PRE- AND POSTSYNAPTIC ACTIONS OF PENTOBARBITAL ON
CORTICOTHALAMIC TRANSMISSION

by

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Abstract

This thesis examined the pre- and postsynaptic actions of an anesthetic barbiturate, pentobarbital, on neurons of the corticothalamocortical system in vitro. The in vivo system mediates conscious and sleep states. The thesis focuses on pentobarbital actions that induce network oscillations, and modify responses of single thalamocortical neurons to corticothalamic stimulus trains. The thesis addressed the following: (1) does pentobarbital induce oscillations in thalamic slices? (2) what receptors contribute to oscillations? (3) how does pentobarbital interact with modulators of excitability? (4) what are pentobarbital effects on post- and presynaptic parameters of glutamatergic transmission during short-term depression (STD)? (5) how do the effects of pentobarbital on STD compare with selective action potential blockade? (6) given the well-known actions of pentobarbital on metabolism, do its effects on STD mimic glucose deprivation?

Pentobarbital at a subanesthetic concentration induced 1-15 Hz oscillations, requiring glutamatergic excitation, but not elevated temperature or low extracellular [Mg$^{2+}$]. Glycine receptors mediated oscillations in ventrobasal nuclei, disconnected from nucleus reticularis thalami (nRT). γ-aminobutyrate (GABA) receptors mediated oscillations in isolated nRT.

By acting on N-methyl-d-aspartate (NMDA) receptors, spermine modulated membrane rectification, firing threshold, and decay of excitatory postsynaptic potentials (EPSPs). These interactions occurred at the polyamine site on NMDA receptors.
Pentobarbital enhanced STD of excitatory postsynaptic currents (EPSCs) by decreasing quantal size. These use-dependent effects persisted during blockade of desensitization and saturation of glutamate receptors and hence, likely were presynaptic. Pentobarbital decreased apparent quantal size and amplitude in the post-stimulus train, evoked miniature EPSCs (minEPSCs) but not ongoing, pre-train minEPSCs, reaffirming a presynaptic action. Pentobarbital eliminated EPSC facilitation early in a train, due to high extracellular $[K^+]_o$. Partial blockade of action potentials by tetrodotoxin reduced the apparent quantal size and evoked minEPSC size, without effect on pre-stimulation minEPSC. Like pentobarbital, glucose deprivation reduced quantal size and rundown of quantal contents. Glucose deprivation abolished STD and intra-train, post-gap jump in EPSC amplitude.

In summary, this thesis describes several new types of synaptic modulation by pentobarbital that complement known postsynaptic mechanisms of anesthesia. The analysis techniques provide a new approach for examining the pre- and postsynaptic drug effects on transmission in the brain.
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# Abbreviations

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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACSF</td>
<td>Artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5methyl-4-isoazolopirionate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APV</td>
<td>2-amino-5-phosphono-valerate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine-5'-triphosphate</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic 3'5'-adenosine-monophosphate</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CNQX</td>
<td>6-cyano-7-nitroquinoxaline</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>Concentration of drug that produces a half-maximal effect</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EGTA</td>
<td>Ethylene glycol-bis-(β-aminoethyl ether) N,N,N'N'-tetraacetic acid</td>
</tr>
<tr>
<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
</tr>
<tr>
<td>EPSC</td>
<td>Excitatory postsynaptic current</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GluR</td>
<td>Glutamate receptor</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-[2-hydroxyethyl]piperazine-N'-(2-ethanesulfonic acid)</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz (s$^{-1}$)</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Concentration of a drug that produces a half-maximal inhibition</td>
</tr>
<tr>
<td>I$_h$</td>
<td>Hyperpolarization activated inward current</td>
</tr>
<tr>
<td>I$_{Kir}$</td>
<td>Inwardly rectifying K$^+$ current</td>
</tr>
</tbody>
</table>
$I_{\text{leak}}$ Voltage-independent leak current

$I_{\text{NaP}}$ Persistent $\text{Na}^+$ current

$I_T$ Low threshold $\text{Ca}^{2+}$ current

IPSP Inhibitory postsynaptic potential

IPSC Inhibitory postsynaptic current

LTS Low threshold $\text{Ca}^{2+}$ spike

MGB Medial geniculate body

min Minute

NMDA N-methyl-D-aspartate

nRT Nucleus reticularis thalami

pH Hydrogen concentration; pH-log[\text{H}^+]

pKa Dissociation constant; pH-log[base]/[cation]

$R_i$ Input resistance

REM Rapid eye movement

SEM Standard error about the mean

$\tau$ Time constant; time required to reach (1-1/e) of a steady state value

TEA Tetraethylammonium

TTX Tetrodotoxin

$V_m$ Membrane potential

VB Ventrobasal complex of the thalamus

VPL Ventral posterior lateral thalamic nucleus
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Chapter 1
INTRODUCTION

1.1. Scope of thesis

The thesis describes in vitro experiments that delineate the modulation of corticothalamic transmission by pre- and postsynaptic actions of the barbiturate, pentobarbital. Introduced in the first half of the 20th century, barbiturates have received extensive clinical use, based on their pharmacological and pharmacokinetic properties. Drugs in this class are sedative-hypnotics, anti-epileptics, and general anesthetics. Occasionally used in humans, pentobarbital is still the most widely used general anesthetic in experimental animals. Pentobarbital produces a wide range of in vivo effects. Unlike other barbiturates, pentobarbital does not have anti-epileptic properties at subanesthetic doses. Like other barbiturates in anesthetic doses, pentobarbital is capable of terminating convulsions. The anesthetic effects of pentobarbital are due to a depression of neuronal excitability in the central nervous system (CNS). This reduced responsiveness occurs in all CNS regions, contributing to the overall loss of awareness of environment during induction of pentobarbital anesthesia.

It is generally accepted that barbiturate-induced depression involves postsynaptic interactions of neurons in the cortico-thalamocortical (CTC) system. As with other drugs, there is limited evidence for presynaptic actions of pentobarbital at central synapses. This gap in knowledge stems from the inability to obtain reliable electrical recording at axon terminals, which regulate neurotransmitter release. This thesis explores the method
of fluctuation analysis of synaptic responses to repetitive stimulation. The methodology described here facilitates the separation of pre- from postsynaptic drug effects. It provides a new approach for assessing actions of drugs, such as pentobarbital. The overall hypothesis of this thesis is that pentobarbital has actions at pre- and postsynaptic sites on neurons; reduction of excitatory transmitter release compromises transmission in the CTC system. If these effects occur at anesthetic doses in vivo, they could contribute to a loss of consciousness.

1.2. Background

1.2.1. EEG activity and brain oscillations

In the conscious brain, sleep and wake states correspond to varying degrees of synchronized oscillations in the electroencephalogram (EEG), correlative with rhythmic electrical activity of networks of neurons in the CTC system. Voltage oscillations of neurons in the CTC network produce this synchrony (Steriade, 2003). During the early stages of sleep, there is prevalent spindling EEG activity, which reflects 6-14 Hz oscillations of cortical neurons. As sleep evolves into deeper stages, this spindling behaviour transforms to the slower delta activity in the 1 - 4 Hz range. Lesions in the thalamus result in a disruption of the characteristic rhythmic EEG pattern in cortical neurons (Villablanca and Salinas-Zeballos, 1972). Thalamic neurons can generate and maintain oscillations that are independent of cortical inputs (Villablanca and Marcus, 1972). In summary, the thalamus is an essential component of the network for generating oscillations during natural sleep.
1.2.2. Receptor mediation of sleep-like oscillations in the CTC system

The synaptic interactions that mediate thalamic oscillations are complex (Jones, 2002). The type of receptors involved in generation of oscillations may alter their frequency range. For example, antagonism of ionotropic receptors for \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) enhances the light stage of non-rapid eye movement (NREM) slow wave sleep resulting in 6-14 Hz oscillations. On the other hand, N-methyl-D-aspartate (NMDA) receptor antagonism increases the deep stages while reducing the lighter stage of NREM sleep, favoring oscillations in the 1-4 Hz range (Juhasz et al., 1990). Activation of thalamic metabotropic glutamate receptors results in 1-4 Hz membrane oscillations (Emri et al., 2003). Thus, the involvement of multiple excitatory receptors in thalamic oscillogenesis provides a mean for regulating the transition between the light and deep stages of sleep.

Reduction in synaptic transmission through ionotropic receptors for the inhibitory transmitter, \( \gamma \)-aminobutyric acid (GABA) results in sustained thalamic oscillations. Blockade of type A GABA (GABA\(_A\)) receptors with the potent antagonist, bicuculline, produces delta (1-4 Hz) oscillations, characteristic of deep sleep (von Krosigk et al., 1993). Penicillin, a much weaker GABA\(_A\) receptor antagonist, is also effective in inducing sleep oscillations in vivo (Vital-Durand et al., 1972). In both cases, the oscillations result, presumably, from a reduced inhibitory receptor-mediated transmission, which heightens neuronal excitability and primes the CTC system for rhythmic activity.
1.2.3. Non-synaptic mechanisms of sleep-like oscillations

Non-synaptic interactions provide an additional mechanism to explain thalamic oscillations. These mechanisms include intrinsic ionic conductances and gap junctions. According to this view, oscillations initiate with a depolarization of thalamic neurons which brings their resting membrane potential above threshold and generates regenerative $\text{Ca}^{2+}$ spikes. Upon inactivation of the $\text{Ca}^{2+}$ channels, activation of voltage sensitive $\text{K}^+$ channels hyperpolarizes the membrane potential, which is then repolarized by the hyperpolarization-activated cation conductance ($I_{\text{H}}$). The contribution of ionic conductances has been demonstrated \textit{in vitro} and was obtained by the use of patch clamp recordings in thalamic neurons (Leresche et al., 1991, McCormick and Pape, 1990). In these studies, oscillations occurred in isolated neurons that express T-type and L-type $\text{Ca}^{2+}$ channels and were independent of synaptic inputs (Alonso and Llinas, 1989; Leresche et al., 1991). Pharmacological blockade of these channels abolished the ability to generate rhythmic patterns.

More recent studies (Hughes et al., 2002) have implicated gap junctions as mediators of thalamic synchrony. This appears to be the case in a small subset of ventrobasal thalamic neurons, but may not be significant for thalamic synchronization. Hence, intrinsic conductances are an essential requirement for generation of synchronized activity in thalamic neurons in addition to synaptic components of the CTC system.
1.2.4. Synaptic composition of the CTC system

The CTC system consists of excitatory glutamatergic and inhibitory GABAergic pathways, as well as 'presumed' glycinergic pathways (Ran et al., 2004). Thalamocortical relay neurons, in the dorsal thalamic nuclei, receive extensive connections from the

![Diagram of the corticothalamocortical circuit](image)

Figure 1.1. Diagram of the corticothalamocortical circuit. Thalamocortical relay neurons in the dorsal thalamus receive excitatory glutamatergic inputs from pyramidal neurons in the neocortex. In the neocortex, pyramidal neurons receive reciprocal excitatory glutamatergic inputs from thalamocortical relay neurons. GABAergic neurons in the reticular thalamic nucleus receive glutamatergic inputs from pyramidal neurons in the neocortex and from thalamocortical relay neurons. Reticular neurons send inhibitory projections to thalamocortical relay neurons in the dorsal thalamus. GABAergic reticular neurons contain local inhibitory collateral and dendro-dendritic synapses.
cortex via glutamatergic synapses which constitute the corticothalamic pathway (Figure 1.1). The projection of thalamocortical neurons back to the cortex is a reciprocal pathway, mediated by glutamate (Jones, 2002). There is also a unidirectional excitatory glutamatergic pathway which originates in the cortex and terminates in the reticular thalamic nucleus. In addition, neurons of the dorsal thalamus excite inhibitory GABAergic reticular thalamic neurons by a glutamatergic pathway (Ohara, 1988). Thus, corticothalamic transmission triggers a loop of excitatory and inhibitory inputs in the CTC system.

Corticothalamic activation occurs at ionotropic and metabotropic glutamate receptors (Jones, 2002). Ionotropic receptors include AMPA/kainate and NMDA receptors that mediate fast and slow excitatory postsynaptic responses, respectively. Slow excitation is also possible by activation of metabotropic glutamate (mGlu) receptors. Overall, a wide spectrum of excitatory receptors that are subject to modulation, together with inhibitory receptors, govern the pattern of CTC rhythmic activity.

1.2.5. **GABA receptors modulate the frequency of sleep-like oscillations**

Inhibitory transmission, mediated by ionotropic GABA\(_A\) receptors and metabotropic GABA\(_B\) receptors, originates from GABA neurons of the nucleus reticularis thalami (nRT) which terminate on dorsal thalamic neurons (reviewed by Llinas et al., 2005). There are inhibitory interneurons intrinsic to the dorsal thalamus that are estimated to make up 1-2 % of the total neuronal population. Activation of GABA\(_A\) and GABA\(_B\) receptors contributes to the transition between the delta and spindle rhythms (Bal et al.,
GABA receptors have been implicated in the generation of spindle rhythms (von Krosigk et al., 1993). GABA receptors are thought to exist extra-synaptically and mediate the slower delta (1-4 Hz) oscillations (Bal et al., 1995a). The different durations and locations of GABA and GABA receptor mediated responses provide frequency modulation.

1.2.6. Glycine receptors contribute to thalamic oscillogenesis

A few studies have implicated glycine receptors in thalamic inhibition in addition to GABA receptor mediated inhibition (Tebecis, 1974; Ghavanini et al., 2005). Glycine receptors have been shown to mediate theta-like oscillations (6-15 Hz) induced by pentobarbital in isolated thalamic ventrobasal slices (Ran et al., 2004). This novel pathway is quite intriguing, in light of the fact that glycine receptors mediate oscillations independent of GABA transmission in spinal neurons. Glycine receptor antagonism induces oscillations in other systems, such as in spinal cord neurons (Bracci et al., 1996). Thus, the rhythmic activity of the CTC network may be shaped by at least two types of inhibitory ionotropic receptors.

1.2.7. Barbiturate anesthetics induce sleep-like oscillations

Barbiturates induce cortical as well as thalamic oscillations, in vivo, similar to those during natural sleep. This ability is attributable to changes in membrane properties of neurons in the CTC system. The oscillations occur at frequencies in the delta (1-4 Hz) and theta (7-14 Hz) ranges, similar to deep and/or light natural sleep. The most commonly used barbiturates with this property include pentobarbital and thiopental. Less
commonly, volatile anesthetics induce \textit{in vivo} oscillations (Keifer et al., 1994). One of the mechanism by which barbiturates induce oscillations may depend on the excitable state of thalamic neurons, involving modulation of membrane properties and receptor interactions at corticothalamic synapses.

\textbf{1.2.8. Polyamine modulation of barbiturate action}

The anesthetic potency of barbiturates depends on actions on corticothalamic transmission through N-methyl-D-aspartate (NMDA) receptors, subject to polyamine modulation. For example, polyamines decrease the rate at which NMDA receptors desensitize (Lerma, 1991) whereas pentobarbital has the opposite effect (Charlesworth et al., 1995). However, the effects of polyamines at NMDA receptors include both enhancement and inhibition (Benveniste and Mayer, 1993). Spermine, among other polyamines, enhances the property of barbiturates to induce general anesthesia. This enhancement may result from dual inhibitory actions of spermine and pentobarbital at the Mg\textsuperscript{2+} site on NMDA receptors (Daniell, 1992). However, pentobarbital depression of corticothalamic transmission may prevent the prolongation of NMDA responses caused by spermine.

\textbf{1.2.9. Polyamine enhancement of corticothalamic transmission: Relevance to thalamic oscillogenesis}

Polyamines are endogenous biogenic amines that interact with synaptic and nonsynaptic targets and may modulate corticothalamic transmission or enhance thalamic excitability. The CTC circuit may be primed for oscillatory activity as a result of polyamine-mediated
prolongation of EPSPs. Similar to other polyamines, spermine acts at both sides of neuronal membrane. These interactions influence ion channels and transmitter-gated channels (reviewed by Williams, 1997). Intracellular spermine enhances membrane rectification by blocking inward rectifier K\(^+\) (K\(_{IR}\)) channels (Schuber, 1989) and confers an inwardly rectifying property on receptor-gated channels activated by acetylcholine (Haghighi and Cooper, 2000) and AMPA (Koh et al., 1995). Extracellularly, spermine interacts with receptors for NMDA resulting in the prolongation of postsynaptic responses (reviewed by Rock and Macdonald, 1995). Low concentrations of spermine enhance NMDA-evoked currents whereas high concentrations produce a voltage-dependent block of these currents in hippocampal neurons (Benveniste and Mayer, 1993). The effects of spermine and its ability to influence barbiturate actions are unknown in the CTC system.

Spermine and other polyamines are present at micromolar extracellular concentrations in the brain, including the thalamus (Harman and Shaw, 1981). This may indicate a possible role as a modulator of membrane excitability. Recent binding studies have challenged the validity of the previous measurements and estimate the extracellular concentration of spermine at < 1 \(\mu\text{M}\) (Dot et al., 2000). Neurons and glia release spermine during electrical stimulation, depolarization by high external [K\(^+\)], and activation of NMDA-receptors (Harman and Shaw, 1981; Fage et al., 1992). Uptake of spermine maintains low extracellular concentrations, presumably resulting from a constitutive release of spermine (Dot et al., 2000). Spermine uptake is regulated by various transporters in the dendrites, cell bodies, and nerve terminals, as well as by a high
affinity transporter expressed in glia (Laube and Veh, 1997). Spermine, at concentrations > 50 μM, enhances long-term potentiation (Pussinen et al., 1998; Toth et al., 2000) and neuroprotection (Trout et al., 1993; Muir and Lees, 1995; Ferchmin et al., 2000). Hence, alterations in extracellular spermine concentration can provide a unique mechanism for modulation, prolonging or shortening the duration of excitatory responses to corticothalamic stimulation.

A deficiency of spermine may have important consequences in dysfunctional states, whereas excessively high extracellular concentrations may predispose neurons in the CTC network to hyperexcitable states. For example, pharmacological inhibition of polyamine synthesis decreases polyamine concentrations in the cochlea (Schweitzer et al., 1986). This deficiency induces a temporary hearing loss in humans and rats (reviewed by McCann and Pegg, 1992), possibly due to decreased spermine-regulation of NMDA-receptor-mediated activities in cochlear neurons (Petralia et al., 2000). On the other hand, exceptionally high concentrations of spermine may exist in several neurological disorders, including neurodegenerative diseases (Yatin et al., 2001), stroke (Lukkarinen et al., 1997), global and focal ischemia (Baskaya et al., 1997; Dogan et al., 1999) and kindling epilepsy in an animal model (Hayashi et al., 1992; Herberg et al., 1992; De Sarro et al., 1993; Halonen et al., 1993). Hence, the alterations of excitable states imposed by endogenous modulators may determine the outcome of barbiturate actions on thalamic neurons. However, barbiturates may modify corticothalamic transmission independent of the excitable state of thalamic neurons during short-term alterations of synaptic strength or, short-term plasticity.
1.2.10. Pre- and postsynaptic mechanisms of short-term depression (STD)

Synaptic plasticity has long been the focus of many neuroscientists, in view of its potential roles in signal processing, learning, and memory (Fortune and Rose, 2000; Zucker and Regehr, 2002). Plastic changes in synaptic efficacy include both enhancement and reduction of neurotransmission, depending upon experimental conditions and stimulation paradigms. Enhancement can be observed both on short- and long-time scales, termed facilitation and long-term potentiation (LTP), respectively, and reduction also can be short- (seconds to minutes) or long-lasting (minutes to hours), termed short- and long-term depression (STD and LTD), respectively (von Gersdorff and Borst, 2002; Voronin, 1994). Both facilitation and depression are demonstrable on short time scales in synaptic responses evoked by pairs of evoked stimuli, generally referred to as paired-pulse facilitation or paired-pulse depression. These plastic alterations also occur during and after trains of stimuli at certain frequencies of stimulation. Although paired-pulse studies have shed light on short time scale modifications of synaptic strength, the dynamics and progression of these phenomena are rather limited. Studies that use intermediate (5-20 pulses) to long (>20 pulses) trains of stimulation pulses have provided a more comprehensive view of short-term depression and a fuller expression of these processes (reviewed by von Gersdorff and Borst, 2002).

1.2.10.1. Presynaptic mechanisms of STD

STD reflects reductions in the number of quanta released per stimulus (Elmqvist and Quastel, 1965a). This could be because of depletion of a releasable presynaptic store of quanta, consequent to release, as proposed by Liley and North (1953). However, there are
other possibilities: inactivation of presynaptic Ca$^{2+}$ channels, changes in presynaptic action potential configuration, or decreased rate of endocytosis (see below). A reduction in the amount of transmitter per quantum would also be classified as presynaptic (Elmqvist and Quastel, 1965b).

It should be noted that it is often assumed that there is a 1:1 relationship between quantum and vesicle (del Castillo and Katz, 1954). This relationship has been confirmed by increases in membrane capacitance measured during vesicular fusion (Almers and Neher, 1987). However, the relationship is in fact controversial (Matthews, 1996; Vautrin and Barker, 2003). For the present purposes, a quantum is defined as an elementary pulse of a packet of transmitter which generates a brief synchronized postsynaptic current.

