutions:

The external solution consisted of: 145 mM NaCl, 5 mM KCl, 10 mM HEPES, 2 mM CaCl₂, 2 mM MgCl₂, and 10 mM glucose (300-310 mOsm, pH adjusted to 7.3). Action potentials and AMPA/Kainate and NMDA receptor mediated excitatory synaptic transmission were blocked using 250 nM tetrodotoxin, 50 μM DL-2-amino-5-phosphono-pentoanic acid (APV), and 10 μM 6,7-dinitro-5-xaline-2,3-dione (DNQX) respectively in order to isolate the inhibitory synaptic currents. These blockers were dissolved from 1000x stocks in Ringer solution to achieve final concentrations. The
electrode solution was comprised of: 145 mM CsCl, 10 mM NaCl, 10 mM HEPES, 1 mM Na₂EDTA, and 20 mM glucose (adjusted to 300-320 mOsm with sucrose, pH 7.3, free Ca²⁺ 80 nM).

**Electrophysiology:**

An Axon Instruments Axopatch200B amplifier was used to record mIPSCs (5 kHz low pass filtered) that were digitized (10 kHz) using an Axon Instruments Digidata 1200A interface. Electrode placement was accomplished using a Sutter Instruments MP-285 micromanipulator. All recordings were performed at room temperature (20-22°C) on visually identified pyramidal neurons after a minimum 20 days *in vitro* (20DIV). Neurons were clamped at a membrane potential of ~60 mV. Electrodes were pulled using a Sutter Instruments P-97 horizontal electrode puller and Sutter Instruments borosilicate glass (OD. 1.5mm, I.D. 0.86mm) and coated with paraffin wax to reduce capacitive noise. Electrode resistance ranged from 3-6MΩ. Series resistance was typically in the range of 7-15MΩs with compensation up to 80%. Series resistance was monitored constantly and recordings were abandoned if it reached >20MΩ.

**Data analysis:**

mIPSCs were acquired using Axon Instruments Clampex version 7 for Microsoft Windows and subsequently analyzed offline using Synaptosoft’s Minianalysis version 6.0.1. Offline sorting of the mIPSCs for further analysis used the following criteria: rise time less than 2 ms and the peak amplitude was greater than 25 pA to avoid spurious events. Exponential fits were done on all mIPSCs meeting these criteria and they were
classified as either mono- or bi-exponential according to the criteria used in Hutcheon et al (2000).

For peak scaled non-stationary noise analysis (psNSNA), the mIPSCs were additionally software filtered at 1 kHz (low pass). psNSNA was performed by first classifying mIPSCs according to exponential deactivation kinetic (mono- or bi-exponential). Once grouped, the mIPSCs free of artifacts and excessive noise were individually selected. The non-stationary noise parameters used were synaptic current NSNA, 30 bins, averaging of 2 mIPSCs with subtraction of baseline variance. The relationship between current and variance is described by the equation:

$$\sigma^2 = i \bar{I} - I^2 / N$$

where:

- $\sigma^2$ is the variance
- $i$ is the unitary current
- $I$ is the mean current
- $N$ = channel number

In order to estimate $i$ a parabolic relationship is fit where channel number is free to vary as well. However, this variable is not reliable due to the peak scaled paradigm that is used to scale and subsequently sort the mIPSCs. Thus these estimates only reliably determine the unitary current $i$ (Traynelis and Jaramillo 1998).

Statistical Analysis

Drugs effects were assessed by taking the median attributes (amplitude, time constant) for each recording and subtracting this value from the matched attribute with drug present. The median differences from each recording were averaged to generate
average median differences for each drug treatment. A paired t-test was then used to test for significant differences. Data are presented as the average of medians and therefore is expressed as the mean ± standard error of the mean (S.E.M.) unless otherwise stated.
Results:

The results were obtained from 95 patch recordings taken from primary cortical cultures of minimum 20 days in vitro (DIV). In the presence of 250 nM tetrodotoxin, 50 μM DL-2-amino-5-phosphonopentanoic acid (APV), and 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX), miniature inhibitory post synaptic currents (mIPSCs) were observed as negative deflections from the baseline holding current. These events were between -20 and -100 pA. These events have been shown to occur as either mono- or biphasic mIPSCs (Hutcheon et al., 2000). However, the biophysical properties of these responses has not be determined nor has the subunit identities of these different receptors been examined. Thus, in the results described below, we first estimate the unitary channel conductances in each synaptic type and then investigate the effects of various GABA_A receptor modulators that are reportedly selective for different α subunits.

Peak scaled non-stationary noise analysis reveals different unitary currents for mono- and biphasic synapses

We determined if the single-channel conductance of mono- and biphasic GABA_A receptors differed by employing peak scaled non-stationary noise analysis (Traynelis et al., 1993). Briefly, this involved scaling the average response to the peak of each individual mIPSC and then sorting these scaled events into 30 bins of equal amplitude to calculate the mean current as described by De Koninck and Mody (1994). Only uncontaminated mIPSCs free from external noise and large shifts in baseline current were accepted for analysis. Fig. 1 shows an example of this analysis from one recording, showing the fitted parabolic relationship that estimates the unitary channel current. The
parabolic shape is expected if the assumption that low channel variance at the peak holds (De Koninck and Mody, 1994). In 25 cells, psNSNA yielded a mean unitary current of $-1.5 \pm 0.3$ pA for the monophasic receptors; in 10 cells, psNSNA gave a unitary current of $-2.4 \pm 0.2$ pA for biphase receptors; these values corresponded to estimated conductances of 25 pS and 40 pS respectively.

**Figure 1:** The unitary currents for mono- and bi-exponential mIPSCs differ.

In the upper trace A, the unitary conductance of a monophasic synapse was $-1.7$ pA. The lower trace calculates a unitary conductance of $-2.1$ pA

Pharmacological studies reveal both similarities and differences in sensitivities to various $\alpha$ subunit selective compounds

*Effects of zolpidem on mIPSCs at high and low concentrations*

Zolpidem, a positive benzodiazepines-like modulator recognized for its $\alpha_1$ subunit selectivity, was employed to test for the presence of the $\alpha_1$ subunit in the different
synapses. Bath application of zolpidem consistently enhanced the decay time constant at three different concentrations in the mono-exponential mIPSCs (Table 1). In 12 cells, 0.5 \( \mu M \) zolpidem increased decay time constant by 29\% \((p<0.01)\) while having no effect on peak amplitude (Fig. 2A; Table 1). However, in 2 cells, a clear potentiating effect on the mIPSCs was observed (Fig. 2B, 2C) that reversed upon washout (Fig. 2C). In 10 cells, where sufficient bi-exponential mIPSCs could be collected, 0.5 \( \mu M \) zolpidem had no effect on either deactivation time constant but it caused a decrease in their amplitude by 26\% \((p<0.01); \) Fig. 3; Table 2). In contrast to the mono-exponential mIPSCs, 0.5 \( \mu M \) zolpidem did not have a potentiating effect on the bi-exponential mIPSCs.
Figure 2: The effects of 0.5 μM zolpidem on decay time constant and peak amplitude of averaged mono-exponential mIPSCs.

In panel A, 0.5 μM zolpidem increased the decay time constant and did not change the peak amplitude. However, in B and C panels, 0.5 μM zolpidem had a potentiating effect on peak amplitude of 15% and 28% respectively. Note in panel C this effect is reversible.
Figure 3: The effects of 0.5 μM zolpidem on decay time constants and peak amplitude of averaged bi-exponential mIPSCs.

In panel A, 0.5 μM zolpidem had no effect on the deactivation but decreased amplitude.

Figure 4: The effects of 1.0 μM zolpidem on decay time constant and peak amplitude of averaged mono-exponential mIPSCs.