Presynaptic mechanisms contribute to, or dominate, STD at low to moderate stimulus frequencies ($\leq 10$Hz ; Elmqvist and Quastel 1965a,b; Charlton et al., 1982; Emptage et al., 2001). However, at higher stimulation frequencies, postsynaptic mechanisms such as receptor desensitization or saturation, may also contribute to STD (Figure 1.4).

1.2.10.1.1. Depletion of quantal store

A simple model that assumes the release of a constant fraction of 'available quanta' with each action potential, at a constant rate of 'refill', might explain STD on the basis of store depletion (Vere-Jones, 1966). These assumptions are too simplistic for neuromuscular (Elmqvist and Quastel, 1965a) or hippocampal synapses (Rosemund and Stevens, 1996) and the calyx of Held (Wu and Borst, 1999; Schneggenburger et al., 1999). For example,
the rate of refill of transmitter packets is evidently accelerated at high frequencies of stimulation (Elmqvist and Quastel, 1965a; Wang and Kaczmarek, 1998), resulting in a faster recovery from depletion of the readily releasable pool of transmitter packets. The adjustment of the rate of refill of transmitter packet may provide a regulatory mechanism that diminishes STD in response to high frequency inputs.

Figure 1.2: Depression of end-plate potentials (EPPs) during tetanic stimulation. Extracellularly recorded EPPs decrease and reach a plateau after 8 stimuli. Plateau reflects a steady-state between depletion of transmitter packets and refill of quanta and of transmitter per quanta. Stimulation frequency was 180 Hz (From Liley and North 1953).

The release probability, presumably the fraction of 'available quanta' that are released per stimulus, varies in different preparations. Release probabilities have a heterogeneous distribution at different release sites (Wu and Borst, 1999; Sakaba and Neher, 2001). The heterogeneity of release probabilities could depend on the position of transmitter packets with respect to Ca\(^{2+}\) channels (positional heterogeneity) and/or their sensitivity to Ca\(^{2+}\) (biochemical heterogeneity). These observations led to the following modifications in the depletion model (Liley and North, 1953; Elmqvist and Quastel, 1965a, Miledi and
Thies, 1967): 1) a $\text{Ca}^{2+}$- and activity-dependent enhancement of refill (Elmqvist and Quastel, 1965a; Zimmermann and Whittaker, 1977); 2) the existence of multiple groups of transmitter packets with varying probabilities of release (Auger and Marty, 1997); and, 3) a decrease in the number of participating release sites (Weis et al., 1999).

An increase in presynaptic intracellular $\text{Ca}^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) accelerates the uptake of transmitter into packets or increases the number of releasable packets to the same extent in the absence or presence of an exogenous $\text{Ca}^{2+}$ buffer (Wu and Borst, 1999). This implies that the actions of internal $\text{Ca}^{2+}$ are indirect and may involve second messengers. Indeed, interactions of $[\text{Ca}^{2+}]_i$ with calmodulin activate protein kinases which accelerate vesicular refill and reduce store depletion (Sakaba and Neher, 2001).

The existence of groups of packets with varying release probabilities does not alter the rate of store depletion at any one site. Instead, there is a heterogeneous distribution of groups of transmitter packets in different release sites (Figure 1.2; Auger and Marty, 1997).

![Intrasite quantal variance](image1)

![Intersite quantal variance](image2)

Figure 1.3: Intra- and intersite variability of transmitter release.
1.2.10.1.2. Reduction in transmitter content

A second possible mechanism for STD is reduction in amount of transmitter per quantum (Elmqvist et al., 1965b; Naves and Van Der Kloot, 2001; reviewed by Vautrin and Barker, 2003). Blocking acetylcholine synthesis reduces the transmitter content early (Van der Kloot and Molgo, 1994) or only late (Elmqvist et al., 1965b) after prolonged neuromuscular stimulation. At CNS synapses, there are both supporting (Ishikawa et al., 2002) and contradicting (Sakaba and Neher, 2001) reports about the correlation between reduction in transmitter content and depression. Studies on rates of transmitter refill, recycling and endocytosis would clarify these discrepancies.

1.2.10.1.3. Modification of the presynaptic action potential

A third presynaptic mechanism of STD is modification of presynaptic action potential configuration (Brigant and Mallart, 1983; Smith, 1983). Also, in cultured hippocampal neurons, Na$^+$ channel inactivation produces failure in nerve conduction at presynaptic branch points, enhancing depression (Debanne et al., 1997; Brody and Yue, 2000; He et al., 2002). However, these changes occur in conjunction with changes in Ca$^{2+}$ currents. At the calyx of Held, action potentials are reduced in amplitude and increased in duration much to the same degree as Ca$^{2+}$ currents, leading to STD (Borst and Sakmann, 1999). Hence, this mechanism may co-exist with Ca$^{2+}$-dependent aspects of STD.

1.2.10.1.4. Inactivation of Ca$^{2+}$ channels

A fourth mechanism of STD involves enhanced inactivation of Ca$^{2+}$ channels at synaptic terminals. At calyx of Held neurons, the inactivation of release sites contributes to STD
during prolonged high frequency stimulation (Forsythe et al., 1998). The inactivation of Ca\(^{2+}\) channels is subject to modulation and coupling to various G-proteins by glutamate-(mGluRs), GABA\(_{B}\)-, adenosine-, and noradrenaline-receptors (Barnes-Davis and Forsythe, 1995; Isaacson, 1998; Kajiwara, 1997; Takahashi et al., 1996; Wu et al., 1998). In calyx of Held neurons, modulation by mGluRs seems to contribute to up to 10 % of depression due to inhibition of Ca\(^{2+}\) currents (von Gersdorff et al., 1997). In summary, inactivation of Ca\(^{2+}\) channels might impair the release process enhancing STD at corticothalamic synapses.

1.2.10.1.5. Interference with endocytosis

The fifth mechanism, and last to be discussed here, is the regulation of endocytosis, discovered in shibire mutant flies. These mutants suffer from severe STD and paralysis due to defective endocytosis at neuromuscular synapses (Poodry and Edgar, 1979). Recent investigations show an enhanced STD in response to genetic interference with endocytosis (Delgado et al., 2000; Luthi et al., 2001). Blockade of the action of dynamin, a regulator of vesicle endocytosis, markedly enhances STD and prolongs the recovery time at calyx of Held synapses (Takahashi et al., 2000).

1.2.10.2. Postsynaptic mechanisms of STD

1.2.10.2.1. Receptor desensitization

At high stimulation frequencies, postsynaptic receptor desensitization and saturation contribute to STD. Receptor desensitization of AMPA receptors was observed during paired-pulse depression at retinogeniculate synapses (Chen et al., 2002; Kielland and
Figure 1.4: Mechanisms of short-term depression and their presumed site of action. Different mechanisms of synaptic depression, acting at distinct points in the synaptic vesicle cycle have been proposed and can be directly studied in synapses with large synaptic terminals (Adapted from von Gersdorff and Borst, 2002).
Heggelund, 2002). However, the long recovery time (>4 s) of postsynaptic responses after STD induced by 5 or 10 Hz stimulus trains implies that receptor desensitization does not contribute to STD at all stimulation frequencies. The faster time scale of recovery from desensitization of AMPA and NMDA receptors means that these receptors do not likely contribute to STD at low frequencies of stimulation. Since AMPA and NMDA receptors desensitize on different time scales, a postsynaptic mechanism also would depend on their recovery times. However, the recovery time from STD is identical in the responses evoked on activation of both receptors. This means that receptor desensitization makes minimal contribution to STD at low frequencies of stimulation.

1.2.10.2.2. Receptor saturation

A saturation of postsynaptic AMPA receptors might also contribute to STD. Although a single quantum does not saturate AMPA receptors (Ishikawa et al. 2002), intensive stimulation of glutamate release leads to significant receptor saturation at calyceal synapses (Wu and Borst, 1999). Non-stationary fluctuation analysis methods reveal a contribution of receptor saturation to STD (Scheuss et al., 2002). Like receptor desensitization, saturation is not significant at low stimulation frequencies and is minimal at the onset of repetitive stimulation when release of transmitter is maximal (Matveev and Wang, 2000). Hence, receptor saturation does not likely contribute to depression at low stimulation frequencies.
Pentobarbital effects on a wide variety of synaptic and non-synaptic targets might provide a window of corticothalamic transmission for examining the mechanisms mentioned above.

1.2.11. Pre- and postsynaptic effects of pentobarbital

1.2.11.1. Postsynaptic effects

1.2.11.1.1. Effects on receptor systems

The most studied postsynaptic receptor target for pentobarbital action is the receptor for GABA of subtype A (GABA\(_A\)R; Macdonald and Olsen, 1994). By prolonging its decay, pentobarbital enhances the GABA-mediated Cl\(^-\) current, in a wide variety of brain preparations, including ventrobasal thalamic neurons (Wan and Puil, 2002; Table 1.1). Pentobarbital can also directly activate Cl\(^-\) currents through GABA\(_A\)R (Mathers, 1987).

In hippocampal neurons, pentobarbital has use-dependent postsynaptic actions of promoting AMPA receptor desensitization (Jackson et al., 2003), which would contribute to corticothalamic STD. Other studies have demonstrated an action of pentobarbital to promote the desensitization of the GluR2 subtype of AMPA receptors (Taverna et al., 1994). The actions of pentobarbital at this receptor subtype, also expressed in thalamic neurons (Spreafico et al., 1994), are very sensitive to the actions of cyclothiazide (CTZ), a blocker of AMPA receptor desensitization (Jackson et al., 2003).
Pentobarbital has depressant actions on NMDA receptor channels (Charlesworth et al., 1995). These actions include a reduction in the probability of channel opening, a shortening of mean open time, and a decrease of burst length.

1.2.11.1.2. Effects on non-receptor systems

The postsynaptic effects of pentobarbital on K$^+$ currents have been studied in cerebellar and hippocampal neurons (Carlen et al., 1985), and extensively studied in ventrobasal thalamic neurons (Wan et al., 2003; Table 1.1). These effects include: 1) increasing the input conductance by activating a leak current, 2) activating a voltage-dependent K$^+$ conductance, and 3) decreasing the hyperpolarization-activated Na$^+$/K$^+$ inward current (I$_h$).

Pentobarbital reduces Ca$^{2+}$ currents by increasing channel inactivation in dissociated spinal cord (Werz and Macdonald, 1985) and hippocampal neurons (ffrench-Mullen et al., 1993). These observations may explain pentobarbital effects in decreasing low-threshold spike firing in thalamic neurons (cf. Wan and Puil, 2002).

1.2.11.2. Presynaptic effects of barbiturates

Sherrington (1906) initially suggested that anesthetics reduce synaptic transmission rather than decreasing nerve conduction. Larrabee and Posternak (1952) showed that general anesthetics depress transmission through sympathetic ganglia, without effects on nerve conduction. Most investigations have dealt with the postsynaptic effects (Franks and Lieb, 1994). The contribution of presynaptic components to barbiturate anesthetic
properties has been neglected due to the difficulty in recording from axon terminals and distinguishing between their pre- and postsynaptic effects. Only two reports have indicated a reduction of transmitter release without changes in action potential configuration in CNS neurons (Mathews and Quilliam, 1964; Weakly, 1969).

1.2.11.2.1. Effects on ion channels

1.2.11.2.1.1. Na$^+$ channels

At anesthetic doses, barbiturates broaden action potentials at the frog neuromuscular junction (Thompson and Turkanis, 1973). The broadening of the action potential might result from a hyperpolarizing shift in activation (Wartenberg et al., 1999) or a use-dependent block of the Na$^+$ channel (Rehberg et al., 1995). In principle, a broader action potential should promote transmitter release, by prolonging the depolarization of the terminal and Ca$^{2+}$ influx.

1.2.11.2.1.2. Voltage gated Ca$^{2+}$ channels

Ca$^{2+}$ imaging studies show pentobarbital suppression of Ca$^{2+}$ entry into terminal branches of hippocampal neurons (Baudoux et al., 2003). These observations suggest that pentobarbital enhancement of Ca$^{2+}$ channel inactivation (ffrench-Mullen et al., 1993) may promote STD by reducing Ca$^{2+}$ entry into the nerve terminals.

1.2.11.2.1.3. K$^+$ channels

K$^+$ channels are abundant at presynaptic terminals and are highly involved in regulating transmitter release (reviewed by Dodson and Forsythe, 2004). However, there is little
Table 1.1: Summary of synaptic and non-synaptic actions of pentobarbital

<table>
<thead>
<tr>
<th>Site</th>
<th>Effect</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; or IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Neuron</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMPA</td>
<td>use-dependent inhibition</td>
<td>20 µM</td>
<td>Hippocampal (culture)</td>
<td>Jackson et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Decreased EPSP Amplitude</td>
<td>50 µM</td>
<td>Thalamocortical (slice)</td>
<td>Wan et al., 2003</td>
</tr>
<tr>
<td>NMDA</td>
<td>reduced mean channel open time</td>
<td>250 µM</td>
<td>Olfactory (culture)</td>
<td>Charlesworth et al., 1995</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;</td>
<td>prolonged current decay time</td>
<td>53 µM</td>
<td>Thalamocortical (slice)</td>
<td>Wan et al., 2003</td>
</tr>
<tr>
<td></td>
<td>increased mean channel open time</td>
<td>100 µM</td>
<td>Thalamocortical (slice)</td>
<td>Wan et al., 2003</td>
</tr>
<tr>
<td>Glycine</td>
<td>prolonged current decay time</td>
<td>30 µM</td>
<td>Spinal dorsal horn (culture)</td>
<td>Lu and Xu, 2002</td>
</tr>
<tr>
<td><strong>Ion channel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voltage-dependent Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Conduction block</td>
<td>3 mM</td>
<td>Lobster (slice)</td>
<td>Blaustein, 1968</td>
</tr>
<tr>
<td>Voltage-dependent K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>decreased I&lt;sub&gt;h&lt;/sub&gt;, I&lt;sub&gt;KIR&lt;/sub&gt;</td>
<td>8 µM</td>
<td>Thalamocortical (slice)</td>
<td>Wan et al., 2003</td>
</tr>
<tr>
<td>Voltage-independent K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Increased I&lt;sub&gt;leak&lt;/sub&gt;</td>
<td>8 µM</td>
<td>Thalamocortical (slice)</td>
<td>Wan et al., 2003</td>
</tr>
<tr>
<td>Low-threshold Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>no effect on I&lt;sub&gt;T&lt;/sub&gt;</td>
<td>≥ 100 µM</td>
<td>Thalamocortical (slice)</td>
<td>Wan et al., 2003</td>
</tr>
<tr>
<td>Voltage-dependent Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Enhanced inactivation</td>
<td>3 µM</td>
<td>Hippocampal (culture)</td>
<td>ffrench-Mullen et al., 1993</td>
</tr>
</tbody>
</table>
direct evidence for anesthetics acting on $K^+$ channels at nerve terminals. The function and properties of $K^+$ channels vary widely at presynaptic locations. These include: 1) dampening of the presynaptic action potential by low-voltage-activated $K^+$ channels; 2) faster repolarization (termination of the action potential) by presynaptic high-voltage-activated $K^+$ channels; and, 3) activity-dependent modulation of transmitter release by interplay of $K^+$ - and $Na^+$-current activation. The structure of presynaptic voltage-gated $K^+$ channels which mediate inactivating transient and non-inactivating delayed rectifier currents ($I_A$ and $I_{KDR}$) shows some similarity to $K^+$ channels located in the soma (Dodson and Forsythe 2004), suggesting possible interactions with pentobarbital.

1.2.11.2.2. Effects on the release machinery

There are no reports on pentobarbital interactions with the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) proteins, which mediate exocytotic transmitter release at active zones (cf. Duman and Forte 2003). However, imaging techniques demonstrated a pentobarbital effect of suppression of transient increases in intracellular $[Ca^{2+}]$ at axon terminals associated with a decrease in the amplitude of spontaneous EPSPs (Baudoux et al., 2003). Since SNARE proteins are sensitive to intracellular $Ca^{2+}$ alterations, one can assume an indirect effect that could impair or increase release.

1.2.11.2.3. Effects on energy metabolism

Pentobarbital impairs respiratory metabolism as well as glutamate synthesis, presumably at presynaptic terminals. Early studies have shown that pentobarbital reduces cellular
Chapter 1. Introduction

1.2.1. Effects on transmitter release

1.2.1.2. Evoked release

The presynaptic actions of pentobarbital were initially studied in spinal motoneurons (Weakly, 1969), and involve a reduction in evoked transmitter release. Subanesthetic concentrations of pentobarbital decreased the number of released quanta, without changing the input resistance and firing threshold (quantal content; Figure 1.1). Hence, the study demonstrated for this site a presynaptic effect of pentobarbital with a negligible postsynaptic effect.

In later studies, pentobarbital was found to enhance evoked transmitter release at the neuromuscular junction by increasing the number of quanta released by nerve stimulation (Thomson and Turkanis, 1973; Seyama and Narahashi, 1975; Weakly and Proctor, 1977). The contrasting enhancement and reduction of transmission were dose-dependent and attributable to the combined pre- and postsynaptic actions of pentobarbital (Proctor and Weakly, 1976). The enhanced quantal release was assessed from the ratio of evoked to
spontaneous end-plate potentials (EPPs), whereas postsynaptic effects were identified as decreased miniature amplitudes. Although initially the increased quanta was presumed to result from the increased duration of a broader presynaptic action potential (Thompson and Turkanis, 1973), subsequent studies demonstrated no such effect (Weakly and Proctor, 1977). In summary, barbiturates have dose-dependent effects attributed to dual pre and postsynaptic actions that include both increase and decrease of quantal release and reduction of quantal size (Figure 1.2).

1.2.11.2.4.2. Spontaneous release

Studies on spontaneous release provided greater detail about the complex nature of barbiturate effects. Barbiturate application increased the frequency and decreased the amplitude of spontaneous acetylcholine release, independent of extracellular $[\text{Ca}^{2+}]$ (Pincus and Insler, 1978). These observations were quite similar to the $[\text{Ca}^{2+}]$-independent enhancement of miniature EPP frequency by ethanol (Quastel et al., 1971). These results implied that an effect of barbiturates on $\text{Ca}^{2+}$ uptake determined the magnitude of the changes in quantal content (Rahamimoff et al., 1976). In summary, barbiturates have opposing effects at the neuromuscular junction – a presynaptic action that increases miniature EPP frequency and quantal content and a postsynaptic action that increases membrane conductance, reducing amplitude of spontaneous and evoked EPPs. Due to lack of reliable methods of assessment, the effects of barbiturates on transmitter release have not received study at central synapses. As investigated in this thesis, the use of fluctuation analysis can provide an accurate measure of barbiturate modulation of quantal parameters during short-term synaptic plasticity.
1.2.12. Theory of depletion model and fluctuation analysis

Statistical estimation of synaptic plasticity dates back to the work of Del Castillo and Katz (1954) who analyzed amplitude fluctuations of spontaneous and evoked responses at the neuromuscular junction. The resemblance of incremental amplitude fluctuation to the mean amplitude of spontaneous miniature synaptic events formed the basis of a ‘quantal hypothesis of transmitter release’ (Del Castillo and Katz, 1954). According to this hypothesis, 3 parameters describe transmitter release at a given synapse: (1) the average amplitude of the postsynaptic response (Q); (2) total number of independent release sites at the synapse (N); and, (3) the average probability of release across all sites (p). Changes in p and Q constitute respectively the pre- and postsynaptic strength of synaptic connectivity and either one or the other must be altered whenever there is plastic modification in synaptic transmission. Alterations in these parameters reflect a drug’s mechanism of action, e.g., a drug that acts exclusively at postsynaptic receptors must modify Q without an effect on p or N.

Since the classical approach to fluctuation analysis (del Castillo and Katz, 1954; Boyd and Martin, 1955) of the synaptic response depends upon quantal content being low enough that there are many 'unit' responses, alternative statistical methods for analyzing quantal release, collectively called variance-mean analysis are often employed (Elmqvist and Quastel, 1965a; Vere-Jones, 1966; Clements, 2003). The variance-mean analysis uses the relation between the mean and the variance of iterated synaptic responses. The binomial model of transmission is valid if the variance has a parabolic relation to the mean of the response (Clements, 2003); the variance/mean ratio gives a measure of Q
(Elmqvist and Quastel, 1965a,b; Clamann et al., 1991; Silver et al., 1998; Scheuss and Neher, 2001). A major limitation of the variance mean approach is that it is restricted to steady-state responses under stationary conditions of release, i.e., all release sites are assumed to be independent and have the same release probability. When applied to long trains of synaptic responses, the variance-mean analysis is useful for the study of short- and long-term modification of synaptic plasticity (Elmqvist and Quastel, 1965a). The present study utilized a modification of the variance-mean analysis (see later in this section).

The use of the classical quantal analysis presents some difficulties in interpreting changes in $p$, $Q$, and $N$. This method necessitates the use of clearly detectable mean response amplitudes, plotted in an amplitude histogram. In many preparations and various CNS neurons, however, the quanta are not easy to detect and do not form clear peaks in the histogram. Such difficulty may result from sampling error or low signal to noise ratio. Additional factors that interfere with detection of quantization include high quantal content and variability in quantal size. In some preparations, quantal size is highly variable in the range of 44-90%, as observed in distributions of miniature excitatory postsynaptic currents (mEPSCs; Frerking and Wilson, 1996). The heterogeneity in the probability of transmitter release is in the range of 22-71% in spinocerebellar tract neurons (Walmsley et al., 1988) and > 50% in hippocampal neurons (Murthy et al., 1997). The variability in quantal size and release probability necessitate modifications of the binomial model, such as the compound binomial, multinomial and compound
multinomial models (Brown et al., 1976; Redman, 1990; Quastel, 1997; Silver et al., 1998).