Here, 1.0 μM zolpidem reduced the peak amplitude but increased the decay time constant. A shows the traces scaled appropriately and in B the peaks have been scaled to show the decay time constant has been increased in the presence of zolpidem.

Because there was no clear potentiating effect of zolpidem, we increased the concentration employed to see if a higher dose would be efficacious. As shown, in Fig. 3, 1 μM zolpidem decreased the amplitude of mono-exponential mIPSCs in 7 cells tested (p<0.03) while enhancing the decay time constant (p<0.002) (Fig. 4; Table 1). This
concentration again had no significant effect on either decay time constants, but surprisingly the peak amplitude was unaffected in the bi-exponential mIPSCs (Fig. 5; Table 2).

![Graph showing control and 1 µM zolpidem effects on mIPSCs](image)

**Figure 5:** The effects of 1 µM zolpidem on decay time constants and peak amplitude of averaged bi-exponentials mIPSCs.

1 µM zolpidem had no effect on the time course or peak amplitude.

At yet a higher concentration, 10 µM zolpidem increased the mono-exponential decay time constant by 32% in 17 of 19 cells (p<0.0004) while having no significant effect on amplitude (Fig. 6A; Table 1). However, like 0.5 µM zolpidem, 10 µM zolpidem also demonstrated a potentiating effect on amplitude in a minority of cells (Fig. 6B, 6C). In 6C, the potentiating effect on peak amplitude persists despite 10 minutes of washout. Overall, 10 µM zolpidem did not significantly affect the decay time constants or peak amplitude in the bi-exponential mIPSCs in 14 cells (Fig. 7; Table 2). However, in 1 cell, a potentiating effect on peak amplitude was observed, increasing by 18% (Fig 7B).
Figure 6: The effects of 10 μM zolpidem on decay time constant and peak amplitude of averaged mono-exponential mIPSCs.

In A, 10 μM zolpidem increased decay time constant with no concomitant effect on amplitude. In B and C, 10 μM zolpidem showed a potentiating effect on peak amplitude of 19% and 23% respectively. In C, the potentiating effect persists after washout.

The α₃ subunit selective compound SB-205384 increased the slow decay time constant of the bi-exponential mIPSCs

To test for the contribution of the α₃ subunit to the synaptic behaviour, the α₃ subunit selective compound SB-205384 was used (Meadows et al., 1997, 1998). Bath perfusion of 5 μM SB-205384 was immediately followed by baseline instability (data not shown). 5 μM SB-205384 did not significantly affect the decay time constant or
amplitude in mono-exponential mIPSCs (Fig. 8; Table 1). In the bi-exponential mIPSCs, 5.0 μM SB-205384 increased the slow time constant by 41% (p<0.004; Table 2).

**Figure 7:** The effects of 10 μM zolpidem on decay time constants and peak amplitude of averaged bi-exponential mIPSCs.

In A, 10 μM zolpidem does not affect either the amplitude or decay time constant. In B, 10 μM zolpidem has a potentiating effect on peak amplitude of 18%.
Figure 8: The effects of 5 μM SB-205384 on averaged mono- and bi-exponential mIPSCs.

In the top trace, 5 μM SB-205384 does not affect either amplitude or the deactivation. In the lower trace, 5 μM SB-205384 increases the slow time constant.

The α₆ subunit selective compound furosemide effects on mIPSCs

The non-competitive GABA_A receptor antagonist furosemide was used to test for the presence of α₄ in the different synapses. In both mono- and biphasic mIPSCs, 100 μM furosemide reduced the peak amplitude (Table 1 & 2). It also increased the rate of deactivation by decreasing the magnitude of the fast time constant (Fig. 9; Table 2). Similarly, the mono-exponential mIPSCs were also reduced in amplitude by 20% (p<0.0003) in 10 cells while having no effect on decay time constant (Table 1). In the bi-exponential mIPSCs, 100 μM furosemide reduced amplitude by 12% (p<0.016) and increased the rate of deactivation by 11% (p<0.04; Table 2). Three cells displayed a potentiating effect (data not shown).
Figure 9: The effects of 100 μM furosemide on averaged mono- and bi-exponential mIPSCs.

In the upper trace, 100 μM furosemide significantly reduced peak current amplitude in averaged mono-exponential mIPSCs. After 10 minutes of washout, this effect persisted. In the lower trace, 100 μM furosemide reduced peak amplitude in averaged bi-exponential mIPSCs.

The α₅ subunit selective compound L-655,708 decreased both peak amplitude and decay time constant in mono-exponential mIPSCs and fast decay time constant and peak amplitude in bi-exponential mIPSCs

The α₅ subunit selective compound L-655,708 (Quirk et al., 1996) was used to assess the presence of α₅ in the different synapses. 5 μM L-655,708 consistently reduced the amplitude in both populations of mIPSCs and affected the time constant parameters differently (Fig. 10; Table 1 & 2). In the mono-exponential mIPSCs, 5 μM L-655,708 reduced the amplitude by 28% (p<0.001) and decay time constant by 27% (p<0.003) in
13 cells (Table 1). In the bi-exponential mIPSCs, L-655,708 reduced the amplitude by 24% (p<0.01) and the fast decay time constant by 25% (p<0.0002) in 11 cells (Table 2).

![Graph showing the effects of L-655,708 on mIPSCs](image)

**Figure 10:** The effects of 5 μM L-655,708 on averaged mono- and bi-exponential mIPSCs.

In the upper trace, 5 μM L-655,708 reduced peak amplitude in averaged mono-exponential mIPSCs. The reduction persisted after 10 minutes of washout. In the lower trace, 5 μM L-655,708 reduced peak amplitude in averaged bi-exponential mIPSCs.

**Pregnenolone sulphate (PS) reduced peak amplitude in both mono- and bi-exponential mIPSCs**

We wished to determine if the neurosteroid pregnenolone sulphate (PS) differentially affected the mIPSCs. While α subunit selectivity with PS has not been established, we tested whether PS could discriminate mono- from biphasic receptors. In both the mono- and bi-exponential mIPSCs, 10 μM PS reduced the peak amplitude in a partially reversible fashion (Fig.11; Table 1 & 2). Mono-exponential mIPSCs taken from
19 cells showed peak amplitude reductions by 13% (p<0.0003; Table 1). There was no effect on the decay time constant. Bi-exponential mIPSCs taken from 19 cells had peak amplitude reductions of 22% (p<0.00002) with no effect on either decay time constant (Table 2). In another 8 cells, PS either increased the amplitude or produced no effect (data not shown).

Figure 11: The effects of 10 μM PS on averaged mono- and bi-exponential mIPSCs.

In the upper trace, 10 μM PS reversibly reduced peak amplitude of averaged mono-exponential mIPSCs.

In the lower trace, 10 μM PS reversibly reduced peak amplitude of averaged bi-exponential mIPSCs.

Tonic baseline experiments suggest an extrasynaptic localization of the α1, α3, and α4 subunits

To test for a contribution of the subunits α1, α3, and α4, we applied the α subunit selective compounds to baseline currents (Fig. 12). As 1 μM GABAZINE has been reported to block the phasic inhibition (mIPSCs), we employed it to assay for the effects of zolpidem on the tonic current. As shown in Fig. 12, GABAZINE blocked the mIPSCs
without altering baseline. The subsequent application of 1.0 μM zolpidem produced an increase in holding current of 189.5 pA (average 61.9 pA ± 15.6, n = 7), suggesting an extrasynaptic presence of the α1 subunit.