Despite limitations, the variance-mean method provides an independent approach for comparing the experimental means and variances with the model-based fits. An advantage is that changes in synaptic parameters due to deviations from the simple assumptions of the binomial model are reflected in the slope of the linearized variance-mean plot (Silver et al. 1998). The variance-mean method also is useful for analysis of initial synaptic responses in long trains at different frequencies, and at various probabilities of release due to systematic variations in the external [Ca$^{2+}$]. In cases where quantal content varies within the train, however, the variance-mean method, per se, cannot follow gradations of the synaptic parameters (p and Q) within the train.

Another difficulty in the classical quantal hypothesis is the assumption that there is constant number of participating release sites, defined as stationary. During repetitive stimulation, however, this assumption is not valid as the number of participating sites is continuously altered and hence is non-stationary. Vere-Jones (1966) and Quastel (1997) pointed out an inherent variation of N that occurs during release. Such variations can be estimated by using covariance analysis methods derived by Vere-Jones (1966), Quastel (1997), and Scheuss and Neher (2001) from the mathematical model of Vere-Jones (1966). These methods essentially correct the variance/mean ratio for the effect of p and provide, in principle, estimates of Q at successive responses in iterated trains. The methods depend upon the validity of the binominal model, which is indicated by the
existence of negative correlations between responses to successive stimuli (Elmqvist and Quastel, 1965a; Scheuss and Neher, 2001). Assuming the maximal release of one packet of transmitter per stimulus per release site and little 'refill', a negative correlation between successive responses occurs because no release by one stimulus precludes release at the next (Vere-Jones, 1966). The correlation between successive stimuli might be influenced by presynaptic modulators of release (Vizi and Somogyi, 1989). A sudden increase in the rate of refill should show up as a positive interstimulus correlation (Matveev and Wang, 2000).

By using the binomial depletion model discussed above, one can assess the effects of barbiturates on quantal parameters of transmission in the thalamus during short-term alterations in synaptic plasticity such as STD.

1.3. Rationale

Why study STD in the thalamus? STD contributes to the generation of oscillations, an essential behaviour of thalamic neurons (Steriade 1999; Castro-Alamancos and Calcagnotto, 1999). For example, the effects of STD on thalamic firing behaviour depend on the composition, desensitization, and saturation of postsynaptic receptors in thalamic neurons (Chen et al., 2002; Bartlett and Smith, 2002). There are no reports on the presynaptic mechanisms that mediate short-term plasticity at corticothalamic synapses. Not surprisingly, most neuroscientists have assumed that thalamic plasticity is entirely postsynaptic (Castro-Alamancos and Calcagnotto, 1999). This view has received
challenge by a demonstration of a presynaptic form of LTP at corticothalamic synapses (Castro-Alamancos and Calcagnotto, 1999). A frequency- and Ca$^{2+}$- dependence and decreased paired-pulse facilitation characterized this form of LTP. However, the previous study lacked a continuous measure of the plastic alterations, which would emerge during longer stimulation train, en route to LTP.

Why study the effects of barbiturates on STD? A barbiturate-induced depletion of transmitter packets at nerve terminals might promote STD during corticothalamic transmission. The best support for such a mechanism is based on the enhanced quantal release observed in spinal motoneurons (Weakly, 1969). The increase in released quanta depletes the store of transmitter packets, which could exaggerate STD.

The interactions of barbiturates at the neuromuscular junction and on spinal motoneurons provide some rationale for studying the pre- and postsynaptic aspects of corticothalamic transmission. Thalamocortical neurons in vivo are indeed sensitive because barbiturates disrupt their characteristic firing behaviour (Contreras and Steriade, 1996). Current theory maintains that these effects are postsynaptic and are due to enhanced GABAergic inputs. In some studies (Castro-Alamancos and Calcagnotto, 1999), thalamocortical neurons display postsynaptic mechanisms of plasticity. Recent studies have shown that NMDA receptors may enhance, whereas GABA receptors may reduce the synaptic strength of corticothalamic responses. The plastic effects of STD would contribute to synaptic connectivity of the thalamus during development (Bartlett and Smith, 2002). Both NMDA and GABA receptors are well-documented postsynaptic targets for
anesthetics but there is little or no information about potential presynaptic mechanisms that may explain the observed barbiturate-depression of synaptic responses (Richards, 1971; Sawada and Yamamoto, 1985).

1.4 Objectives and research approach

One of the objectives of the present study was to assess the changes in quantal content and size, number of release sites, and rate of vesicular refill during repetitive stimulation, by using a corrected version of the variance-mean method. The correction of the variances was obtained using the covariances between successive synaptic stimuli (Vere-Jones, 1966; Quastel, 1997; Scheuss and Neher, 2001). The present study examined how barbiturate anesthetics alter these corrected parameters. The hypothesis was that any effect of pentobarbital on corticothalamic transmission, manifested in a change of STD during repetitive stimulation, must be reflected in changes of quantal content and/or quantal amplitude (Q).

The study examined the anesthetic effects on synaptic transmission during repetitive stimulation of corticothalamic axons, in order to allow an expression of short-term depression. The binomial model was used to estimate changes in synaptic parameters such as quantal size and content, number of release sites, and rate of refill of transmitter packets. These include STD, observed as decreases in amplitude ('rundown') of the initial synaptic responses and increases in amplitude of synaptic responses observed after a recovery from intra-train gap between stimuli. The findings enabled an assessment of the
interactions of endogenously released glutamate with AMPA receptors while differentiating pre- from postsynaptic mechanisms of STD. By pharmacological inhibition of AMPA receptor desensitization, it was possible to distinguish a postsynaptic contribution to STD during repetitive stimulation.

The validity of the covariance-corrected variance/mean method of determining quantal amplitude was established in control experiments which showed consistency with the binomial model - in particular, negative correlations between responses to successive stimuli that conformed with predictions of the model. These investigations represent a determination of anesthetic effects on both pre- and postsynaptic aspects of excitatory synaptic transmission, for the first time in neurons of the CNS.

The anesthetic interactions with corticothalamic transmission presumably pertain to the mechanism of barbiturate anesthesia. The investigations validated a method for estimating the pre- and postsynaptic contributions to synaptic plasticity. Hence, the present study obtained new knowledge about anesthetic interactions with the mechanisms of plasticity, perhaps relevant to drug-induced amnesia as well as unconsciousness.

The effects of pentobarbital on axonal conduction (Blaustein, 1968) and shunting of thalamocortical neuron firing (Wan and Puil, 2002), suggested a presynaptic blockade of action potential. Thus, it was worthwhile to compare the effects of Na\(^+\) channel blockade with tetrodotoxin to those of pentobarbital.
The high energy demand of repetitive stimulation and the effects of pentobarbital on metabolism (Quastel and Wheatley, 1932; Crane et al. 1978) provided rationale to examine whether conditions of energy shortage would promote STD. For this reason, STD was examined during conditions of energy shortage imposed by glucose deprivations.

The known depressant actions of barbiturates provided rationale to examine whether pentobarbital could reduce the effects of drugs that heighten excitability. The present studies investigated the effects of pentobarbital on thalamic hyperexcitability due to extracellular spermine. First, it was necessary to examine whether extracellular spermine would heighten excitability by actions on membrane electrical properties, synaptic activation, and firing modes of thalamic neurons. Only a few investigations have addressed this issue in CNS neurons, finding that millimolar concentrations of spermine depress population excitatory postsynaptic potentials (EPSPs) mediated by NMDA and non-NMDA-receptors, as well as inhibitory postsynaptic potentials (IPSPs) in hippocampal CA1 neurons (DiScenna et al., 1994; Eterovic et al., 1997). Secondly, I examined the interactions of pentobarbital with spermine for the presumed opposing actions of the drugs on corticothalamic transmission, which modulated thalamic excitability.

The in vivo effects suggested that pentobarbital might promote oscillogenesis in slice conditions. The present investigations also addressed pentobarbital effects on oscillatory behaviour in the corticothalamocortical network of neurons. In vitro oscillations were
induced by corticothalamic stimulation in combination with pentobarbital application at sub- and anesthetic concentrations. Participating receptors were identified by pharmacological blockade as well as surgical separation of VB nuclei from the nRT. The frequency distribution of pentobarbital oscillations was determined by using spectrocorrelograms, obtained for continuous extracellular multi-unit recordings. These investigations facilitated the identification of a pro-oscillatory action of a subanesthetic concentration of pentobarbital on the CTC network.

1.5. Major questions

Alterations in corticothalamic transmission may have a crucial role in oscillogenesis and modulatory mechanisms of anesthesia. This thesis will focus on actions of pentobarbital on thalamocortical excitability, including the modification of pre- and postsynaptic aspects of synaptic transmission. The studies addressed the following questions:

1. Is pentobarbital capable of inducing thalamic oscillations under slice conditions?

2. What receptors contribute to pentobarbital oscillations in vitro?

3. Does spermine enhance neuron excitability and does pentobarbital modify spermine action?

4. Are the assumptions made by the binomial depletion model valid during STD at corticothalamic synapses? Does the analysis reveal changes in quantal parameters that are consistent with the binomial depletion model?
5. Does pentobarbital alter STD? How do plastic alterations relate to changes in pre- and postsynaptic parameters?

6. During STD, in what ways are the actions of pentobarbital similar to selective Na$^+$ channel blockade? Does pentobarbital affect energy metabolism? Does glucose deprivation mimic pentobarbital actions?
Chapter 2

METHODS

2.1. Slice preparation

The Animal Care Committee at The University of British Columbia approved the procedures for these experiments. Experiments were performed on young adult Sprague-Dawley rats or gerbils (age 12-15 days) since they lack extensive myelination and are therefore ideal for proper formation of patch-clamp seals. Animals were decapitated while under deep isoflurane anesthesia. The cerebral hemispheres were quickly removed (~1 min) from the cranial vault and immersed for 1-2 min in ice-cold (0-2 °C) sucrose solution. The sucrose solution contained (in mM): sucrose, 248; NaHCO₃, 26; glucose, 10; KCl, 2.5; CaCl₂, 2; MgCl₂, 2; and Na₂HPO₄, 1.25. The brain was quickly transferred to artificial cerebrospinal fluid (ACSF), which had the same composition except for 124 mM NaCl instead of sucrose. In repetitive stimulation experiments, glucose concentration was increased from 10 to 25 mM and the following drugs added (in mM): myo-inositol 3, Na-pyruvate 2, and ascorbic acid 0.4. This altered ACSF composition (320 mOsm) enhanced to the vitality of repetitively stimulated neurons. The ACSF, on saturation with 95% O₂ and 5% CO₂, was adjusted to a pH of 7.3-7.4. The brain was trimmed into a cube (~0.5 cm³) containing the cortex and thalamus. A Vibroslicer (Campden Instruments, London, England) was used to cut 250 µm slices, for whole-cell recording, or 500 µm slices, for extracellular recordings. The slices were cut in a hybrid coronal plane that formed a 45° angle with a sagittal plane. The slices used for extracellular recordings were somewhat thinner than similar slices described by Tancredi et al. (2000).
The increased thickness (500 μm compared to 250 μm) facilitated the induction of pentobarbital oscillations in vitro. The use of a hybrid cut slice maximized the corticothalamic fiber content and enhanced the ability to evoke excitatory postsynaptic potentials (EPSPs) or currents (EPSCs). In a series of extracellular recording experiments, a razor blade was used to surgically separate the ventrobasal (VB) nuclei from nRT. The slices were electrically stimulated by using a bipolar tungsten electrode (tip diameter ~100 μm), placed in the slice at 0.2–0.3 mm from the recording electrode. For experiments performed in gerbils, horizontal slices were cut at 250 μm thickness that contained medial geniculate (MGB) and inferior collicular nuclei. In these slices, EPSPs were evoked by stimulating at a position mediodorsal to the MGB and near corticothalamic axons. Stimulation at this position resulted only in EPSPs. The stimuli consisted of single pulses of approximately 30 V in amplitude (range, 10–100 V) and 100–200 μs in duration. The stimulation rate was 0.5 Hz. Using these stimulus parameters, it was possible to evoke inhibitory postsynaptic potentials (IPSPs) when the electrode was placed in the brachium, midway between the inferior colliculus and MGB. In experiments performed in rat thalamus, slices included portions of the VB thalamus, internal capsule, and nucleus reticularis thalami (nRT). The postsynaptic potentials and currents were averaged and fitted with an α- function (pClamp 8 software), yielding the rise and decay time constants. Finally, the slices were incubated for 2-3 h in ACSF at room temperature (22-25 °C), until required for recording, which was carried out at 21-25°C.
2.2. Drug application

The slices were perfused on a nylon mesh with oxygenated ACSF and drugs. The drugs were prepared in distilled water, firstly as stock solutions at ~1000 times the required concentration and then frozen. Just before the experiment, the stock solutions were thawed and diluted in ACSF for application. Pentobarbital, Mg\textsuperscript{2+}-adenosine 5'-triphosphate salt (MgATP), the Ca\textsuperscript{2+} chelators, ethylene glycol-bis-(\(\beta\)-aminoethyl-ether)-\(N,N,N',N'\)-tetraacetic acid (EGTA) or 1,2-bis(2-aminophenoxy)ethane-\(N,N,N',N'\)-tetaacetate (BAPTA), Na\textsuperscript{+}-guanosine 5'-triphosphate (NaGTP), HEPES, QX-314, CsCl, D-2-amino-5-phosphono-valerate (APV), cyclothiazide (CTZ), kynurenate (KYN), and the inorganic chloride salts were obtained from Sigma (St. Louis, MO). The drugs were diluted in ACSF and the pH adjusted in the range of 7.3-7.4. Extracellular solutions were delivered in two ways: 1) bath applications performed using a roller-type pump at a rate of 2 ml/min through a submersion-type of chamber with a volume of ~ 0.3 ml, and 2) local application by a glass pipette (~100-200 \(\mu\)m tip diameter) connected by polyethylene tubes to the various drug reservoirs. The local application approach allowed for a rapid switch (within < 5 s) between the various drugs (Quastel et al., 1971).

2.3. Extracellular recording

Extracellular multiunit activity was recorded in lateral and medial portions of ventral posterior thalamus of submerged slices. The glass electrodes had tip diameters of ~1 \(\mu\)m and resistances of 2-5 M\(\Omega\) when filled with 4M NaCl. Aided by light microscopy (Zeiss Axioskop, Jena Germany), the electrode was positioned at 25-50 \(\mu\)m under the slice
surface and recorded multiple unit potentials with an isolation amplifier (World Precision Instruments, Sarasota, FL, USA). The signals were bandpass filtered with series, low and high-pass 8 pole Bessel filters (cutoffs, 1 and 16 Hz). The signals were digitized at 5 kHz, and stored and analyzed (in part) with Axoclamp 8.2 software (Axon Instruments, Foster City, CA).

2.4. Whole-cell recording

The electrical recordings were made in the current- and voltage-clamp modes of an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). A pClamp 8.2 software (Axon Instruments) was used on a Pentium computer for data acquisition, storage and analysis. The voltage values were corrected for a measured junction potential of -11 mV. For voltage-clamp recordings, the recording electrode were coated with Sylgard and the volume of the bath solution lowered in order to minimize electrode and bath capacitance, respectively. In current-clamp experiments, the intracellular patch solution contained (in mM): K-gluconate, 140; N2-hydroxyethylpiperazine-N2-ethanesulfonate (HEPES), 10; KCl, 5; NaCl, 4; adenosine 5-triphosphate (disodium salt), 3; guanosine 5-triphosphate (trisodium salt), 0.3; EGTA, 10; and CaCl$_2$, 1. This combination of EGTA and Ca$^{2+}$ yielded a final [Ca$^{2+}$] of 10 nM (calculated with Max Chelator software). In experiments with BAPTA, EGTA was substituted with an equimolar concentration of BAPTA, which yielded a final [Ca$^{2+}$] of 1 nM. In voltage-clamp experiments, the patch solution contained (in mM): Cs-gluconate 125; TEA-Cl, 20; the lidocaine derivative QX-314, 3; HEPES, 10; Na-phosphocreatine, 5; Mg-ATP, 4; GTP, 0.4; and EGTA, 10, pH 7.3, 295-
300 mOsm. The intracellular presence of these drugs minimized the contribution of postsynaptic Na\(^+\), K\(^+\), and Ca\(^{2+}\) channels while achieving an improved space clamp (Cahalan and Almers 1979; Konishi 1990; Budde et al. 1994).

2.5. Detection of signals

2.5.1. Direct method

An even number of points (typically 4-10) were averaged around the point of largest value (Figure 2.1). This approach reduced the error due to noise at the local maximum point. The amplitude of the initial response was subtracted from baseline. Overlapping responses were obtained by subtraction of the single exponential fit of the late component from the preceding response.

![Figure 2.1: Direct method of peak detection](image)

2.5.2. Deconvolution method

Another method for obtaining peak amplitude was deconvolution. The recorded signal represents the convolution of the time course of release of multiple quanta by the time course of a single quantum (Figure 2.2). Deconvolving the signal by the long \(\tau\) of a quantum will yield a value independent of superposition on the tail of a previous signal.
If $a = e^{-1/\tau}$ where $\tau$ is the time constant of decay of individual quanta, then the deconvolution of a signal $y_i$ to produce: $y'_i = y_i - a \cdot y_{i-1}/(1-a)$ excludes the components of signal amplitude due to the 'tail' of a previous signal. The resulting peaks are, however, much noisier than the original signal.

![Deconvolution Diagram]

Figure 2.2: Peak detection obtained by using deconvolution method.

2.5.3. *First and second derivative method*

Peak were also detected by looking for zero crossings of the first and second derivatives (Figure 2.3). In this method, the zero crossing of the first derivative occurs at the location of the peak. The second derivative crosses zero at the point of maximal rise of the signal. The two points were used to obtain a single exponential fit that contained the peak estimate.

![Derivative Diagram]

Figure 2.3: First and second derivative method for peak detection. The first and second derivatives were used to detect the location of the peak (1) and the maximal rate of rise (2), respectively. The single exponential fit contained the peak estimate (1), above the zero crossing of the first derivative (Chen and Regehr, 1999).
Table 2.1: Comparison of peak detection methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Direct</th>
<th>Deconvolution</th>
<th>1st and 2nd derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$ (nA)</td>
<td>1.25 ± 0.35</td>
<td>1.25 ± 0.24</td>
<td>1.23 ± 0.27</td>
</tr>
<tr>
<td>$S_{10}$ (nA)</td>
<td>0.56 ± 0.11</td>
<td>0.53 ± 0.08</td>
<td>0.55 ± 0.09</td>
</tr>
<tr>
<td>$S_1/S_2$</td>
<td>0.58 ± 0.08</td>
<td>0.56 ± 0.06</td>
<td>0.57 ± 0.09</td>
</tr>
<tr>
<td>$S_{12}/S_{10}$</td>
<td>1.35 ± 0.12</td>
<td>1.31 ± 0.13</td>
<td>1.34 ± 0.10</td>
</tr>
<tr>
<td>Var($S_1$)</td>
<td>0.023 ± 0.007</td>
<td>0.021 ± 0.005</td>
<td>0.024 ± 0.009</td>
</tr>
<tr>
<td>Cov($S_1$, $S_2$)</td>
<td>- 0.010 ± 0.003</td>
<td>- 0.012 ± 0.004</td>
<td>- 0.009 ± 0.005</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; $S_i$ is EPSC amplitude where $i$ corresponds to EPSC number. Var – variance, Cov – covariance (averages from 5 neurons).

Table 2.1 compares EPSC parameters obtained by the 3 peak detection methods. There were no significant differences between the 3 methods ($P < 0.05$, ANOVA test). Values obtained by the deconvolution method were normalized to the 1st peak value and rescaled by the mean amplitude obtained by the direct method. The direct and 1st and 2nd derivative methods did not require any scaling. Since a choice of the peak detection method did not produce major errors in parameter values, the direct method was used throughout the study, for the data presented in the RESULTS section.
2.6. Repetitive stimulation

Excitatory post-synaptic potentials (EPSPs) or currents (EPSCs) were evoked by stimulating a small portion of corticothalamic projections to VB thalamic neurons. For stimulation, a bipolar tungsten electrode was placed in the internal capsule (IC). The IC was first stimulated with a strong (100V, 50-400 μs) stimulus, the amplitude of which was reduced as the electrode approached the surface of the fibers. Once the electrode was just above the IC fibers the amplitude of the stimulus was reduced to a value that was twice the minimal required to evoke EPSCs (or EPSPs). This indicated a contribution of a small number of stimulated fibers. Under these conditions of stimulation, trains of 20 stimuli were evoked at various frequencies (2.5-20 Hz) and statistical analysis performed using a binomial model.