In the middle trace, bath application of 5 μM SB-205384 was followed by an increase in holding current by 131 pA (average 78.4 pA ± 25.6, n = 6). This increase was slow to recover requiring more than 20 minutes. As seen in the lowest trace, following 100 μM furosemide application, only a small decrease in holding current was observed of 17 pA (average 22.1 pA ± 4.9, n = 4). Although small, this effect was again poorly reversible.
Figure 12: The effects of 1 μM zolpidem, 5 μM SB-205384, and 100 μM furosemide on holding current.

In the top trace, 1 μM zolpidem increased holding current in a partially reversible fashion. 1 μM GABAZINE was applied to eliminate synaptic mIPSCs. In the middle trace, 5 μM SB-205384 increased holding current in a partially fashion. In the bottom trace, 100 μM furosemide decreased holding current but was poorly reversible.
Table 1: Summary of pharmacological effects on mono-exponential mIPSCs

<table>
<thead>
<tr>
<th></th>
<th>Control Tau (ms)</th>
<th>Control Amplitude (pA)</th>
<th>Drug Tau (ms)</th>
<th>Drug Amplitude (pA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μM zolpidem</td>
<td>24.5 ± 2.2</td>
<td>-59.4 ± 4.0</td>
<td>34.1 ± 4.1*</td>
<td>-55.5 ± 5.3</td>
</tr>
<tr>
<td>1 μM zolpidem</td>
<td>19.3 ± 1.2</td>
<td>-52.8 ± 2.7</td>
<td>28.5 ± 2.2**</td>
<td>-45.8 ± 2.5*</td>
</tr>
<tr>
<td>10 μM zolpidem</td>
<td>17.3 ± 1.7</td>
<td>-61.7 ± 4.5</td>
<td>25.8 ± 2.7***</td>
<td>-61.6 ± 6.4</td>
</tr>
<tr>
<td>5 μM SB-205384</td>
<td>18.3 ± 1.3</td>
<td>-57.7 ± 4.7</td>
<td>20.1 ± 2.0</td>
<td>-51.5 ± 2.6</td>
</tr>
<tr>
<td>100 μM furosemide</td>
<td>17.3 ± 2.1</td>
<td>-73.2 ± 3.6</td>
<td>16.3 ± 1.4</td>
<td>-58.4 ± 2.2***</td>
</tr>
<tr>
<td>5 μM L-655,708</td>
<td>22.3 ± 2.1</td>
<td>-80.1 ± 3.6</td>
<td>16.2 ± 2.2***</td>
<td>-57.4 ± 3.0***</td>
</tr>
<tr>
<td>10 μM pregSO4</td>
<td>18.5 ± 1.2</td>
<td>-68.5 ± 4.2</td>
<td>20.1 ± 1.3</td>
<td>-57.4 ± 2.3***</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001
Table 2: Summary of pharmacological effects on bi-exponential mIPSCs

<table>
<thead>
<tr>
<th></th>
<th>Control Tau Fast (ms)</th>
<th>Control Tau Slow (ms)</th>
<th>Control Amplitude (pA)</th>
<th>Drug Tau Fast (ms)</th>
<th>Drug Tau Slow (ms)</th>
<th>Drug Amplitude (pA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5μM zolpidem</td>
<td>7.7 ± 1.1</td>
<td>77.5 ± 9.3</td>
<td>-65.3 ± 5.2</td>
<td>6.8 ± 0.9</td>
<td>77.7 ± 12.6</td>
<td>-48.7 ± 3.7*</td>
</tr>
<tr>
<td>1μM zolpidem</td>
<td>6.0 ± 0.6</td>
<td>67.8 ± 7.2</td>
<td>-56.9 ± 3.6</td>
<td>7.9 ± 0.9</td>
<td>96.8 ± 18.2</td>
<td>-52.0 ± 3.9</td>
</tr>
<tr>
<td>10μM zolpidem</td>
<td>6.5 ± 0.4</td>
<td>76.3 ± 7.7</td>
<td>-62.3 ± 5.4</td>
<td>7.3 ± 0.6</td>
<td>91.0 ± 9.2</td>
<td>-59.4 ± 5.0</td>
</tr>
<tr>
<td>5μM SB-205384</td>
<td>6.7 ± 0.4</td>
<td>81.5 ± 5.2</td>
<td>-60.3 ± 3.3</td>
<td>6.3 ± 0.4</td>
<td>139.0 ± 18.7**</td>
<td>-57.7 ± 3.6</td>
</tr>
<tr>
<td>100μM furosemide</td>
<td>6.8 ± 0.4</td>
<td>65.6 ± 3.8</td>
<td>-75.5 ± 5.2</td>
<td>5.7 ± 0.4*</td>
<td>59.9 ± 7.4</td>
<td>-67.1 ± 4.0*</td>
</tr>
<tr>
<td>5μM L-655,708</td>
<td>7.6 ± 0.2</td>
<td>76.1 ± 4.8</td>
<td>-89.8 ± 8.6</td>
<td>5.7 ± 0.4***</td>
<td>70.3 ± 8.5</td>
<td>-67.9 ± 3.7*</td>
</tr>
<tr>
<td>10μM pregSO4</td>
<td>7.1 ± 0.5</td>
<td>73.2 ± 8.2</td>
<td>-76.3 ± 4.3</td>
<td>6.7 ± 0.5</td>
<td>66.3 ± 5.5</td>
<td>-59.3 ± 2.9***</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001
Table 3: Summary of effects on mIPSCs

<table>
<thead>
<tr>
<th></th>
<th>Mono(tau)</th>
<th>Mono(amp)</th>
<th>Bi(tau fast)</th>
<th>Bi(tau slow)</th>
<th>Bi(Amp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μM zolpidem</td>
<td>Increased</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Decreased</td>
</tr>
<tr>
<td>1 μM zolpidem</td>
<td>Increased</td>
<td>Decreased</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10 μM zolpidem</td>
<td>Increased</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5 μM SB-205384</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Increased</td>
<td>No</td>
</tr>
<tr>
<td>100 μM furosemide</td>
<td>No</td>
<td>Decreased</td>
<td>Decreased</td>
<td>No</td>
<td>Decreased</td>
</tr>
<tr>
<td>5 μM L-655,708</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
<td>No</td>
<td>Decreased</td>
</tr>
<tr>
<td>10 μM pregSO4</td>
<td>Decreased</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

Table 4: Summary of synaptic and extrasynaptic α subunit localization:

<table>
<thead>
<tr>
<th></th>
<th>Synaptically (mono)</th>
<th>Synaptically (bi)</th>
<th>Extrasynaptically</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>α3</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>α4</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>α5</td>
<td>yes</td>
<td>yes</td>
<td>?</td>
</tr>
</tbody>
</table>
Discussion:

*Non-stationary noise analysis:*

While there seems to be no general consensus as to the single-channel conductance of GABA$_A$ receptors, our data falls within the 8-85 pS range of other laboratories using hippocampal (Robinson *et al.*, 1991; Eghbali *et al.*, 1997; Birnir *et al.*, 2000; De Koninck and Mody, 1994), cerebellar granule cells (Farrant and Brickley, 2003; Kaneda *et al.*, 1995), visual cortex (Perrais and Ropert, 1999), and dissociated neurons preparations (Lorez *et al.*, 2000). Using hippocampal and dorsal root ganglia preparations, Lorez *et al.* (2000) demonstrated $\gamma_2$ knockout mice had lower conductance states of 12 pS with an infrequent 24 pS state. In wildtype mice, subconductance states of 18 and 12 pS were also observed. These different conductance states likely reflect different receptor subtype combinations (Brickley *et al.*, 1999).