2.7. Induction of plastic modifications of corticothalamic synaptic responses

In early experiments, short-term depression was induced in a long train of repetitive stimulation (50 pulses) at 2.5, 5, 10, and 20 Hz. Trains were applied with a 20 s inter-train interval to allow complete post-stimulation recovery. In most experiments, trains were 20 pulses; in preliminary experiments, the time course of post-stimulation recovery was assessed by applying trains with alternating inter-train intervals of 1 and 10 s or more. Trains at the various frequencies were applied in a sequence that included all possible combinations. This approach avoided biasing results by possible 'memory' in the response from the previous frequency. For example, for frequencies 2.5, 5, 10 and 20 Hz the sequence cycle was as follows: 10, 10, 20, 20, 5, 5, 2.5, 2.5, 10, 2.5, 10, 20, 5, 20, 2.5, 5. These stimulation protocols avoided time dependent correlations at a particular
frequency. By omitting the 11th stimulus in trains of 20 stimuli, it was possible to assess the jump in response that reflects more time for 'refill' in the doubled gap between stimuli.

2.8. Analysis of extracellular recordings

For multi-unit analysis, signal-to-noise ratio (SNR) was used for comparing the relative power density of voltage fluctuations (Gabbiani and Koch, 1998). Fast Fourier Transforms were applied to filtered voltage traces, binned at 20 ms over 1-15 Hz range. Dominant frequencies were visualized with spectrocorrelograms (MATLAB 6.1). Autocorrelation functions (MATLAB 6.1) of consecutive data sweeps (10 ms bin width) were used to assess stationarity.

2.9. Fluctuation analysis of corticothalamic synaptic responses

Amplitudes of EPSCs at various locations in the train were summarized in tables showing their descriptive parameters. Namely, the EPSC amplitudes of the 1st ($S_1$), 2nd ($S_2$), and averaged responses 15th – 20th ($\text{Plateau}(S_{15-20})$). The descriptive tables also contained EPSC amplitude ratios early ($S_2/S_1$) in the train, around the intra-train omitted stimulus ($S_{12}/S_{10}$), and between the plateau and the 1st response ($\text{Plateau}/S_1$).

The following equations were used to estimate the variance and covariance. For 2 successive EPSCs of amplitude $S_i$ and $S_{i+1}$ that are repeated r times:
\[ \text{Var}(S_i) = \frac{1}{r-1} \sum_{r=1}^{r-1} (S_{i,r} - S_{i,r+1})^2 / 2 \]

Var is calculated using sequential pairs of repeats.

\[ \text{cov}(S_i, S_{i+1}) = \frac{1}{r-1} \sum_{r=1}^{r-1} (S_{i,r} - S_{i,r+1})(S_{i+1,r} - S_{i+1,r+1}) / 2 \]

cov is calculated using sequential pairs of repeats of successive responses.

**Theory of corrected variance/mean as a measure of quantal amplitude**

Assuming a binomial behavior (Vere-Jones, 1966; Quastel, 1997):

The release probability \( p \) is the product of the output probability \( p_o \), determined by the readily available pool, and the eligibility probability \( p_A \), which depends on the rate of refill and the stimulation frequency. Hence, \( p = p_o \cdot p_A \)

where \( p_o \) is the probability for an output from an 'available' site and \( p_A \) is the probability for a site to be available.

Also, \( p = \frac{m}{N} = \frac{m \cdot Q}{N \cdot Q} \) \( m \) - quantal content; \( N \) - number of release sites.

Var is expected to be:

\[ \text{Var} = m \cdot Q^2 \cdot (1 - p) \cdot (1 + CV_b^2) \]

Between site variations

\[ \text{Var} = m \cdot Q^2 \cdot (1 - p) \cdot (1 - p + CV_w^2) \]

Within site variations

where CV - coefficient of variation

The mean is always simply \( m \cdot Q \). With predominantly between-site variation in \( Q \) (Auger and Marty, 2000), the variance to mean ratio gives:

\[ \frac{\text{Var}}{\text{mean}} = Q \cdot (1 + CV_Q^2) \cdot (1 - \frac{m}{N}) = Q \cdot (1 + CV_Q^2) \cdot (1 - \frac{\text{mean}}{N \cdot Q}) \]
Consider now covariances.

At any one site if there is no refill, if there is a response at any one stimulus then there is none at the next, and vice versa

\[
\text{cov}(S_i, S_j) = \langle S_i S_j \rangle - \langle S_i \rangle \langle S_j \rangle \\
\text{cov}(S_i, S_j) = 0 - \langle S_i \rangle \langle S_j \rangle \\
\text{while } \text{Var}(S_i) = \langle S_i \rangle Q (1 + CV_Q^2) - \langle S_i \rangle^2
\]

For \(N\) sites, \(S\)'s, \(\text{Var}\)'s, and \(\text{cov}\)'s are multiplied by \(N\), hence

\[
\text{Var}(S_i) = \langle S_i \rangle Q (1 + CV_Q^2) - \langle S_i \rangle^2 / N \\
\text{cov}(S_i, S_j) = - \langle S_i \rangle \langle S_j \rangle / N
\]

That is, \(\text{cov}(S_i, S_j)\) multiplied by \(\langle S_i \rangle / \langle S_j \rangle\) is the same as the term in \(\text{Var}(S_i)\) that involves \(N\). Thus, one can define an apparent quantal size, \(Q'\), that is the same as if quantal release were Poisson distributed, for each (average) member of the train (Scheuss and Neher, 2001)

\[\text{"apparent quantal size"} = Q' = Q (1 + CV_Q^2) = \text{Var}(S_i) / \langle S_i \rangle - \text{cov}(S_i, S_j) / \langle S_j \rangle\]

The same result is obtained if variation of quantal size is within sites.

If refill (\(\alpha\)) is appreciable, \(\langle S_i S_j \rangle\) becomes positive and covariances become less negative with increased separation of \(j\) from \(i\) (Vere-Jones, 1966; Quastel, 1997). A corollary is that correction of variance/mean ratio to give \(Q'\) is incomplete, and the best one can do is use \(j = i + 1\) for choosing which covariance to use.
If, and only if, a covariance is non-zero, one can in principle estimate \( N \) from any two responses:

\[
N_{\text{cov}} = -\frac{\langle S_i, S_j \rangle}{\text{cov}(S_i, S_j)}
\]

valid only for \( \text{cov}(S_i, S_j) < 0 \) in the absence of refill.

However, \( 1 / N_{\text{cov}} = -\frac{\text{cov}(S_i, S_j)}{\langle S_i, S_j \rangle} \) is a derived number that should correspond to the \( N \) calculated from the decline in calculated quantal contents of responses in trains.

It is notable also that if there is nonstationarity of \( Q \) between trains, e.g. if local changes in conditions change 'shunting' between genesis and recording of signals, one obtains

\[
\text{Var}(S_i) = < S_i > Q (1 + CV_Q^2) (1 + \gamma^2) - \frac{< S_i >^2}{N} + S_i^2 (\gamma^2)
\]

(no refill)

\[
\text{cov}(S_i, S_j) = \frac{< S_i, S_j >}{N} + < S_i > < S_j > (\gamma^2)
\]

where \( \gamma^2 \) is the between-train variance/mean\(^2 \) which can be large relative to \( 1/N \) although much less than 1 (Quastel, personal communication). The net result is that between-train variation can lead to a diminished, nulled, or positive covariance, obscuring the negative covariance due to the binomial nature of the system. However, corrected variance-mean, \( Q' \), comes out the same as in the absence of between-train non-stationarity. The same result is obtained with between-train non-stationarity of \( N \).

**Estimation of quantal content of signals**

The number of quanta, also referred to as the quantal content \((m)\), was obtained separately from the initial five responses where EPSCs decline, and the subsequent
plateau. From the 6th to 10th stimulus, the quantal contents were obtained from the ratio of the size response to the corresponding quantal size:

\[ 'quantal\ content' = m_i = \frac{<S_i>}{Q_i}, \quad \text{where } i \geq 10 \]

The quantal contents of the 1st response were obtained by dividing the response size by an average of the quantal size at the first stimulus of various frequencies (2.5, 5, 10, and 20 Hz). The quantal contents of response 2-5 were obtained by dividing the response size by the quantal size averaged between the 2nd and 5th response.

In the absence of refill, theoretical covariances are, for any pair of outputs, in terms of S's, \( \text{cov}(S_i,S_{i+1}) = -\frac{<S_i>-<S_{i+1}>}{N} \) (see above), but with refill this becomes (Vere-Jones, 1966; Quastel, 1997):

\[ \text{cov}(S_i,S_j) = -\frac{<S_i>-<S_j>}{N} + f(\text{refill} = \alpha) \]

That is, the negative covariance becomes smaller the higher \( \alpha \), the probability of refill between stimuli. Evidently, this probability is greater the more stimuli are separated in time, causing \( \text{cov}(S_i,S_j)/(<S_i>-<S_j>) \) to be most negative when \( j = i + 1 \), and progressively less negative with \( j = i + 2, i + 3, \text{etc.} \)

**The presynaptic 'releasable store' and 'refill'**

According to the depletion model, signals fall in a train (STD) because each release of quanta from a pre-existing store leaves the next stimulus with fewer quanta to draw upon. If release probability is constant, this model gives an exponential decline of signals to a near steady state at which outputs balance refill of the store. Because the data of Elmqvist and Quastel (1965a) showed consistently higher \( S_2 \)'s than expected on this basis, they
therefore used a plot of \( <S> \) versus accumulated sum of previous \( <S>'s \) to obtain by extrapolation a number representing a total 'apparent presynaptic store' presumably equivalent to \( QN \), 'refill' being apparently small early in the train. This method was also used by Scheuss and Neher (2001). However, given negligible refill, one may also reason as follows: the first stimulus releases \( m_1 \) quanta leaving \( N - m_1 \), the second \( m_2 \) quanta, leaving \( N - m_1 - m_2 \), etc. Soon, few are left if fractional release \( (P_0) \) is more than \( \sim 0.3 \). Then the release is \( P_0 \) multiplied by \( (N - m_1 - m_2 - m_3 \ldots \text{etc.}) \). The store \( (N) \) is therefore

\[
\overline{m}_1 + \overline{m}_2 + \overline{m}_3 / P_0, \quad \text{or} \quad \overline{m}_1 + \overline{m}_2 + \overline{m}_3 + \overline{m}_4 / P_0, \quad \text{or} \quad \overline{m}_1 + \overline{m}_2 + \overline{m}_3 + \overline{m}_4 + \overline{m}_5 / P_0 \text{ etc.}
\]

Therefore, given an estimate of \( P_0 \), one has various estimates of \( N \), which become overestimates, because there is some refill, the further one goes along the train. A fair compromise between underestimation of \( N \) (at low \( P_0 \)) and overestimation (because of refill) is at \( \overline{m}_1 + \overline{m}_2 + \overline{m}_3 + \overline{m}_4 / P_0 \), or of \( QN \), using \( S \)'s instead of \( m \)'s, with \( P_0 \) estimated as \( S_1 / (S_1 + S_2 + S_3 + S_4) \) or \( S_2 / (S_2 + S_3 + S_4) \) whichever is larger (Quastel, personal communication), with the same assumption that \( Q \)'s are constant. The result is less overestimation of \( QN \), when refill (\( \alpha \)) is not negligible, than with the method of Elmqvist and Quastel (1965).

A still better estimate of the 'releasable store' is \( QN' = QN/(1 + \alpha) \) and in theory \( \alpha \) is obtainable (Vere-Jones, 1966) since at equilibrium,

\[
\overline{S}_{eq} = Q \cdot \alpha \cdot P_0 \cdot N / (\alpha + P_0 - \alpha \cdot P_0) \quad \text{while} \quad \overline{S}_1 = Q \cdot P_0 \cdot N \quad \text{giving} \quad P_0 = \overline{S}_1 / (Q \cdot N)
\]
Therefore, defining \( x = \frac{S_{\text{eq}}}{S_1} = \alpha/(\alpha + P_o - \alpha \cdot P_o) \), rearranging gives
\[
\alpha = x \cdot P_o / (1 - x + x \cdot P_o)
\]

However, this estimate is highly sensitive to error in the estimate of QN, and therefore of \( P_o \) and if \( \alpha \) is not small in the early part of STD will result in an underestimate of \( P_o \) and \( \alpha \) that cannot be corrected without information as to how \( P_o \) and \( \alpha \) change during the train. In the tables summarizing the data, QN was used simply as a descriptive measure, equal to \( \frac{S_1 + S_2 + S_3 + S_4}{p} \), with \( p \) being \( \frac{S_1}{(S_1 + S_2 + S_3 + S_4)} \) or \( \frac{S_2}{(S_2 + S_3 + S_4)} \) whichever is larger.

**The jump after an omitted stimulus**

In the plateau phase, outputs are the same. The number of quanta present at stimulus \( j \) is \( m_j \) and release is \( m_j = p \cdot n_j \) where \( p \) is fractional release. This leaves a store of \( n_j - p \cdot n_j \) and refill is \( \alpha \cdot (N - n_j \cdot (1 - p)) \). Since the next store is the same,
\[
n_{j+1} = n_j = n_j \cdot (1 - p) + \alpha \cdot (N - n_j \cdot (1 - p))
\]
and rearranging,
\[
n_j - n_{j} \cdot (1 - p) + \alpha \cdot n_j \cdot (1 - p) = \alpha \cdot N
\]
\[
n_j \cdot (p + \alpha - \alpha \cdot p) = \alpha \cdot N
\]
\[
n_j = \frac{\alpha \cdot N}{(p + \alpha - \alpha \cdot p)}
\]
This expression, derived by Vere-Jones (1966), was used above to obtain \( \alpha \).

Now, if stimulus \( j+1 \) is omitted, there is further filling, namely, \( \alpha \cdot (N - n_j) \).
After the gap, the store is

\[ n'_{j+2} = n_j + \alpha \cdot (N - n_j) \]

\[ = n_j \cdot (1 - \alpha) + \alpha \cdot N \]

\[ = n_j \cdot (1 - \alpha) + n_j \cdot p + \alpha - \alpha \cdot p \]

\[ = n_j \cdot (1 + p - \alpha \cdot p) \]

On the assumption that \( p \) does not change after the gap,

\[ m_{j+2}/m_j = n'_{j+2}/n'_j = 1 + p \cdot (1 - \alpha) \]

2.10. Statistical comparisons

A Student's t-test was used for comparing two groups and an analysis of variance (ANOVA) test for comparing more than 2 groups. In some cases, a Wilcoxon signed rank test was used for matched pairs of groups. \( P < 0.05 \) was considered significant.
Chapter 3
RESULTS

Parts of the results in the foregoing section have been published (Ran et al., 2004).

Part I. Pentobarbital oscillations in vitro in ventrobasal thalamus

3.1. Extracellular effects of pentobarbital

3.1.1. Pentobarbital application and internal capsule stimulation

Pentobarbital (PB) application (20 or 200 µM) did not produce oscillations in 6 out of 6 VB slices. Electrical stimulation of the internal capsule also did not produce oscillations in 12 out of 12 slices. Spontaneous oscillations were not observed during these conditions. However, PB at 20 µM in combination with stimulation at 0.05 Hz induced oscillations at 0.3-1 s intervals (Figure 3.1A) in all slices (n = 69). The oscillations typically lasted < 20 s from the stimulus onset and had a frequency distribution that remained approximately stationary (Figure 3.1B), as confirmed by autocorrelation analysis. Figure 1C shows that PB increased the SNR near 3 and 8 Hz, with smaller increases near 11 and 13 Hz. Since electrical stimulation was essential, PB application was a necessary, but not a sufficient condition for evoking oscillations. For convenience, these oscillatory responses will be referred to as “PB-induced oscillations”.

3.1.2. Effects of reduced extracellular Mg^{2+} ([Mg^{2+}]_e)

Application of PB increased the oscillations in slices made hyperexcitable in low [Mg^{2+}]_e media (cf. Tancredi et al. 2000; Jacobsen et al. 2001). Perfusion with low [Mg^{2+}]_e (0.65 mM) with, or without combined PB application did not result in oscillations
Figure 3.1. Pentobarbital induces extracellular oscillations in ventrobasal nuclei during electrical stimulation of internal capsule at 0.05 Hz. A) Pentobarbital (PB) reversibly evoked oscillatory discharge at 0.3-1 s intervals. B) Spectrocorrelogram of activity in slice (A) illustrates that PB (horizontal bar) induced oscillations in the 1-13 Hz range. The greyscale gradations correspond to the magnitude (black, highest; white, zero) of oscillations as a function of frequency. C) Signal-to-noise (SNR) is shown as a function of frequency, averaged from n = 6 slices in control (open circles) and n = 6 slices bathed in 20 μM PB (closed circles).
Chapter 3. Results

Figure 3.2. Pentobarbital-induced oscillations in low Mg\(^{2+}\) medium. A) Experiment similar to Figure 3.1 was conducted in a slice bathed in 0.65 mM [Mg\(^{2+}\)] (low [Mg\(^{2+}\)]\(_{e}\)). Oscillations appeared in low [Mg\(^{2+}\)]\(_{e}\) during electrical stimulation. B) PB application reversibly extended the frequency range of firing from 5-10 Hz, to 1-15 Hz. C) SNR versus frequency plots were averaged from 6 slices in low [Mg\(^{2+}\)]\(_{e}\) medium, before (open circles) and after 20 μM PB (closed circles). \(P < 0.001\) in C.
in 6 out 6 slices (Figure 3.2). Perfusion with low [Mg$^{2+}$]$_e$ (0.65 mM) and electrical stimulation resulted in oscillations at 5 to 9 Hz in 14 out of 14 slices (cf. Figure 3.1). Under these conditions, PB application increased oscillatory activity in 10 out of 10 additional slices. Figure 3.2B shows that PB application reversibly intensified the oscillations and extended their frequency range from 5-9 Hz, to 1-15 Hz. Application of PB increased the SNR predominantly near 8 Hz, and to lesser extent near 3, 11, and 13 Hz (Figure 3.2C). PB-induced oscillations did not apparently depend on the hyperexcitability due to low extracellular [Mg$^{2+}$]$_e$.

3.1.3. Effects of raised temperature.

It was necessary to determine if the frequencies of PB-induced oscillations were temperature dependent, as shown in vivo (Andersen and Andersen, 1968). Spontaneous oscillations were not observed in slices at 34 °C. Figure 3.3 illustrates a 10 °C increase in temperature did not produce significant alterations in the SNR over the 1-15 Hz range (n = 6). Since the higher temperature did not significantly affect PB-induced oscillations, the study was conducted using slices maintained near 24 °C, promoting slice viability.

3.1.4. Application of high dose pentobarbital

A 10-fold increase in PB concentration induced oscillations which subsequently ceased, despite continuing PB application with internal capsule stimulation (n = 6). Figure 3.4 shows this biphasic effect for PB at 200 μM. The frequency range (1-15 Hz) at 3 min was similar to that observed with PB at 20 μM (Figure 3.4B). The frequency range
Figure 3.3. Effects of raised temperature on pentobarbital oscillations. An increase in temperature from 24 °C to 34 °C did not alter the discharge frequency in a slice (A) or the mean SNR (B) during oscillations induced by PB (20 μM). Electrical stimulation (0.05 Hz) was applied throughout, as in Figure 3.1. n = 6, P > 0.05 in B.
Figure 3.4. Time dependence of effects of increasing concentrations on pentobarbital oscillations. A) At 200 μM, PB application evoked oscillations in a slice (24 °C) at 3 min which disappeared within 9 min of the application. B) SNR (n = 6) showed an increase at 3 min (closed circles) which disappeared within 9 min of the application (open circles). Electrical stimulation (0.05 Hz) applied throughout, as in Figure 3.1. $P < 0.01$ in B.
narrowed to 1-4 Hz before disappearance of the oscillations at ~9 min. The biphasic effect of PB at 200 μM occurred in 6 out of 6 additional slices under low [Mg²⁺] conditions and electrical stimulation (not shown). Hence, subsequent studies focused on the stable oscillations induced by PB at 20 μM under normal [Mg²⁺] conditions.

3.1.5. Effects of synaptic receptor blockade

A possible involvement of glutamate receptors in PB-induced oscillations was examined, as found for the oscillations induced by electrical stimulation and low [Mg²⁺] conditions (Tancredi et al., 2000; Jacobsen et al., 2001). Application of kynurenate (1 mM), an ionotropic glutamate receptor blocker, reversibly abolished PB-induced oscillations in 5 out of 5 slices (not shown). Hence, glutamatergic transmission was essential for the PB-induced oscillations.

Experiments using applications of GABA antagonists showed that GABA-receptors likely participate in PB-induced oscillations. During electrical stimulation, bicuculline methiodide (50 μM) reversibly induced oscillations at 1-4 Hz (n = 3, not shown). In comparison with PB, co-application of bicuculline with PB resulted in reduced oscillogenesis at 5-15 Hz in 6 out of 6 slices, without apparent changes in the 1-4 Hz oscillations (Figure 3.5A). However, recent studies have shown that bicuculline methiodide may have effects in addition to GABAₐ receptor blockade (Debarbieux et al., 1998; Seutin et al., 1997) that could account for the depression of oscillations.
The question of receptor specificity was further examined by applying another GABA_A antagonist, gabazine (Uchida et al., 1996; Seutin et al., 1997). Application of gabazine (20 μM) reversibly induced oscillations at 1-4 Hz in 6 out of 6 slices, similar to bicuculline (not shown). In comparison with PB, co-application of gabazine (20 μM) with PB resulted in reduced oscillogenesis at 5-15 Hz in 6 out of 6 slices, without apparent changes in oscillations at 1-4 Hz (Figure 3.5B). Therefore, it seemed likely that gabazine- and bicuculline-sensitive GABA_A receptor interactions participated in PB-induced oscillations at frequencies above 4 Hz.

Application of the GABA_B receptor antagonist, CGP 35348 (100 nM) during electrical stimulation, reversibly induced oscillations at 5-15 Hz in 3 slices (not shown). Co-application of CGP 35348 with PB resulted in reduced oscillogenesis at 1-4 Hz and 11-15 Hz, sparing the 5 to 10 Hz range in 6 out of 6 slices (Figure 3.5C). Thus, GABA_B antagonism induced oscillations centred near 8 Hz, and during PB application, resulted in suppressed oscillations at lower and higher frequencies.

Combined antagonism by bicuculline and CGP 35348 did not evoke oscillations during electrical stimulation in 5 slices (not shown). Unexpectedly, oscillations in the 5-10 Hz range persisted during co-application of GABA receptor antagonists with PB in 6 out of 6 slices (Figure 3.5D). Hence, GABA_A and GABA_B mediated inhibition was apparently not an absolute requirement for oscillations during PB application.
Figure 3.5 Antagonists of GABA, and glycine receptors modulate frequency of pentobarbital oscillations. A-F (n = 6 in each panel) show mean SNR as a function of frequency during application of 20 μM PB. A) Bicuculline (BIC, 50 μM) decreased the SNR of the oscillations at middle, and especially at high frequencies (P < 0.01). B) Gabazine (GBZ, 20 μM) also decreased the SNR of the oscillations in the middle and high frequencies, similar to bicuculline in A (P < 0.01). C) CGP 35348 (CGP, 100 μM) decreased the SNR of the oscillations at low and high frequencies (P < 0.01). D) Combined application of BIC and CGP 35348 did not eliminate PB enhancement of the SNR in the middle frequency range (P < 0.01). E) Strychnine (STR; 1 μM) decreased the SNR of the oscillations at low and high frequencies, similar to CGP 35348 application (P < 0.01). F) STR (1 μM) combined with BIC and CGP 35348 abolished PB enhancement of SNR (P < 0.01). In A-F, the SNR for control PB oscillations is shown as a dotted reference plot. Horizontal line, SNR = 1.
In view of the incomplete blockade of all oscillation frequencies by GABA receptor antagonism, it was necessary to apply picrotoxinin, which blocks at a different site on the GABA<sub>A</sub> receptor than bicuculline or gabazine, as well as inhibiting homomeric glycine receptors (Yoon et al., 1998). Picrotoxinin (50 μM) during electrical stimulation did not evoke oscillations in 6 out of 6 slices. Application of picrotoxinin (50 μM) (n = 4), alone, or co-applied with CGP 35348 (n = 2), eliminated PB-induced oscillations (not shown). These results are consistent with picrotoxinin blockade of GABA<sub>A</sub> and glycine receptors.

The next experiment determined the effects of strychnine, a glycine-receptor antagonist, on PB-induced oscillations. By itself, strychnine (1 μM) induced oscillations, but not during GABA receptor antagonism. These effects were not studied further in these investigations. During PB application, strychnine (1 μM) reversibly decreased the oscillations at low and high frequencies, sparing those at 5-10 Hz in 5 out of 5 slices (Figure 3.5E). This effect was similar to that of CGP 35348. During GABA receptor antagonism, co-application of strychnine with PB resulted only in rudimentary oscillations in 5 out of 5 slices (Figure 3.5F). Hence, both GABA and glycine receptors apparently modulated PB-induced oscillations.

3.1.6. Pentobarbital-induced oscillations in separated thalamic nuclei.

The possibility that GABAergic connections from nRT were necessary for PB-induced oscillations was examined by studying the effects of PB in VB nuclei and nRT, isolated from each other (Figure 3.6). In VB nuclei surgically isolated from nRT (Figure 3.7A), PB application evoked oscillations at > 5 Hz during electrical stimulation at a VB
site that was 1-2.5 mm from the recording electrode (Figure 3.6). GABA receptor blockade by co-applied bicuculline and CGP 35348 had no significant effect on the oscillations (not shown, n = 5). Hence, receptors for GABA did not appear to have a modulatory role in VB nuclei, isolated from nRT.

In view of the persisting oscillations during GABA receptor blockade, the effects of strychnine in VB nuclei were determined, after isolation from nRT. Strychnine (1 μM) reversibly eliminated PB-induced oscillations (Figure 3.7; n = 6). This implied that PB actions on glycine receptor systems were essential for the oscillations in VB nuclei, deprived of GABAergic inputs.

Since disconnection from nRT altered the frequency of oscillations in VB nuclei, the effects of PB in nRT were determined, before and after its disconnection from VB nuclei. Before disconnection, PB application (20 μM) induced oscillations at 1-12 Hz in nRT during electrical stimulation of the internal capsule in 5 out of 5 slices (1-10 Hz in Figure 3.8). Surgical disconnection from VB nuclei in these 5 slices did not significantly affect the ability of PB application to induce nRT oscillations in a similar frequency range (Figure 3.8). Hence, PB can induce oscillations in nRT, at a site distinct from VB nuclei.

GABA receptor blockade by co-applied bicuculline and CGP 35348 abolished the oscillations induced by PB in nRT (not shown). This occurred with (n = 6), or without
Figure 3.6. Photomicrograph of sagittal slice shows complete separation (asterisk) of VB nuclei from nRT. Cx, cortex; Hipp, hippocampus; IC, internal capsule; nRT, nucleus reticularis thalami; Po, posterior thalamic nucleus. Vertical arrows: rostral, R; caudal, C.
Isolated VB (no nRT)

Figure 3.7. Pentobarbital oscillations in electrically stimulated VB nuclei, after surgical separation from nRT. **Top:** In a slice (top and middle), PB (20 μM) evoked 6-15 Hz oscillations in VB nuclei, after separation from nRT. **Middle:** Co-application with STR (1 μM) abolished these oscillations. **Bottom:** Plot of SNR as a function of frequency quantifies the effects of STR in 11 slices ($P < 0.01$, $t$-test).
Figure 3.8. Pentobarbital oscillations in electrically stimulated nRT, before and after its surgical separation from VB nuclei. A) PB (20 μM) induced oscillations at 1-10 Hz in nRT during stimulation of the internal capsule. B) In another slice, PB (20 μM) induced oscillations at 1-9 Hz in nRT, electrically stimulated at 0.05 Hz after surgical separation from dorsal thalamic nuclei.
(n = 6) connections to VB nuclei. Strychnine application had no significant effect on the oscillations induced by PB in nRT, with (n = 5) or without (n = 5) connections to VB nuclei (not shown). Hence, PB can induce purely GABAergic oscillations in nRT, separated from VB nuclei.

3.1.7. Discussion

Pentobarbital oscillations required ionotropic glutamate excitation, but not elevation of temperature from 24° to 34° or low [Mg$^{2+}$] conditions. Although they can occur spontaneously under different conditions (Jacobsen et al., 2001), oscillations were never observed without electrical stimulation, in the present study. The oscillations had a broader frequency range than seen with low extracellular [Mg$^{2+}$]. Hence, pentobarbital likely acted on Mg$^{2+}$ independent sites.

Receptors for GABA modulated pentobarbital-induced oscillations. GABA$_A$ antagonism with bicuculline methiodide or gabazine eliminated oscillations at 10–15 Hz, but not in the lower frequency range. Bicuculline and gabazine had equivalent effects, with and without co-applied pentobarbital. The modulation of pentobarbital-induced oscillations was not likely due to unselective actions of bicuculline methiodide (cf. Debarbieux et al., 1998). Gabazine blocks GABA$_A$ receptors (Uchida et al., 1996) without producing the effects of bicuculline methiodide on intrinsic membrane currents in CNS neurons (Seutin et al., 1997). The above results imply that GABA$_A$ receptors modulated the oscillations in the high frequency range.
Picrotoxinin abolished oscillations induced by pentobarbital, in marked contrast to the other GABA$_A$ antagonists. Picrotoxinin itself did not induce oscillations, in agreement with previous reports (Jacobsen et al., 2001). In addition to blocking GABA$_A$ receptors, picrotoxinin inhibits homomeric glycine receptors (Yoon et al., 1998). Thus, a picrotoxinin-blockade of both GABA$_A$ and glycine receptors apparently mimicked the effects of co-applied strychnine and bicuculline. Apparently, GABA$_B$ receptors modulated the oscillations in both low and high frequency ranges. The oscillations persisted in the 5–10 Hz range during CGP 35348 (Jacobsen et al., 2001 and Ziakopoulous et al., 2000) or strychnine applications.

Unexpectedly, GABA receptor blockade did not abolish the oscillations, rather they persisted in a 5–10 Hz range. This reflected an altered network function because pentobarbital-induced oscillations in the same frequency range in ventrobasal nuclei deprived of reticular GABAergic inputs. After this surgical isolation, or during GABA receptor blockade, strychnine application eliminated the oscillations. In nRT disconnected from ventrobasal nuclei, strychnine did not affect the oscillations mediated by glutamate and GABA. In summary, pentobarbital induced oscillations in isolated networks of the ventrobasal and reticular nuclei, mediated by glutamate receptors and modulated by overlapping interactions at GABA$_A$, GABA$_B$, and glycine receptors.
Parts of the results in the following section have been published (Ran et al., 2003).

**Part II. Pentobarbital modulation of NMDA receptors in corticothalamic transmission**

The present section of the thesis addressed the issue of how modulation of NMDA receptors affects the excitability of thalamic neurons. NMDA receptors are located at distal synapses on thalamic neurons and receive extensive cortical inputs. Abnormal modulation of NMDA receptors may result in thalamic hyperexcitability, which contributes to some forms of epilepsy. A depressant action of pentobarbital may reduce the effects caused by such abnormal modulation. The following experiments examined how pentobarbital affects the modulation of thalamic excitability by spermine, an endogenous polyamine with extracellular modulatory effects on NMDA receptors.

**3.2.1 Effects of spermine**

**3.2.1.1. Tonic firing**

Spermine application reversibly increased the number of action potentials in all neurons depolarized from rest by current pulse injection. Spermine (100 μM) applied for 3 - 6 min induced tonic firing of action potentials on top of subthreshold responses. When action potentials were present in the control, spermine application increased the rate of firing (Figure 3.9A). Long recovery times of 35 - 45 min characterized spermine’s effects on thalamic firing modes after 6 min applications. In the neuron of Figure 3.9A, substantial recovery occurred at ~ 32 min after discontinuing the spermine application.
The spermine-induced increase in the firing frequency was concentration-dependent over the range of 50 and 500 μM (n = 19, Figure 3.9B). In addition, application of 1 μM spermine did not affect firing (n = 2); however, at 1 mM, there was a marked increase in the firing rate (n = 2), without any apparent recovery (data not shown). Spermine, applied at an ED\textsubscript{50} of 100 μM (cf. Figure 3.9B), reversibly increased the number of action potentials per pulse by an average of ~ 80% in nine neurons (control, 1.8 ± 0.3 action potentials/pulse and spermine application, 3.3 ± 0.4 action potentials/pulse, paired t-test, \( P < 0.01 \)).

3.2.1.2. Passive membrane properties

The increased firing due to spermine did not likely result from changes in the passive membrane properties which did not greatly change during 3 to 6 min spermine applications (cf. subthreshold responses in Figures. 3.9A and 3.11A). The average resting potentials were -67 ± 4 mV during the control period, and -66 ± 5 mV during applications of spermine at 50 - 500 μM (n = 19). Spermine application did not significantly change the mean membrane time constant (\( \tau_m = 64 ± 6 \) ms in control, and 76 ± 6 ms during spermine application, paired t-test) and mean input resistance (\( R_i = 772 ± 38 \) MΩ in control, and 756 ± 61 MΩ during spermine application, paired t-test), computed from the responses to hyperpolarizing current pulse injections in 19 neurons held at -65 mV. The lack of effect on passive membrane properties may result from spermine actions on distal dendrites. Hence, the spermine-induced effects on the passive properties could not account for the changes in firing threshold.
Figure 3.9. Spermine enhanced tonic firing in a concentration-dependent manner in MGB neurons. A) Spermine application (100 μM, 3 min) enhanced action potential firing evoked by current pulses (25 and 50 pA, 500 ms, 1.5×threshold). Holding potential, -65 mV. Vertical upper bar, 30 mV and lower bar, 60 pA; horizontal bar, 150 ms. B) Increase in number of action potentials per pulse was concentration dependent. The control firing was 1.8 ± 0.3 action potentials/pulse (n=9). The ED50 was ~100 μM for spermine-enhanced firing which approached saturation at 200 μM.
3.2.1.3. Action potential threshold

Spermine (100 μM) decreased the latency to tonic firing by decreasing the threshold (Figure 3.10). Spermine decreased the firing threshold from $-51.0 \pm 0.6$ mV to $-57.1 \pm 2.2$ mV (Figure 3.10B). Significant changes in action potential amplitude did not accompany the decreased threshold. Figure 3.10B summarizes the effects of spermine on firing threshold for six neurons.

The possibility that NMDA receptors mediated the effects on the firing threshold and tonic firing rate was examined by determining the interactions of spermine and the competitive antagonist, APV (50 μM). As shown in Figure 3.10B, spermine reduced the threshold voltage for an action potential evoked with a 500 ms current pulse, by an average of $6.2 \pm 1.1$ mV. On recovery from spermine (washout, Figure 3.10B), application of APV alone, or in combination with spermine, did not significantly change the firing threshold (during APV, $-52.1 \pm 1.9$ mV and APV + spermine, $-53.0 \pm 1.7$ mV; $n = 6$) or changes in membrane properties that could account for the blockade of the spermine-induced reduction in firing threshold. This signified that APV acted on NMDA receptors to completely block spermine action. Since these neurons had received a spermine application prior to APV, APV was also applied to five neurons that had not previously received a drug application in order to assess the possibility of constitutive release of glutamate in the slice. Here, APV produced an increase in threshold, which remained largely unaltered by a subsequent, combined application with spermine (Figure 3.10C). All neurons showed substantial recovery at 15 min after discontinuing the
Figure 3.10. Spermine (100 μM) decreased the action potential threshold. (A) Spermine application produced a leftward shift in action potential latency (current pulse duration, 500 ms). Arrows point to threshold in control and spermine. (B) NMDA receptors mediated spermine effects on action potential threshold. The control threshold was -50.9 ± 0.6 mV, which spermine reduced to -57.1 ± 2.2 mV (n = 6). Partial recovery was observed after 15 min (-52.4 ± 0.6). Blockade of NMDA receptors by APV (50 μM) reduced the threshold by < 1 mV. A reduction in threshold was not observed during co-application of APV and spermine (-0.9 ± 0.6 mV, n = 6). (C) APV (50 μM) increased firing threshold in five neurons from -52.3 ± 0.7 to -48.7 ± 0.5 mV. Subsequent spermine application did not alter the increased threshold (-49.1 ± 0.6 mV). Two-way ANOVA; * in (B) indicates P < 0.01 and in (C) P < 0.05. Vertical bar in (A), 15 mV; horizontal bar, 50 ms.
application. These data implicated an NMDA receptor mechanism in the spermine-induced decrease in the threshold.

NMDA receptors also mediated the spermine-induced increase in firing rate. In the neuron of Figure 3.11A, spermine application (100 μM, 3 min) reversibly increased the number of action potentials during a 500 ms current pulse injection from one action potential in the initial control, to three action potentials. In the neurons that had not previously received a spermine application, an increase in the current pulse amplitude during action potential blockade due to APV application, produced a return of the action potential (Figure 3.11B). The APV blockade of spermine-induced increase in firing also was overcome by an increase in the current amplitude. The APV-induced blockades of action potentials and spermine enhancement of firing were not attributable to an increased input conductance and were completely reversible. The graph of Figure 3.11C summarizes the data that implicated NMDA receptor mediation.

It was necessary to examine the possibility that non-NMDA receptors for glutamate contributed to the increased firing during spermine application. These studies determined the interactions of spermine with an AMPA receptor antagonist, CNQX. Application of CNQX (30 μM) for 6 min did not result in significant changes in evoked action potential firing, configuration, or membrane electrical properties. Subsequently, combined CNQX and spermine application did not greatly alter the reduction in threshold and ~200% increase in firing rate evoked by current pulses (amplitude ~1.5 × threshold), as observed with prior spermine application in all five neurons (CNQX, 1.4 ± 0.3 action potentials per
Figure 3.11. Spermine increased tonic firing by interacting with NMDA receptors. (A) Spermine application (100 μM, 3 min) reversibly induced firing. After a 15 min washout from spermine, APV (50 μM, 6 min), an NMDA receptor antagonist, blocked the evoked action potential. Firing was not observed during co-application of APV and spermine (3 min). Washout shows recovery at 10 min after discontinuing the co-application. Lower traces show hyperpolarizing tests for input resistance. (B) Application of APV (50 μM, 6 min) abolished firing induced by just-threshold current pulse (40 pA). A subsequent 3 min co-application of spermine and APV did not alter this suppression (lower superimposed traces in middle panel). A two-fold increase in current amplitude overcame the blockade during APV application, alone, or during co-application with spermine (upper superimposed traces in middle panel). Recovery was observed after 10 min washout. (C) Summary of spermine effects on firing in six neurons. ANOVA; *P < 0.01 – significantly different from control, **P < 0.05 – significantly different from spermine. Vertical bar, 30 mV; horizontal bar, 200 ms.
pulse, and CNQX+spermine, 4.2 ± 0.4 action potentials/pulse; data not shown). Hence, the increase in tonic firing rate due to spermine application did not likely involve AMPA receptor interactions.

3.2.1.4. Membrane rectification

The following experiments examined the possibility that the spermine-induced increase in tonic firing involved voltage-dependent membrane properties. For example, thalamocortical neurons exhibit larger voltage responses to depolarizing, compared to hyperpolarizing current pulses (Tennigkeit et al., 1996; Parri and Crunelli, 1998; cf. Figure 3.12B). Spermine (100 μM) application for 3 min increased inward rectification in a range between the rest and firing threshold, but did not appreciably change the responses at hyperpolarized potentials, down to -100 mV (n =19; Figure 3.12A). Quantification of the increase in rectification on depolarization was difficult because spermine application shortened the latency to firing (cf. arrows in Figures. 3.10A and 3.12A). Application of APV (50 μM, 6 min) completely blocked the rectification in the upper right quadrant of the voltage - current (V - I) relationship. A subsequent co-application with spermine (100 μM) did not greatly change this curve. The graph of Figure 3.12 A (right) summarizes these findings for six neurons.

There was little or no involvement of AMPA receptors in the spermine-induced (100 μM, 3 min) enhancement of rectification produced by depolarizing current pulses. CNQX (30 μM, 6 min) did not alter spermine’s effects on the rectification in five neurons. The average voltage response during co-application of CNQX and spermine (15.9 ± 0.6 mV)
was significantly different from control (11.5 ± 0.5 mV) or CNQX application (11.3 ± 0.4 mV; ANOVA, \( P < 0.05 \)). Hence, the spermine-induced increase in the depolarizing responses involved NMDA receptors, but likely not AMPA receptors.

The next investigations examined whether spermine increased the rectification in the upper right quadrant of the \( V - I \) relationship by interacting with a persistent \( \text{Na}^+ \) conductance. The rectification observed on depolarization from \( \sim -70 \) mV to threshold involved a persistent \( \text{Na}^+ \) conductance, sensitive to TTX blockade (Tennigkeit et al., 1996; Parri and Crunelli, 1998). Blockade of voltage-dependent \( \text{Na}^+ \) channels with TTX (0.6 \( \mu \)M, 6 min) decreased the slope of the \( V - I \) relationship, more in the depolarizing quadrant than in the hyperpolarizing quadrant. The blockade with TTX nullified the ability of spermine (100 \( \mu \)M, 3 min) to increase rectification on depolarization (\( n = 6 \); Figure 3.12B). The results imply that spermine increased rectification in the upper right quadrant of the \( V - I \) relationship by increasing a TTX-sensitive, voltage-dependent \( \text{Na}^+ \) conductance.

The spermine-induced enhancement of rectification on depolarization of the neuron also may depend on extra or intracellular \( \text{Ca}^{2+} \), as in neocortical neurons (Crill, 1996). Hence, the spermine-induced enhancement of voltage responses to depolarizing current injections were measured during intracellular application of BAPTA (10 mM) and extracellular perfusion with nominally \( \text{Ca}^{2+} \)-free ACSF. In the neuron of Figure 3.13A, perfusion of \( \text{Ca}^{2+} \)-free ACSF did not greatly alter these depolarizing responses. In four neurons, a 50 pA current pulse evoked average responses of 9.8 ± 0.6 mV in control
Figure 3.12. Effects of spermine (100 μM, 3 min) on membrane rectification. (A) Voltage-current (V - I) relationship of a neuron shows that spermine increased depolarizing response which was abolished during combined application (3 min) with APV. APV (50 μM, 6 min), alone, reduced rectification in upper right quadrant. V - I curve after 15 min washout shows substantial recovery. Graph at right summarizes effects of spermine, APV, and their co-application on rectification. The response on depolarization increased from 12.5 ± 0.5 mV (control) to 17.5 ± 0.8 mV during spermine application, and after 15 min washout, recovered to 12.9 ± 0.7 mV. Subsequent APV application reduced the response on depolarization from 12.9 ± 0.7 mV (first washout) to 9.2 ± 0.4 mV (APV). Co-applied APV and spermine did not greatly alter rectification (8.7 ± 0.3 mV). Recovery from APV and spermine occurred after 15 min (12.7 ± 0.5 mV). Holding potential, -70 mV. (B) V - I relationship for a neuron shows that TTX application (0.6 μM, 6 min) decreased rectification on depolarization over a ~10 mV range. Co-applied with TTX, spermine did not alter rectification in upper right quadrant. A 20 pA pulse was sufficient to observe rectification on depolarization, whereas a - 50 pA pulse produced little or no rectification on hyperpolarization. Graph at right summarizes effects of TTX and spermine on rectification. Rectification on depolarization decreased from 13.1 ± 0.8 mV (control) to 10.2 ± 0.8 mV during TTX application. A subsequent co-application with spermine did not greatly alter depolarizing responses (10.8 ± 0.7 mV). Inserts in upper left quadrants of (A) and (B) show superimposed responses (7 mV) to depolarizing and hyperpolarizing current pulses (duration 500 ms) of 60 and - 60 pA, during control (C), spermine (S), and at 3 min of co-application of TTX and spermine (TTX+S). Bar graph values are mean ± S.E.M. ANOVA test , n = 6; * and **P < 0.05.
ACSF and 10.0 ± 0.5 mV in 0 mM [Ca$^{2+}$]. The response increased to 15.2 ± 1 mV during spermine application (in 2 mM Ca$^{2+}$ ACSF; ANOVA, $P < 0.01$) which did not significantly change during combined application of spermine and 0 mM [Ca$^{2+}$] (10.2. ± 0.3 mV, $n = 4$, $P > 0.05$, ANOVA).

In contrast, the intracellular application of BAPTA, a more rapid Ca$^{2+}$ chelator than EGTA, eliminated the spermine-induced enhancement of voltage responses, observed on depolarization (Figure 3.13B). In neurons recorded with BAPTA-containing pipettes, spermine application did not alter the responses to depolarizing currents (average of 12.4 ± 1 mV in control and 12.4 ± 0.7 during spermine; $n = 4$). This implied that spermine induced either Ca$^{2+}$ entry into the neuron or a Ca$^{2+}$-dependent conductance which enhanced the subthreshold depolarizing responses and promoted rectification in the upper right quadrant of the $V$-$I$ relationship.

The spermine-induced increase in rectification on depolarization would reduce firing threshold. This provided rationale to determine if the spermine-induced reduction of the action potential threshold also depended on extra-and intracellular [Ca$^{2+}$]. The omission of Ca$^{2+}$ from the ACSF, which normally contained 2 mM Ca$^{2+}$, decreased the threshold from -53.7 ± 2.9 mV to -60.6 ± 3.1 mV ($n = 3$, ANOVA, $P < 0.05$). On application of spermine in ACSF that was nominally Ca$^{2+}$-free, there was no change in the threshold (control, -60.6 ± 3.1 mV, and spermine, -60.2 ± 3.2 mV; $n = 3$). Recovery of the threshold from the effects of the Ca$^{2+}$-free solution occurred at 10 min after returning to
Figure 3.13. Alterations in extra- and intracellular Ca\(^{2+}\) influence spermine effects on depolarizing current - voltage (\(V - I\)) relationships in MGB neurons. (A) Voltage responses to current pulses (80 pA, 500 ms in upper traces) and \(V - I\) diagram show that removal of extracellular Ca\(^{2+}\) from ACSF abolished the increase in voltage responses induced by spermine during internal application of EGTA (10 mM). Perfusion of Ca\(^{2+}\)-free media for 6 min (0 Ca\(^{2+}\)), alone, and with spermine (100 \(\mu\)M, 3 min) did not alter voltage response. After a 10 min washout, and perfusion with control media containing 2 mM Ca\(^{2+}\) (10 min), spermine application (100 \(\mu\)M, 3 min) increased the voltage response. \(V - I\) diagram in same neuron shows that spermine did not change the slope of the voltage responses during Ca\(^{2+}\)-free perfusion. When applied during extracellular perfusion with normal [Ca\(^{2+}\)], spermine increased the voltage responses to current pulses that were > 25 pA. (B) Voltage responses to current pulses (80 pA, 500 ms in upper traces) and \(V - I\) diagram show that spermine did not increase the voltage responses in a neuron recorded during internal BAPTA (10 mM). Vertical bar, 10 mV; horizontal bar, 100 ms.
normal Ca\textsuperscript{2+} perfusion. A subsequent application of spermine for 3 min in normal solution (2 mM Ca\textsuperscript{2+}) reversibly reduced the firing threshold to \(-60.0 \pm 2.6\) mV (n = 3, ANOVA, \(P < 0.05\)). These effects, observed when the pipette solution contained 10 mM EGTA, were largely reversible (recovery, \(-55.3 \pm 2.3\) mV). The spermine-induced reduction in action potential threshold was re-examined using the fast Ca\textsuperscript{2+} chelator, BAPTA (10 mM), applied internally. A 3 min application of spermine did not significantly change the threshold in four neurons recorded with BAPTA-containing pipettes (control, \(-49.3 \pm 2.1\) mV, and spermine, \(-49.3 \pm 2.3\) mV). These experiments demonstrated that the effects of spermine on action potential threshold depended on Ca\textsuperscript{2+} entry.

3.2.1.5. Low threshold Ca\textsuperscript{2+} spike (LTS) firing

Application of Ca\textsuperscript{2+}-free ACSF abolished the transient, low threshold spike (LTS), evoked at the offset of hyperpolarizing current pulses or on step depolarization in neurons held at hyperpolarized potentials. This blockade confirmed the Ca\textsuperscript{2+} mediation of the LTS (Tennigkeit et al., 1996). Spermine application increased action potential firing on top of a LTS in only 10 out of 19 neurons, in contrast to the increased tonic firing rate on spermine application, observed in all neurons. As shown in Figure 3.14A, spermine induced an action potential on the rebound depolarizing response at the termination of hyperpolarizing current pulses. In 5 out of the 10 neurons hyperpolarized with DC to -80 mV, spermine application induced one or two action potentials on a subthreshold rebound response to hyperpolarizing current pulses. Spermine application increased the amplitude
of the rebound LTS that did not reach action potential threshold in the remaining 5 neurons. The effects of spermine were reversible, requiring 20 to 40 min for recovery.

Blockade of voltage-dependent Na\textsuperscript{+} channels with TTX did not significantly alter the ability of spermine to enhance the LTS in 6 neurons (cf. Figure 3.14A and B). During TTX blockade, the spermine enhancement of the LTS depended on the holding potential. In these experiments, the LTS was evoked by injecting hyperpolarizing currents of different amplitude into neurons held at different membrane potentials (Figure 3.14C).

Co-application of spermine (100 μM) and TTX induced an LTS in neurons at potentials that caused marked inactivation of the LTS. At potentials where an LTS was present, a spermine application increased its amplitude and rate of rise (dV/dt). There was a greater increase in the dV/dt of the LTS when the neuron was held at -55 mV than at -85 mV (Figure 3.14C). After spermine application, the LTS evoked in a neuron held at -55 mV had an average dV/dt of $3.1 \pm 0.2$ mV/ms, compared to $1.5 \pm 0.3$ mV/ms in the control during TTX application. The average rate of decay was $1.7 \pm 0.3$ mV/ms (n = 6) with fast ($26 \pm 5$ ms, n = 5) and slow ($146 \pm 12$ ms, n = 5) components (trace 2, Figure 3.14B).

Figure 3.14C summarizes the effects of spermine on the dV/dt of the LTS, showing a maximal effect at a holding potential ($V_h$) = -55 mV and a minimal increase at $V_h$ = -85 mV in 6 neurons (paired t-test, $P < 0.01$).

Since the hyperpolarization-activated current influences the rate of rise of the LTS, the next experiments examined whether spermine affected the voltage sag, mediated by this current (Tennigkeit et al., 1996). The voltage sag was not prominent in the majority of
neurons. Spermine application (100 μM) produced no change in the voltage sag induced by a hyperpolarizing current pulse in three neurons. Hence, the increase in the rate of rise of the LTS did not likely involve interactions with this hyperpolarization-activated conductance.

Due to the previous observations, it was necessary to establish if the spermine potentiation of the LTS involved interactions at NMDA receptors (n = 8). In six of these neurons, APV application decreased the amplitude of the subthreshold responses to current pulses (cf. Figure 3.14D, lower traces). As shown for the neuron of Figure 3.14D, APV application also decreased an LTS burst to a single action potential and LTS rate of rise, increasing the latency to the first action potential on top of the LTS. Despite APV antagonism of NMDA receptors, spermine application transformed the subthreshold response into an LTS, as well as decreased the latency to an action potential on top of the LTS (cf. APV and spermine traces in Figure 3.14D). In eight out of eight neurons, APV application (50 μM, 6 min) reduced the average rate of rise of the LTS from 1.6 ± 0.3 mV/ms in naive controls to 1.2 ± 0.2 mV/ms. A subsequent co-application of APV and spermine caused a significant increase in the rate of rise of the LTS to 2.2 ± 0.1 mV/ms (ANOVA, P < 0.05).

In light of the previous observations, it was necessary to determine if the spermine potentiation of the LTS involved interactions with AMPA receptors. During blockade of AMPA receptors with CNQX (30 μM, 6 min), spermine significantly increased the dV/dt of the LTS (control, 1.8 ± 0.1 mV/ms and spermine, 3.6 ± 0.2 mV/ms; n = 5, ANOVA, P
Chapter 3. Results

Figure 3.14. Effects of spermine (100 μM, 3 min) on the low threshold Ca$^{2+}$ spike (LTS) firing. (A) Superimposed voltage responses (control, spermine, and recovery) show that spermine induced an LTS on termination of a hyperpolarizing current pulse (-40 pA, 500 ms). (B) Spermine increased the rate of rise and amplitude of the LTS at the end of a hyperpolarizing current pulse (-80 pA) just before (1), during (2), and after (3) spermine application during TTX blockade (0.6 μM) of voltage-dependent Na$^{+}$ conductances. Holding potential, -55 mV. (C) Bar graph summarizes spermine effects on dV/dt of the LTS at the end of hyperpolarizing responses and during TTX blockade. Spermine increased dV/dt in neurons held at -85, -75, -65, and, maximally, at -55 mV (n = 6, paired t-test, *P < 0.01, **P < 0.005). (D) APV (50 μM) did not block the effects of spermine on the LTS, as shown by sub-and suprathreshold responses to current pulses (60, 120 pA) during application of APV, alone, and co-application with spermine. Application of APV reduced the subthreshold response, LTS rate of rise, and number of action potentials. Co-application (3 min) of APV and spermine transformed a subthreshold response to an LTS, increased LTS rate of rise, and shortened the latency to the action potential. Recovery (in APV) was observed after a 10 min washout. Vertical bar, 15 mV in (A) and (B); 30 mV in (D). Horizontal bar, 150 ms. Insert in (B) (right): Vertical bar, 3 mV; horizontal bar, 30 ms.
< 0.05). Hence, the effects of spermine on the LTS did not likely involve NMDA or AMPA receptor interactions.

3.2.1.6. Excitatory and inhibitory postsynaptic potentials

Spermine application (100 μM) to 18 neurons resulted in bursts of action potentials on EPSPs evoked by electrical stimulation of corticothalamic projections (Figure 3.15A). Spermine had little or no effects on the rate of rise of the EPSP, but always prolonged the decay phase. The EPSP amplitude increased slightly (3 - 5 mV) during spermine application to five neurons, but this was not a consistent finding in the 18 neurons. The spermine-induced action potentials on the EPSPs were reversible in all neurons. Complete recovery was observed in 13 of 18 neurons at 35 min after terminating the application.

Spermine prolonged the EPSP decay time constant ($\tau_{\text{decay}}$), as estimated with an $\alpha$-function fit of the EPSPs (Figure 3.15B). This promoted the occurrence of action potentials on top of the EPSPs (Figure 3.15A). The ED$_{50}$ for the spermine-induced increase in $\tau_{\text{decay}}$ of EPSPs was ~ 100 μM which was approximately the same for the spermine-induced increase in firing (cf. Figure 3.9B). Recovery to the control value occurred after 30 min (148 ± 15 ms). Figure 3.15B summarizes these results for 15 neurons. Table 3.1 summarizes the effects of single or cumulative applications of spermine on the 90 - 10% decay time in 18 neurons. Spermine application (100 μM) did not significantly affect the amplitude or time course of depolarizing potentials evoked by
Figure 3.15. Spermine (100 μM, 3 min) prolonged late component of corticothalamic EPSPs mediated by NMDA receptors. (A) Spermine increased EPSP amplitude and duration, resulting in three action potentials. (B) Spermine delayed the late component (2) of the EPSPs. The bar graph summarizes the spermine-induced prolongation of EPSP decay time constant (τdecay), expressed as % of the control. Control τdecay was 142 ± 8.5 ms (n = 15, paired t-test, *P < 0.01). (C) Spermine did not affect EPSPs during NMDA receptor blockade by 50 μM APV or significantly change remaining EPSP components. Bar graph summarizes the reduction in EPSP τdecay by APV and lack of spermine effect during APV blockade, expressed as % of control. Control τdecay was 143.6 ± 14 ms (n = 8, ANOVA, *P < 0.01). Vertical bar, 5 mV ; horizontal bar, 200 ms.
Table 3.1: Effects of spermine on EPSP variables

<table>
<thead>
<tr>
<th></th>
<th>Amplitude (mV)</th>
<th>Rise (ms)</th>
<th>Decay (ms)</th>
<th>Half-width (ms)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 1.8</td>
<td>37 ± 6</td>
<td>477 ± 11</td>
<td>238 ± 11</td>
<td>19</td>
</tr>
<tr>
<td>Spermine</td>
<td>9.1 ± 2.3</td>
<td>20 ± 11</td>
<td>710 ± 23*</td>
<td>297 ± 85</td>
<td>19</td>
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<tr>
<td>APV</td>
<td>6.4 ± 2.1</td>
<td>12 ± 1.1</td>
<td>147 ± 9.5</td>
<td>59 ± 4.2</td>
<td>9</td>
</tr>
<tr>
<td>APV + Spermine</td>
<td>6.1 ± 1.9</td>
<td>12 ± 1.6</td>
<td>153 ± 12</td>
<td>63 ± 2.4</td>
<td>9</td>
</tr>
<tr>
<td>CNQX</td>
<td>6.2 ± 0.8</td>
<td>90 ± 5</td>
<td>534 ± 18</td>
<td>247 ± 9.5</td>
<td>9</td>
</tr>
<tr>
<td>CNQX + Spermine</td>
<td>6.3 ± 0.9</td>
<td>95 ± 4</td>
<td>925 ± 24**</td>
<td>433 ± 20**</td>
<td>9</td>
</tr>
<tr>
<td>Arcaine</td>
<td>4.5 ± 1.4</td>
<td>36 ± 7</td>
<td>419 ± 11</td>
<td>204 ± 40</td>
<td>5</td>
</tr>
<tr>
<td>Arcaine + Spermine</td>
<td>4.6 ± 0.6</td>
<td>42 ± 11</td>
<td>394 ± 15</td>
<td>205 ± 38</td>
<td>3</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.9 ± 1.1</td>
<td>24 ± 5</td>
<td>190 ± 7</td>
<td>245 ± 41</td>
<td>3</td>
</tr>
<tr>
<td>Glycine + Spermine</td>
<td>9.0 ± 2.8</td>
<td>28 ± 9</td>
<td>400 ± 21*</td>
<td>437 ± 45</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are mean ± SE. * P < 0.05, ** P < 0.01, t-test
stimulation of the brachium colliculi inferioris (n = 6; data not shown). These potentials, 100 to 200 ms in duration, were likely IPSPs mediated by GABA_A receptors because they reversed at potentials near $E_{Cl}$ (-55 mV) and were sensitive to blockade by bicuculline (50 µM, n = 6).

Spermine prolonged the decay of the late EPSP component mediated by an NMDA-type receptor. The application of APV (50 µM), blocked the late component, resulting in shorter rise (10 - 90%) and decay (90 - 10%) times of the EPSPs (Table 3.2). During NMDA receptor blockade, EPSP mean $\tau_{decay}$ was 100 ± 14 ms, compared to 143 ± 15 ms in control (Figure 3.15C; n = 8, ANOVA, $P < 0.01$). This mean value did not change significantly during combined spermine and APV application (Figure 3.15B).

Interactions with AMPA receptors, which might prolong the EPSPs, were examined by applying spermine in combination with CNQX (30 µM, 6 min). Spermine prolonged the EPSP 90 - 10% decay time during CNQX blockade to the same extent as in the absence of AMPA receptor blockade (Table 3.1). In 3 additional neurons, spermine was applied before the co-application with CNQX. The co-application resulted in a significant prolongation of the EPSP to the same extent as in the absence of CNQX blockade of AMPA receptors (Table 3.1). This confirmed that spermine affected only the NMDA-mediated component. Co-application of CNQX (30 µM) and APV (50 µM) then abolished the early and late components of the EPSP which remained absent despite a subsequent spermine application (n = 4). These results suggest that spermine increased the duration of the EPSP decay phase by interacting with NMDA receptors.
The possibility was considered that spermine prolonged the EPSPs by acting on an extracellular polyamine-sensitive site of the NMDA receptor (cf. Benveniste and Mayer, 1993). This required an investigation of the interactions of spermine and arcaine, an antagonist that acts at the polyamine-sensitive site on the NMDA receptor (Reynolds, 1990). In these studies, arcaine (40 μM), spermine (100 μM), arcaine and spermine, were sequentially applied each for 3 min (n = 3). Arcaine, alone, did not greatly alter the configuration of the EPSP (Figure 3.16A) or produce changes in the EPSP amplitude, 90 - 10% decay time, and half-width (Table 3.1). After a 15 min washout from arcaine application, spermine significantly prolonged the EPSP \( \tau_{\text{decay}} \) to 180 ± 32 ms from 101 ± 16 ms in the control (Figure 3.16A). A subsequent co-application of spermine and arcaine abolished the actions of spermine, resulting in \( \tau_{\text{decay}} \) of 118 ± 16 ms (Figure 3.16A). The graph of Figure 3.16A summarizes the spermine-induced increases in EPSP \( \tau_{\text{decay}} \) and arcaine blockade of spermine effects.

A possibility was tested that spermine increased the NMDA-mediated component of the EPSP by potentiating the actions of glycine on the NMDA receptor. In the presence of a saturating concentration of glycine (40 μM), spermine still prolonged the EPSP by ~ 49% (Figure 3.16B). In 3 neurons, spermine increased EPSP \( \tau_{\text{decay}} \) from 255 ± 44 ms to 379 ± 53 ms (ANOVA, \( P < 0.05 \)). In summary, spermine actions on the EPSP likely involved an extracellular polyamine-sensitive site, and not a glycine-sensitive site of the NMDA receptor.
Figure 3.16. Spermine (100 μM) prolonged the EPSPs by interacting with the polyamine-sensitive site on NMDA receptor. (A) Arcaine (40 μM), a blocker at the polyamine-sensitive site on NMDA receptors, almost eliminated the spermine-induced prolongation of the EPSP, expressed as % control $\tau_{\text{decay}}$ which was 100.4 ± 16 ms ($n = 3$, ANOVA, *$P < 0.05$). Note that there was a 10 min washout after arcaine, prior to spermine application. (B) Spermine (100 μM) prolonged the EPSP $\tau_{\text{decay}}$ during co-application with glycine (10 μM). Control $\tau_{\text{decay}}$ (glycine present) was 255 ± 44 ms ($n = 3$, paired t-test, *$P < 0.05$). Bar graphs summarize the effects of spermine on EPSP $\tau_{\text{decay}}$ during arcaine and glycine applications. Holding potential, -60 mV. Vertical bar, 5 mV; horizontal bar, 100 ms.
A contribution of extracellular Ca\(^{2+}\) or Mg\(^{2+}\) to the spermine-induced enhancement of EPSPs was assessed in the next experiments. Spermine application did not alter the amplitude or duration of the EPSPs during a 6 min perfusion of Ca\(^{2+}\) - free ACSF in three neurons (data not shown). Hence, spermine effects on NMDA-mediated EPSPs likely depended on Ca\(^{2+}\) entry.

In 2 neurons, the omission of Mg\(^{2+}\) from ACSF perfusion resulted in subthreshold oscillations of the membrane potential and spontaneous firing of action potentials. These observations were consistent with previous studies on thalamocortical neurons (Jacobsen et al., 2001) which prevented critical assessment of a co-agonist role of Mg\(^{2+}\) at the polyamine-sensitive site on the NR2B receptor subunit (Kew and Kemp, 1998).

3.2.2. Pentobarbital effects on corticothalamic EPSPs

The following experiments were performed in thalamic neurons of the ventroposteriorlateral thalamic nucleus of the rat (see Methods).

In non-thalamic neurons, pentobarbital, at anesthetic doses, inhibits NMDA-mediated currents (Charlesworth et al., 1995). This provided some rationale to test whether pentobarbital could depress evoked corticothalamic responses mediated by NMDA receptors in VB neurons. Pentobarbital at 200 \(\mu\)M, but not at 50 \(\mu\)M shortened the duration of the NMDA-mediated EPSPs (Figure 3.17A). This result was consistent with pentobarbital’s shortening of NMDA receptor mean open time observed in hippocampal neurons (Charlesworth et al., 1995).
The depressant effect of pentobarbital suggested that it would reduce EPSP prolongation caused by spermine. An application of spermine, at 100 µM, resulted in ~ 45% prolongation of EPSPs (Figure 3.17B). This observation was consistent with EPSP prolongation in medial geniculate neurons of gerbils (cf. Figure 3.15). During spermine application, a subsequent co-application of pentobarbital at 50 µM did not produce a significant change in the duration or amplitude of NMDA-mediated EPSPs. However, a subsequent increase in the pentobarbital dose to 200 µM produced a reversal of the spermine-mediated prolongation of the EPSP (Figure 3.17). Pentobarbital reversal of the prolongation of EPSPs caused by spermine implicated an action at specific modulatory sites.

3.2.3. Interactions of Zn$^{2+}$ with spermine and pentobarbital

The first possibility examined was that pentobarbital reversal of spermine prolongation of EPSPs involved interactions at the Zn$^{2+}$ binding site on NMDA receptors. This was done by applying Zn$^{2+}$, a negative modulator of NMDA receptors at a site distinct from that of polyamines (Forsythe et al., 1988). Application of Zn$^{2+}$ (20 µM, 1 min) resulted in a 32% reduction in EPSP decay time constant (Figure 3.17C). Zn$^{2+}$ application also reduced EPSP mean amplitude, however, this was not significant (6.1 ± 1 mV in controls compared to 4.9 ± 0.9 mV after Zn$^{2+}$, n = 5, P > 0.05). During Zn$^{2+}$ application, co-applied spermine (100 µM, 1 min) prolonged the EPSP decay by 58% (137 ± 12 ms). A subsequent co-application of pentobarbital reversed the spermine-mediated prolongation of EPSP to control levels (with Zn$^{2+}$ present, Figure 3.17), similar to the effects in the
Figure 3.17. Pentobarbital effects on NMDA-mediated corticothalamic EPSPs. (A) Pentobarbital at 200 µM, but not at 50 µM shortened the duration of the NMDA-mediated EPSPs. The bar graph summarizes the effects on EPSP decay time constant (τ_{decay}), expressed as % of the control. Control τ_{decay} was 118 ± 13 ms (n = 6, ANOVA test, *P < 0.05 - significantly different from control). (B) Pentobarbital at 200 µM, but not at 50 µM reversed the spermine-mediated (100 µM, 1 min) prolongation of the NMDA-mediated EPSPs. The bar graph summarizes the pentobarbital-reversal of spermine prolongation of EPSP decay time constant (τ_{decay}), expressed as % of the control. Control τ_{decay} was 123 ± 11 ms (n = 5, ANOVA-test, *P < 0.05 - significantly different from spermine 100 µM + pentobarbital 0 µM). (C) Zn^{2+} (20 µM, 1 min) reduced the amplitude and shortened the duration of NMDA-mediated EPSPs. Graph summarizes the effects on τ_{decay} as in A (n = 5, student t-test, *P < 0.05 - significantly different from control). (D) The presence of Zn^{2+} did not alter spermine's ability to prolong the NMDA-mediated EPSP. A subsequent co-application with pentobarbital still reversed the EPSP prolongation caused by spermine. Graph summarizes the effects on prolongation and its reversal by pentobarbital. τ_{decay} as in A (n = 5, ANOVA-test, *P < 0.05- significantly different from control or spermine + pentobarbital). EPSPs were evoked by internal capsule stimulation (50 V, 100µs). Holding potential, - 60 mV. CNQX (30 µM) was applied throughout the experiment. EPSP traces are averages of 5 samples each.
absence of $Zn^{2+}$. These observations suggested that pentobarbital acted at sites distinct from those of $Zn^{2+}$, possibly the polyamine site.

3.2.4. Antagonism of polyamine site

The lack of effect of $Zn^{2+}$ on pentobarbital reversal of spermine prolongation of EPSPs implicated interactions at the polyamine site. To test this hypothesis, spermine and pentobarbital were co-applied during blockade of the polyamine site with arcaine. By itself, arcaine (40 $\mu$M, 1 min) decreased the duration of EPSPs to 63 % of the control (Figure 3.18); an effect which indicated that endogenously-released spermine contributed to EPSP decay. During arcaine application, a subsequent co-application of spermine did not alter the amplitude or duration of EPSPs (Figure 3.18). A subsequent co-application with pentobarbital (200 mM, 3 min) did not change EPSP duration. The results suggested that the reversal of spermine prolongation of EPSPs involved an allosteric modulatory action of pentobarbital at the polyamine site on NMDA receptors.

3.2.5. Discussion

Spermine application increased the decay time constant of corticothalamic EPSPs mediated by NMDA receptors. This finding is consistent with the increased amplitude of NMDA-evoked currents during spermine application to cultured hippocampal and spinal neurons (Lerma, 1992; Benveniste and Mayer, 1993). In the present studies, the actions of spermine were selective and required extracellular $Ca^{2+}$ because they were abolished in three neurons by brief $Ca^{2+}$-free perfusion. Spermine also did not significantly alter the
Figure 3.18. Pentobarbital reversal of spermine EPSP prolongation involves interactions at the polyamine site on NMDA receptor. A) Application of arcaine (40 μM, 1 min) shortened the duration of NMDA-mediated EPSPs without affecting their amplitude. The bar graph summarizes the effects on $\tau_{\text{decay}}$ in five neurons. B) With arcaine present, spermine (100 μM, 3 min), when applied alone, or in combination with pentobarbital (200 μM, 3 min) did not cause any prolongation of the EPSP. Holding potential, -60 mV. The bar graph summarizes this lack of effect ($n = 5$, $P > 0.05$ with or without spermine). EPSP traces in A and B are averages of 5 samples each.
early EPSP component mediated by AMPA receptors, or appreciably affect IPSPs mediated by GABA receptors that were sensitive to bicuculline antagonism. Application of APV completely blocked the spermine-induced increase in the EPSP decay time constant. This implicated NMDA receptors in spermine actions to increase excitation.

The effects of spermine on MGB neurons involved a polyamine-sensitive site on the NR2B subtype of NMDA receptors, as demonstrated with arcaine and glycine applications. Arcaine, itself, did not have significant effects on the passive and active membrane properties but reversed the spermine-induced decrease of the EPSP decay. Previous studies have shown that arcaine blocks spermine actions by inverse agonism, antagonism, and open-channel blockade of the polyamine-sensitive site on NMDA receptors (Reynolds, 1990; Pritchard et al., 1994). The actions of spermine at this site decreased the EPSP decay, despite saturating concentrations of glycine. These observations are consistent with the glycine-independent potentiation of NMDA currents by spermine at the NR2B receptor subunit in cultured hippocampal neurons (Benveniste and Mayer, 1993). In thalamocortical neurons, the persistence during high glycine concentrations and arcaine reversal imply that spermine acted independently of the glycine site at a specific polyamine-sensitive site on the NR2B receptor subunit of the NMDA receptor.
The NR2B subunit may modulate the decay time constant of the NMDA receptor-mediated EPSP during the development in MGB neurons. At the end of the second postnatal week, thalamocortical neurons express an abundance of the NR2B polyamine-sensitive receptor subtype in the MGB and lateral geniculate body (LGB) of the rat (Chen and Regehr, 2000). The duration of EPSPs mediated by NMDA receptors in LGB neurons of the rat is similar at P14 to that in gerbil MGB neurons. The decay time constant in LGB neurons is longer at P14 in rats than at earlier (P7–P13) or later (P16–P28) stages of development (Chen and Regehr, 2000; cf. also rat MGB at P21–P42, Bartlett and Smith, 1999). Hence, spermine modulation of the NR2B subunit may cause the longer EPSP duration in MGB neurons at P14.

Spermine enhanced excitability by increasing inward rectification on depolarization, without greatly affecting the passive properties of MGB neurons. It is not known if the passive and active membrane properties of MGB neurons mature by P14 in gerbil, as in the rat (Tennigkeit et al., 1998). Thalamocortical neurons of the adult guinea pig and P7–P28 rat inwardly rectify because the activation of persistent Na\(^+\) conductance on depolarization results in an amplification of the voltage response (Jahnsen and Llinas, 1984; Tennigkeit et al., 1998; Parri and Crunelli, 1998). In the present studies, blockade of the TTX-sensitive rectification or NMDA receptors eliminated the spermine-induced enhancement of rectification on depolarization. These findings imply that spermine interactions with NMDA receptors led to activation of a persistent Na\(^+\) conductance in MGB neurons.
An elevation in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_i\)]) due to NMDA receptor activation (Jahr, 1992) may have enhanced rectification on depolarization. In the present study, there was no evidence for a spermine-induced increase in voltage responses on depolarization during Ca\(^{2+}\)-free perfusion or rapid chelation of Ca\(^{2+}\) with intracellular BAPTA. It seems likely that an elevation of [Ca\(^{2+}\)\(_i\)] initiated by spermine actions at NMDA receptors activated intracellular messengers and increased this rectifying behavior. In neocortical neurons, transmitter activation of dendritic NMDA receptors increases Ca\(^{2+}\) entry (Schwindt and Crill, 1995) that may increase channel phosphorylation (Siekevitz, 1991) and a persistent Na\(^+\) conductance (Schwindt et al., 1992). Hence, the spermine-induced enhancement of TTX-sensitive rectification on depolarization may result from NMDA-mediated Ca\(^{2+}\) entry in MGB neurons.

The effects of spermine on membrane rectification and firing threshold may involve the recruitment of a Ca\(^{2+}\)-dependent second messenger, subsequent to NMDA receptor activation. Activation of NMDA receptors enhances Ca\(^{2+}\) entry, resulting in a Ca\(^{2+}\) gradient in the dendrites (Connor et al., 1988) and activation of a protein kinase C (PKC) pathway. A rise in intracellular [Ca\(^{2+}\)] also may activate calmodulin kinase II which enhances Na\(^+\) currents (Carlier et al., 2000). PKC activation increases membrane excitability by shifting the activation curve for the persistent Na\(^+\) current along the voltage axis to more hyperpolarized potentials (Astman et al., 1998; Franceschetti et al., 2000). This voltage range is consistent with the range of spermine enhancement of voltage rectification in our experiments.
The increased rectification on depolarization may have reduced the threshold for an action potential in MGB neurons (cf. neocortical neurons, Stafstrom et al., 1982). Antagonism of NMDA receptors, perfusion with Ca\(^{2+}\)-free ACSF or rapid chelation of Ca\(^{2+}\) with BAPTA, eliminated the reduction in threshold and increased tonic firing due to spermine application. Hence, the modulation of NMDA receptor-mediated Ca\(^{2+}\) entry likely increased membrane rectification on depolarization and reduced firing threshold. This mechanism explains the ability of spermine to increase postsynaptic excitability and tonic firing in MGB neurons.

Spermine facilitated LTS firing by a mechanism that did not involve interactions with NMDA receptors. Spermine increased the rate of rise and amplitude of the LTS, despite APV blockade of NMDA receptors. This was evident on depolarization to action potential threshold where there is a smaller gradient for Ca\(^{2+}\) as well as greater inactivation of T-type Ca\(^{2+}\) channels (Hernandez-Cruz and Pape, 1989). Spermine enhanced the LTS during blockade of voltage-dependent Na\(^{+}\) channels by TTX. Hence, a change in some parameter of the T-type Ca\(^{2+}\) current, e.g. voltage dependence of the inactivation–activation relationship, may have increased the LTS.

Pentobarbital modulated NMDA–mediated corticothalamic EPSPs producing more transient responses. At an anaesthetic concentration, pentobarbital shortened the duration of NMDA-mediated corticothalamic EPSPs. Pentobarbital reversed spermine
prolongation of corticothalamic EPSPs by a mechanism that was independent of Zn$^{2+}$ interactions. The lack of pentobarbital effects during arcaine blockade implicated an action at the polyamine site on NMDA receptors. These effects are consistent with pentobarbital shortening of burst durations of NMDA-mediated single channel currents (Charlesworth et al., 1995). Pentobarbital actions on NMDA-mediated corticothalamic transmission may contribute to its anti-epileptic effects.

The depressant effects of pentobarbital on NMDA-mediated synaptic responses provided a rationale to examine its short-term effects on pre- and postsynaptic parameters of non-NMDA mediated transmission presented in the next section.

**Part III. Effects of pentobarbital on short-term depression**

3.3.1. Behaviour of EPSCs in trains during short-term depression

3.3.1.1 Passive membrane properties

For proper assessment of short-term depression (STD), QX-314 and Cs-gluconate were applied intracellularly to block, respectively, Na$^+$ and K$^+$-channels and reduce postsynaptic currents. With this pipette solution, the input resistance ($R_i$) increased by ~81% (380 ± 25 MΩ, n = 10, $P < 0.05$) compared to values obtained using solutions containing K$^+$-gluconate and no QX-314 (210 ± 15 MΩ, n = 9). During combined Cs$^+$ and QX-314 blockade, pentobarbital (200 μM) did not alter the $R_i$ throughout 3 – 5 min of application (365 ± 34 MΩ, n = 10; cf. Wan et al. 2004). Hence, intracellular blockade
of Na\(^+\) and K\(^+\) channels reduced their postsynaptic contributions to stimulus-evoked depression (Cahalan and Almers 1979; Konishi 1990; Budde et al. 1994).

The experiments were performed in neurons voltage clamped at -80 mV in order to minimize postsynaptic contributions of voltage-dependent Ca\(^{2+}\) conductances (Hernandez-Cruz and Pape, 1989). This allowed the study of the frequency-dependent aspect of corticothalamic STD while minimizing postsynaptic temporal summation.

3.3.1.2. Frequency - dependent fade (STD) of corticothalamic EPSCs

Repetitive stimulation in the 2.5 - 20 Hz range produced STD of EPSCs (Figure 3.19). With increasing stimulation frequencies, the train of EPSCs decreased in amplitude to a plateau (S\(_{15:20}\)) that was 49 to 21 % of the 1\(^{st}\) EPSC amplitude (S\(_1\); see Plateau/S\(_1\) ratio in Table 3.2.1A). The relation between the plateau and stimulation frequency is illustrated in their product value (plateau \(\times\) Hz) which increased significantly at stimulation frequencies > 5 Hz (Table 3.2.1A). The apparent QN decreased at stimulation frequencies \(\geq 10\) Hz, indicating substantial refill at lower frequencies (Table 3.2.1A). The ratio of the amplitude of the 12\(^{th}\) EPSC, subsequent to the omitted 11\(^{th}\) stimulus to the 10\(^{th}\) EPSC amplitude (S\(_{12}/S_{10}\)), increased at stimulation frequencies > 5 Hz (Table 3.2.1A), indicating high values of fractional release in the plateau. The frequency-dependent characteristic of STD was similar to other observations at corticothalamic synapses (Li et al. 2003; Reichova and Sherman, 2004).
Figure 3.19. Frequency-dependence of corticothalamic STD. A) Traces of trains of EPSCs during STD. Increasing the frequency of stimulation from 2.5 to 20 Hz enhanced STD. Expanded traces below show the 1\textsuperscript{st} to 5\textsuperscript{th} EPSCs (middle) and the 10\textsuperscript{th} to 14\textsuperscript{th} EPSCs around the omitted 11\textsuperscript{th} stimulus (bottom). B) Normalized EPSC amplitudes at four stimulation frequencies. At 10 Hz, EPSCs reached a plateau of 40% of the 1\textsuperscript{st} response whereas at 20 Hz EPSC amplitudes reached a plateau of 25% of the 1\textsuperscript{st} response. At 20 Hz stimulation, the mean amplitude of the 11\textsuperscript{th} response just subsequent to the missing 10\textsuperscript{th} stimulus nearly doubled, consistent with the depletion model. Traces in A are averages of 6 repeats from 1 neuron. Values in B are averages of 6 neurons. SEM indicates between neuron variations. Neurons were held at -80 mV.
Table 3.2.1A: Summary of parameters of corticothalamic STD at different frequencies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_1 (nA)</td>
<td>1.19 ± 0.35</td>
<td>1.21 ± 0.38</td>
<td>1.18 ± 0.29</td>
<td>1.17 ± 0.41</td>
</tr>
<tr>
<td>S_2 (nA)</td>
<td>0.67 ± 0.23</td>
<td>0.70 ± 0.25</td>
<td>0.74 ± 0.19</td>
<td>0.56 ± 0.32</td>
</tr>
<tr>
<td>S_2/S_1</td>
<td>0.56 ± 0.19</td>
<td>0.57 ± 0.20</td>
<td>0.63 ± 0.16</td>
<td>0.47 ± 0.27</td>
</tr>
<tr>
<td>S_{12}/S_{10}</td>
<td>1.02 ± 0.08</td>
<td>1.06 ± 0.09</td>
<td>1.31 ± 0.10*</td>
<td>1.86 ± 0.11*</td>
</tr>
<tr>
<td>Plateau (S_{15-20}) (nA)</td>
<td>0.58 ± 0.16</td>
<td>0.52 ± 0.19</td>
<td>0.41 ± 0.17</td>
<td>0.25 ± 0.15</td>
</tr>
<tr>
<td>Plateau/S_1</td>
<td>0.49 ± 0.13</td>
<td>0.43 ± 0.15</td>
<td>0.35 ± 0.13</td>
<td>0.21 ± 0.12*</td>
</tr>
<tr>
<td>Plateau × Hz (nA/s)</td>
<td>1.4 ± 0.4</td>
<td>2.6 ± 0.5</td>
<td>4.1 ± 0.6*</td>
<td>5.0 ± 0.6*</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>4.25 ± 0.11</td>
<td>4.22 ± 0.10</td>
<td>4.09 ± 0.13</td>
<td>3.26 ± 0.14*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6, * (relative to 2.5 Hz) P < 0.01, ANOVA test
STD was found to be associated with negative covariances between pairs of the first 5 EPSCs, consistent with the binomial/depletion model (Figure 3.20). Theoretically, in the absence of refill, the covariance divided by the product of the mean amplitudes of the 1st and 2nd EPSCs ($<S_1>-<S_2>$) equals the negative of the reciprocal of the number of release sites (-1/N). The plot of Figure 3.20A shows the negative of the covariance, expressed as $-\text{cov}(S_1,S_2)/<S_1>-<S_2>$ between the 1st EPSC and the 2nd to 5th EPSCs. At each particular frequency, the covariance term decreased as the distance between the pairs of stimuli increased (Figure 3.20). This attenuation of the covariance term is expected from refill which reduces the negativity of the covariance term. As shown in Figure 3.20, an increase in the stimulation frequency worked in the opposite direction, as expected from a lower $\alpha$ between stimuli. The covariance data were entirely consistent with the binomial/depletion model (see Methods). This validated the use of the covariance term to correct the variance to mean ratios and to estimate the apparent quantal sizes during STD, at each stimulus in the train.

The results indicated an inconsistency with the simple model. Namely, STD was also characterized by a frequency-dependent decrease in the apparent quantal size along the EPSC train (Figure 3.20 B, Table 3.2.1B). After 2 stimuli, the apparent quantal size declined to a plateau value lower than the initial. The equivalence with variance/mean ratios indicated no error in the correction, which is intrinsically small when signals are much less than the initial (Figure 3.20A). The apparent quantal size values, in the 26-35 pA range, were not significantly different from the mean amplitudes of evoked miniature
Figure 3.20. Validation of the corrected variance-mean method during corticothalamic STD. A) Negative covariances within the first five EPSCs during train-evoked STD. Attenuation of the covariance term calculated for pairs of the $1^{st}$ EPSC relative to the $2^{nd}$ to $5^{th}$ EPSCs. The attenuation increased with decreasing stimulus frequencies in the 2.5-20 Hz range, likely due to refill of depleted packets. B) Quantal size estimates during STD. Note the frequency-dependent reduction in quantal size early in the train and the lack of change in quantal size after an intra-train gap at the $11^{th}$ stimulus. C) Alterations in quantal content during STD at 2.5 - 20 Hz. Note the similarity in reduction of quanta to the rundown in EPSC amplitude (Figure 3.19) and the post intra-train gap increase in quanta reflecting a presynaptic provenance. Data were obtained from same neurons as in Figure 3.19. SEM indicates between neuron variations. The relative jump after the gap theoretically equals $P_{output} \times (1-\alpha)$. 
Figure 3.21. Pre- and poststimulation miniature EPSCs in a neuron vary in size. A) Sample records of miniature EPSCs (minEPSCs), 5 s in duration, before and after a 10 Hz stimulus train. B) (a) Amplitude histograms of spontaneous minEPSCs counted 5 s before the stimulus train (6 repeats, 30 s in total). (b) Amplitude histograms of evoked minEPSCs counted 1 s after the stimulus train (6 repeats, 6 s total). Total minEPSC count (minis) is indicated above the histograms. Evoked minEPSC sizes were obtained by subtraction of spontaneous from evoked minEPSC histograms ((b) – (a)). Values next to black arrows are the mean size ± SEM. The apparent quantal sizes (Q') were (in pA): 34.6 at 2.5 Hz, 33.1 at 5 Hz, 32.9 at 10 Hz, and 34.1 at 20 Hz.
Table 3.2.1B: Derived parameters of STD at different frequencies

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S₁,S₂) (nA²)</td>
<td>-0.016 ± 0.009</td>
<td>-0.018 ± 0.010</td>
<td>-0.021 ± 0.013</td>
<td>-0.024 ± 0.012</td>
</tr>
<tr>
<td>Q'(S₁) (pA)</td>
<td>36.1 ± 9.1</td>
<td>37.3 ± 7.3</td>
<td>35.3 ± 6.2</td>
<td>36.5 ± 8.2</td>
</tr>
<tr>
<td>Q'(S₂) (pA)</td>
<td>35.5 ± 8.7</td>
<td>34.9 ± 5.5</td>
<td>34.5 ± 6.4</td>
<td>36.1 ± 5.9</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀) (pA)</td>
<td>31.3 ± 6.1</td>
<td>29.0 ± 7.3</td>
<td>26.1 ± 5.7</td>
<td>25.5 ± 5.4</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀) Q'(S₁)</td>
<td>0.85 ± 0.07</td>
<td>0.78 ± 0.10</td>
<td>0.74 ± 0.08</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>Var/Mean (S₁₅-S₂₀) (pA)</td>
<td>30.7 ± 7.4</td>
<td>31.2 ± 6.6</td>
<td>27.2 ± 4.9</td>
<td>26.4 ± 5.6</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>30.5 ± 3.7</td>
<td>31.4 ± 4.1</td>
<td>32.0 ± 5.3</td>
<td>29.1 ± 6.1</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.4 ± 3.1</td>
<td>11.2 ± 2.9</td>
<td>11.1 ± 3.4</td>
<td>10.5 ± 2.5</td>
</tr>
<tr>
<td>m₁</td>
<td>53 ± 4</td>
<td>52 ± 3</td>
<td>52 ± 8</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>m₁₅-₂₀</td>
<td>34 ± 3</td>
<td>31 ± 4</td>
<td>27 ± 3</td>
<td>21 ± 4*</td>
</tr>
<tr>
<td>m₁₂/m₁₀</td>
<td>1.08 ± 0.11</td>
<td>1.13 ± 0.18</td>
<td>1.25 ± 0.20</td>
<td>1.96 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6 ; * (relative to 2.5 Hz) P < 0.05; data from same neurons as Table 3.2.1A.
EPSCs (minEPSC) observed 1 s after the stimulus train (Figure 3.21; Table 3.2.1B). The frequency of these minEPSCs increased with stimulation frequency (Figure 3.21).

During STD, the quantal content \(m\) also decreased to a plateau that depended on the stimulation frequency. At 20 Hz stimulation, \(m\) reached a plateau value \((m_{15-20})\) of \(-40\%\) of the initial \(m\) \((m_1, \text{Figure 3.20C})\). The \(m\) value increased subsequent to the gap at the omitted 11\(^{th}\) stimulus \((m_{12}/m_{10}; \text{Figure 3.20C, Table 3.2.1B})\), consistent with the increased amplitude of the 12\(^{th}\) EPSC amplitude (20 Hz in Figure 3.19, Table 3.2.1B). This observation was indicative of a refill process that restores the apparent number of releasable quanta. In summary, an intra-train reduction in quantal content mediated much of the frequency-dependent component of STD.

3.3.2. Effects of alterations in extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_e\))

3.3.2.1 Low [Ca\(^{2+}\)]\(_e\) perfusion

Since Ca\(^{2+}\) has been proposed to be a key factor for release probability and therefore a mediator of STD, the following experiments examined if STD persisted under conditions of low release probability. Washing extracellular Ca\(^{2+}\) with EDTA resulted in an overall reduction in the amplitude of the EPSC train (Figure 3.22) and a rundown to a plateau of 28\% of the initial EPSC amplitude (Figure 3.22; Table 3.2.2A). A subsequent application of a 0.2 mM Ca\(^{2+}\) media with no EDTA increased the amplitude of the EPSC train, confirming an effective reduction of extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_e\)) by the previous EDTA solution. Contrary to the expectation, there was a significant drop in the apparent QN in low [Ca\(^{2+}\)]\(_e\) with or without EDTA, rather than a fall in fractional release
In the neuron shown in Figure 3.22, an application of DMSO (1%), subsequent to 0.2 mM \([\text{Ca}^{2+}]_e\), produced higher EPSC amplitudes and faster rundown by increasing the quantal contents at the beginning of the train. Hence, a substantial decrease in \([\text{Ca}^{2+}]_e\) did not result in a loss of STD.

The reduction in QN was, apparently, due to a decrease in apparent quantal size throughout the train in low \([\text{Ca}^{2+}]_e\), with or without EDTA (Figure 3.22; Table 3.2.2B). On application of very low \([\text{Ca}^{2+}]_e\) (0.1 mM \(\text{Ca}^{2+}\) in EDTA), the apparent quantal size was decreased already at the 1st response and in the remainder of the train. The subsequent switch to 0.2 mM \(\text{Ca}^{2+}\) (without EDTA) increased the quantal content at the beginning of the train without having significant effect on quantal size (Table 3.2.2B). These effects of low \(\text{Ca}^{2+}\) on the quantal parameters suggested the participation of high probability release sites consistent with a compound binomial model (Brown et al. 1976; Walmsley et al., 1988). However, the high probability release sites were associated with relatively small quantal responses, perhaps at sites on dendrites relatively protected in some way from reduction in local \([\text{Ca}^{2+}]_e\).

### 3.3.2.2. Elevated \([\text{Ca}^{2+}]_e\) perfusion

An increase in \([\text{Ca}^{2+}]_e\) enhanced STD. Raising \([\text{Ca}^{2+}]_e\) from 2 to 8 mM resulted in increased amplitudes of the initial EPSCs (Figure 3.23). The EPSCs plateau increased by \(\sim 50\%\), whereas, the ratio of the plateau to the 1st EPSC (plateau/\(S_1\)) decreased (Table 3.2.2A). The ratio of EPSCs around the intra-train gap (\(S_{12}/S_{10}\)) increased from \(1.31 \pm 0.08\) to \(1.56 \pm 0.07\) \((n = 5, P < 0.05, t\text{-test})\). Consistent with increases in fractional
Figure 3.22. Persistence of STD in media containing low [Ca$^{2+}$]. Top traces: EPSC trains from a neuron during control, after a 1 min application of 1.1 mM EDTA, after a 2 min wash in 0.2 mM Ca$^{2+}$, and subsequent to a 1 min application of 1% (vol/vol) DMSO. Lower traces, a ×3 magnification of the initial five EPSCs at the beginning of the train are for EDTA and 0.2 mM Ca$^{2+}$. Bottom Left: EPSC amplitudes in low Ca$^{2+}$ media. Bottom middle: quantal size estimates. Bottom right: quantal content estimates. Data in bottom plots are averages of 5 neurons. Error bars in 1st response indicate between-neuron variations.
Chapter 3. Results

0.1 mM [Ca$^{2+}$]

Figure 3.23. [Ca$^{2+}$]$_{e}$ modification of STD. Top: Traces showing rundown of the 1$^{st}$ – 5$^{th}$ EPSCs in 0.1 mM Ca$^{2+}$ (light grey), 2 mM Ca$^{2+}$ (dark grey), and 8 mM Ca$^{2+}$ (black). Bottom Left: Scatter plot of 1$^{st}$ EPSC amplitude in control (2 mM), high Ca$^{2+}$ (Hi Ca$^{2+}$; 8 mM), and low Ca$^{2+}$ (Lo Ca$^{2+}$; 0.1 mM). Horizontal lines indicate mean. Bottom right: Normalized EPSC amplitudes in 0.1 mM Ca$^{2+}$ (squares), 2 mM Ca$^{2+}$ (circles), and 8 mM Ca$^{2+}$ (triangles). Note increased amplitude after the intra-train gap in the control and high [Ca$^{2+}$] and lack of increase in amplitude in low Ca$^{2+}$. Data are from 10 neurons. Holdings potential was -80 mV. Values are expressed as Mean ± SEM.
Table 3.2.2A: Summary of effects of altered $[\text{Ca}^{2+}]_e$ on parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2 mM Ca$^{2+}$)</th>
<th>High Ca$^{2+}$ (8 mM)</th>
<th>Control (2 mM Ca$^{2+}$)</th>
<th>Ca$^{2+}$/EDTA (0.1 mM Ca$^{2+}$)</th>
<th>Low Ca$^{2+}$ (0.2mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$ (nA)</td>
<td>1.42 ± 0.41</td>
<td>2.53 ± 0.34</td>
<td>1.32 ± 0.53</td>
<td>0.47 ± 0.40</td>
<td>0.92 ± 0.42</td>
</tr>
<tr>
<td>$S_2$ (nA)</td>
<td>0.74 ± 0.19</td>
<td>1.42 ± 0.35</td>
<td>0.68 ± 0.27</td>
<td>0.34 ± 0.31</td>
<td>0.63 ± 0.19</td>
</tr>
<tr>
<td>$S_2/S_1$</td>
<td>0.52 ± 0.12</td>
<td>0.56 ± 0.13</td>
<td>0.51 ± 0.20</td>
<td>0.72 ± 0.16</td>
<td>0.68 ± 0.18</td>
</tr>
<tr>
<td>$S_{12}/S_{10}$</td>
<td>1.31 ± 0.08</td>
<td>1.56 ± 0.07</td>
<td>1.28 ± 0.11</td>
<td>1.03 ± 0.06</td>
<td>1.13 ± 0.05</td>
</tr>
<tr>
<td>Plateau ($S_{15-20}$) (nA)</td>
<td>0.62 ± 0.09</td>
<td>0.91 ± 0.11</td>
<td>0.59 ± 0.13</td>
<td>0.13 ± 0.10</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>Plateau/$S_1$</td>
<td>0.48 ± 0.05</td>
<td>0.34 ± 0.04</td>
<td>0.44 ± 0.07</td>
<td>0.28 ± 0.04</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>3.93 ± 0.11</td>
<td>7.2 ± 0.25</td>
<td>4.13 ± 0.17</td>
<td>1.07 ± 0.21</td>
<td>1.55 ± 0.19</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; $n = 5$ in high and low Ca$^{2+}$ groups, * $P < 0.05$, $t$-test. ** $P < 0.05$, ANOVA test. The controls were different neurons for high and low [Ca$^{2+}]_e$. 
Table 3.2.2B: Effects of altered $[Ca^{2+}]_c$ on derived parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2mM Ca$^{2+}$)</th>
<th>High Ca$^{2+}$ (8 mM)</th>
<th>Control (2mM Ca$^{2+}$)</th>
<th>Ca$^{2+}$/EDTA (0.1 mM Ca$^{2+}$)</th>
<th>Low Ca$^{2+}$ (0.2 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S$_1$,S$_2$)</td>
<td>-0.018 ± 0.010</td>
<td>-0.026 ± 0.008</td>
<td>-0.021 ± 0.013</td>
<td>-0.010 ± 0.009</td>
<td>-0.015 ± 0.010</td>
</tr>
<tr>
<td>Q'(S$_1$) (pA)</td>
<td>33.4 ± 7.4</td>
<td>31.2 ± 8.6</td>
<td>35.4 ± 8.9</td>
<td>12.6 ± 8.6*</td>
<td>13.5 ± 7.5*</td>
</tr>
<tr>
<td>Q'(S$_2$) (pA)</td>
<td>32.4 ± 6.5</td>
<td>29.5 ± 7.8</td>
<td>33.6 ± 7.5</td>
<td>11.9 ± 6.5*</td>
<td>13.1 ± 4.2*</td>
</tr>
<tr>
<td>Q'(S$<em>{15}$-S$</em>{20}$) (pA)</td>
<td>28.3 ± 6.9</td>
<td>26.9 ± 9.1</td>
<td>27.3 ± 7.7</td>
<td>12.3 ± 7.0</td>
<td>14.2 ± 8.1</td>
</tr>
<tr>
<td>Q'(S$<em>{15}$-S$</em>{20}$)</td>
<td>0.85 ± 0.17</td>
<td>0.87 ± 0.11</td>
<td>0.78 ± 0.21</td>
<td>0.97 ± 0.11</td>
<td>1.05 ± 0.18</td>
</tr>
<tr>
<td>Var/mean (S$<em>{15}$-S$</em>{20}$) (pA)</td>
<td>29.4 ± 4.9</td>
<td>28.6 ± 6.6</td>
<td>27.9 ± 8.5</td>
<td>13.6 ± 9.2</td>
<td>14.6 ± 7.8</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>30.8 ± 4.2</td>
<td>32.7 ± 7.1</td>
<td>33.1 ± 5.7</td>
<td>14.5 ± 6.3</td>
<td>15.1 ± 6.0</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>10.6 ± 2.9</td>
<td>13.2 ± 4.1</td>
<td>12.4 ± 3.7</td>
<td>13.9 ± 2.7</td>
<td>12.7 ± 2.2</td>
</tr>
<tr>
<td>$m_1$</td>
<td>47 ± 9</td>
<td>78 ± 11*</td>
<td>37 ± 13</td>
<td>36 ± 19</td>
<td>68 ± 15*</td>
</tr>
<tr>
<td>$m_{15-20}$</td>
<td>26 ± 5</td>
<td>33 ± 8</td>
<td>19 ± 10</td>
<td>22 ± 7</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>$m_{12}/m_{10}$</td>
<td>1.21 ± 0.17</td>
<td>1.52 ± 0.09*</td>
<td>1.15 ± 0.11</td>
<td>1.02 ± 0.09</td>
<td>1.13 ± 0.10</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5 in high and low $[Ca^{2+}]_c$ groups; data from same neurons as Table 3.2.2A; * $P < 0.05$, t-test . ** $P < 0.05$, ANOVA test.
release, the changes in STD parameters occurred in parallel to an increase in quantal content at the beginning of the train and around the intra-train gap (m12/m10) with no effect on quantal size (Table 3.2.2B). Hence, raising \([Ca^{2+}]_e\) promoted STD by increasing the quantal content, and increasing fractional release. The apparent increase in QN with high \([Ca^{2+}]_e\) was also reported for neuromuscular junction (Elmqvist and Quastel, 1965a) and was consistent with a compound binomial model (Quastel, 1997) in which fractional release varied between sites.

3.3.3. Receptor desensitization and saturation

3.3.3.1. Effects of blockade of receptor desensitization

The reduction in quantal size observed during STD (Figure 3.20B; Table 3.2.1B) suggested that a substantial component arises from receptor desensitization (Scheuss et al., 2002). Application of a blocker of AMPA receptor desensitization, cyclothiazide (CTZ; 50 μM) had little effect on the development of STD (Figure 3.24; Table 3.2.3A), but estimates of quantal size decreased less than in controls reaching a higher plateau (Figure 3.24; Table 3.2.3B). These data demonstrated a moderate contribution of receptor desensitization to the decrease in the apparent quantal size during STD.

3.3.3.2. Combined blockade of receptor desensitization and saturation

To test whether receptor saturation, in addition to desensitization, contributed to the early drop in quantal size during STD, CTZ (50 μM) was co-applied with kynurenate (KYN; 50 μM); an antagonist that rapidly dissociates from glutamate receptors. The result was abolition of the drop in quantal size early in the train (Figure 3.24C; Table 3.2.3B). At the
Figure 3.24. Effects of blockade of receptor desensitization and saturation on STD. A). Traces of EPSCs (averages of 5 repeats) before and after application of 50 μM cyclothiazide (CTZ; 2 min). Traces on right show time expansions of the 1st-5th EPSC. B) Left: Mean EPSC amplitudes (6 neurons) before and during application of CTZ (50 μM). Note that the reduced depression resulted in a higher plateau in CTZ compared to control. Middle: quantal size estimates during CTZ application decreased to lesser extent compared to control reaching a higher plateau. Right: Quantal content estimates during CTZ application showed a decreased use-dependent reduction and a higher plateau. Note increase in content subsequent to the intra-train gap. C) Combined blockade of receptor desensitization and saturation abolished use-dependent alterations in quantal size but not STD. Left: Mean EPSC amplitudes (6 neurons) before and during combined application of CTZ (50 μM) and KYN (50 μM). Note the reduced amplitude of the 1st EPSC which reached a plateau by the 5th response. Middle: quantal size estimates did not change during combined CTZ and KYN application. Right: Quantal content estimates during combined CTZ and KYN showed an increased use-dependent reduction and a lower plateau compared to control. Note increase in content subsequent to the intra-train gap. Error bars in 1st response show between neuron variations. (P < 0.001 in amplitude data; P < 0.05 in quantal size data; P < 0.01 in quantal content data, t-tests).
Table 3.2.3A: Summary of effects of CTZ and KYN on parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CTZ (50 μM)</th>
<th>Control</th>
<th>CTZ + KYN (50 μM) (50 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$ (nA)</td>
<td>1.29 ± 0.23</td>
<td>1.43 ± 0.26</td>
<td>1.21 ± 0.17</td>
<td>0.81 ± 0.13*</td>
</tr>
<tr>
<td>$S_2$ (nA)</td>
<td>1.04 ± 0.21</td>
<td>1.27 ± 0.22</td>
<td>0.97 ± 0.14</td>
<td>0.62 ± 0.14*</td>
</tr>
<tr>
<td>$S_2/S_1$</td>
<td>0.81 ± 0.13</td>
<td>0.73 ± 0.16</td>
<td>0.85 ± 0.10</td>
<td>0.88 ± 0.17</td>
</tr>
<tr>
<td>$S_{12}/S_{10}$</td>
<td>1.31 ± 0.15</td>
<td>1.23 ± 0.11</td>
<td>1.28 ± 0.19</td>
<td>1.21 ± 0.10</td>
</tr>
<tr>
<td>Plateau</td>
<td>0.59 ± 0.21</td>
<td>0.78 ± 0.19</td>
<td>0.55 ± 0.16</td>
<td>0.38 ± 0.24</td>
</tr>
<tr>
<td>($S_{15-20}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plateau/S_1</td>
<td>0.45 ± 0.10</td>
<td>0.54 ± 0.09</td>
<td>0.45 ± 0.14</td>
<td>0.46 ± 0.17</td>
</tr>
<tr>
<td>Apparent QN</td>
<td>3.93 ± 0.24</td>
<td>4.62 ± 0.31*</td>
<td>3.99 ± 0.18</td>
<td>2.63 ± 0.41*</td>
</tr>
<tr>
<td>($nA$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6, * P < 0.05, t-test. Data are from 10 Hz trains.
Table 3.2.3B: Effects of CTZ and KYN on derived parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (50 μM)</th>
<th>CTZ (50 μM)</th>
<th>Control (50 μM)</th>
<th>CTZ + KYN (50 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S₁,S₂)</td>
<td>-0.027 ± 0.008</td>
<td>-0.022 ± 0.011</td>
<td>-0.024 ± 0.013</td>
<td>-0.011 ± 0.008</td>
</tr>
<tr>
<td>Q'(S₁) (pA)</td>
<td>35.2 ± 2.5</td>
<td>38.3 ± 3.0</td>
<td>37.5 ± 5.7</td>
<td>25.3 ± 4.5*</td>
</tr>
<tr>
<td>Q'(S₂) (pA)</td>
<td>29.4 ± 6.4</td>
<td>37.2 ± 2.4</td>
<td>32.6 ± 3.9</td>
<td>26.1 ± 2.1*</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀) (pA)</td>
<td>26.1 ± 5.7</td>
<td>30.3 ± 3.1</td>
<td>27.3 ± 2.8</td>
<td>24.8 ± 3.1</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀)/Q'(S₁)</td>
<td>0.74 ± 0.15</td>
<td>0.79 ± 0.19</td>
<td>0.71 ± 0.13</td>
<td>0.98 ± 0.10*</td>
</tr>
<tr>
<td>Var/mean (S₁₅-S₂₀) (pA)</td>
<td>27.2 ± 4.9</td>
<td>32.2 ± 4.1</td>
<td>29.4 ± 3.7</td>
<td>25.2 ± 5.1</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>32.0 ± 5.3</td>
<td>34.5 ± 3.9</td>
<td>31.0 ± 2.6</td>
<td>27.6 ± 4.6</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.1 ± 3.4</td>
<td>10.5 ± 2.2</td>
<td>12.3 ± 1.9</td>
<td>8.9 ± 2.4</td>
</tr>
<tr>
<td>m₁</td>
<td>52 ± 8</td>
<td>53 ± 12</td>
<td>34 ± 14</td>
<td>37 ± 16</td>
</tr>
<tr>
<td>m₁₅-2₀</td>
<td>23 ± 5</td>
<td>35 ± 7</td>
<td>24 ± 4</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>m₁₂/m₁₀</td>
<td>1.32 ± 0.21</td>
<td>1.16 ± 0.19</td>
<td>1.21 ± 0.18</td>
<td>1.28 ± 0.21</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6; data from same neurons as Table 3.2.3A. * P < 0.05, t-test.
beginning of the train, the quantal size decreased significantly compared to the control and remained unchanged throughout the train (Figure 3.24C; Table 3.2.3B). Despite a significant reduction in the amplitudes of the 1\textsuperscript{st} and 2\textsuperscript{nd} EPSCs, the characteristic EPSC rundown during STD (Plateau/S\textsubscript{1}) was unaffected by co-application of CTZ and KYN (Table 3.2.3A). Given the lack of effects on the quantal content, it was concluded that the effects of co-applied KYN and CTZ on STD were predominantly postsynaptic.

The contribution of receptor desensitization and saturation to STD seems paradoxical since postsynaptic receptors cannot desensitize or saturate unless release has already occurred at a given site, whereas the negative covariances indicated that sites activated by the 2\textsuperscript{nd} and 3\textsuperscript{rd} stimuli are those that were not involved previously. This paradox could