As a consequence of peak scaled non-stationary noise analysis, we were unable to estimate the channel number and open probability. There is data available from other labs to suggest channel number can be as low as 45 (De Koninck and Mody, 1994). Here based on the average median amplitude of the two types of mIPSCs we estimate the same number of channels open for both types of synapses (25). If $P_{\text{open}}$ is modest (0.7) then this suggests there are approximately 35 channels per synapse. This is well within the physical limits for the number of channels situated in the synaptic membrane.
Pharmacology:

Effect of 0.5, 1.0, and 10 μM zolpidem

The effect of 0.5 μM zolpidem decreasing rather than increasing peak amplitude at various concentrations is contrary to our expectations of zolpidem as a GABAergic potentiating agent. As shown by various groups, application of zolpidem at concentrations ranging from 100 nM to 10 μM at room temperature increased mIPSC amplitude (Defazio and Hablitz, 1998; Perrais and Ropert, 1999; De Koninck and Mody, 2001; Goldstein et al., 2002). It is not clear at this juncture why our data differ from previous reports. One explanation is since zolpidem enhances the observed tonic activation of GABA receptors and this tonic current is a mixture of both the activation of extrasynaptic and synaptic receptors, the synaptic receptors may be partially desensitized and therefore respond less to the phasic release.

Nevertheless some cells did exhibit enhanced amplitude suggesting that a subpopulation of mIPSCs may be zolpidem-sensitive due to a low probability of becoming desensitized. Alternatively, even though partially desensitized, the increased receptor affinity for GABA overcomes this effect in synapses where GABA is not saturating. We should also emphasise that previous studies did not separate mIPSCs into distinct groups of mono- or bi-exponential mIPSCs and our analysis may reveal aspects that are missed when this heterogeneity is not considered. Despite these difference, the observed prolonged decay time constant in the mono-exponential mIPSCs agrees with Perrais and Ropert (1999), who demonstrated 10 μM zolpidem increases both amplitude and decay time constant in mono-exponential mIPSCs.
From our mIPSC analysis, it seems the α₁ subunit receptors are present in the monophasic synapses as they are potentiated and they may nevertheless be present in the bi-phasic ones as well as evidenced by the weak reduction in amplitude produced by 0.5 μM zolpidem. The lack of effect at higher concentrations may be due to a combination of enhanced desensitization and enhanced affinity that effectively cancel each other out. In addition to synaptic localization, our tonic current experiments suggest α₁ may be present at the extrasynaptic level. This contrasts with the results of Caraiscos et al. (2004), who show 100 nM zolpidem had no effect on tonic current.

*Effect of 5 μM SB-205384*

Similar to the published results of Hutcheon et al. (2000), our experiments show SB-205384 has no significant effects on mono-exponential mIPSCs. In contrast to their results, we identified a slow time constant effect in the bi-exponential mIPSCs. This may be explained by the different methodologies used to analyse the data. Hutcheon et al. (2000) did sort and average the two populations and thus small changes in the decay time constants are obscured. Our only explanation is that this extra time constant may reflect the potentiation of GABA spillover onto perisynaptic α₃ subunit containing receptors. As α₃ receptors have the lowest affinity (Verdoorn et al., 1994) they would not be stimulated under physiological conditions but when potentiated by the SB-205384, their stimulation by spillover becomes evident. This interpretation is in line with the evidence that α₃ may be localized extrasynaptically during development to the exclusion of synaptic placement. In effect, the resulting mIPSCs may be a combination of synaptic and extrasynaptic activity. This cannot be confirmed experimentally as there are no
compounds capable of blocking the tonic current while sparing synaptic currents. We should also note this phenomena has been reported to occur under physiological conditions when there is high activity at the synapse (Dittman and Regehr, 1997).

These data are also corroborated by another study that used 1 µM bretazenil as an α3 subunit selective compound (Bosman et al., 2002). While there are some non-selectivity issues with bretazenil, the results of Bosman et al. (2002) showed 1 µM bretazenil increased the time constant of mIPSCs. Hence, a prolonged time constant may be associated with α3 activation. The window for α3 subunit expression appears to be very small however, as the bretazenil effect was robust at postnatal day (PND) 6 and virtually absent by PND21.

Effect of 100 µM Furosemide

In the literature, furosemide is cited as either an α4 (Bosman et al., 2002) or α6 (Tia et al., 1996; Wall, 2002) subunit selective compound. Bosman et al. (2002) used 100 µM furosemide to test for the presence of α4 in developing visual cortex. Furosemide was without effect in ‘immature’ neurons but had a greater effect in mature ones. Wall (2002) and Tia et al. (1996) applied furosemide at cerebellar granule cells, which highly express the α6 subunit and found that it blocked this subunit combination as well. Our data showed that 100 µM furosemide was effective at reducing peak amplitude in both populations of mIPSCs, similar to published results of other groups. Furosemide also weakly blocked the tonic current, indicating α4 is present extrasynaptically in agreement to similar effects that have been seen in the cerebellum where α6 and not α4 is present (Wall 2002).
Effect of 5 μM L-655,708

While identified as an α₅ subunit selective compound, there is a surprising lack of functional studies using this compound. L-655,708 has a documented selectivity for α₅ over the other α subunits by as much as 50 times (Quirk et al., 1996). Caraiscos et al. (2004) found 50 μM L-655,708 did not have any effect on the mIPSCs, but was effective in decreasing the tonic current. Here we found that it did affect the mIPSCs by reducing both amplitude and increasing the rate of deactivation in both type of mIPSCs. We have not fully documented the effects of L-655,708 on holding current but in a few recordings the holding current was seen to increase following L-655,708. Hence, our data suggests a synaptic role for the α₅ subunit, in contrast with Caraiscos et al. (2004). A role for α₅ in tonic inhibition is still not clear.

Effect of 10 μM pregnenolone sulphate (PS)

Our data suggests PS does not discriminate between mono- and bi-exponential mIPSCs as it effectively reduced peak amplitude in both groups. While PS produced antagonistic effects in 70% of the cells, a small percentage experienced no change or an increase in amplitude. PS has been shown to be present in the brain in the nanomolar range (Baulieu and Robel, 1990; Corpechot et al., 1997; Wang et al., 1997). Sulphated steroids have been shown to reverse the effects of GABAₐergic potentiating compounds (Wang et al., 2002). This is clinically relevant as potentially low concentrations of these steroids can be used to treat drug overdose or reverse anaesthesia (Wang et al., 2002). PS has been well documented as an inhibitor of GABAₐergic synaptic transmission
(Majewska et al., 1988). While our results generally agree with that of others, we did not obtain complete block of mIPSCs in the fashion of Poisbeau et al. (1997). Further, they saw the rate of decay decrease following 10 μM PS administration. While we observed a reduction in peak amplitude, complete abolishment of the mIPSCs and an accelerated time constant were never seen. Eisenman et al. (2003) showed PS possesses some similarities to picrotoxin actions, suggesting a similar binding site.

Conclusions:

We have presented biophysical evidence that demonstrates the existence of kinetically distinct receptors. Indeed, mono- and biphasic receptors were shown on average to have different unitary currents. However, as previously mentioned, a shortfall of psNSNA is the inability to accurately estimate channel number and open probability. While our data fall within an acceptable range of GABA<sub>A</sub> receptor unitary currents, the next step is to estimate the number of channels activated at these receptors; this requires a different analytical approach.

The α<sub>1</sub>, α<sub>3</sub>, and α<sub>4</sub> subunits appear involved in both synaptic and extrasynaptic membranes with the α<sub>5</sub> subunit appearing synaptically. In addition, the α<sub>3</sub> subunit appears to be preferentially located perisynaptically in the biphasic synapses. The fact that these α subunits are involved in both synaptic and extrasynaptic spaces indicates a dual role for these subunits and further intimates at the diverse heterogeneity of GABA<sub>A</sub> receptors. Although our results show α<sub>1</sub>, 4, and 5 are present in both mono- and biphasic GABA<sub>A</sub> receptors, there is still the possibility of other subunits being different between them.
References:


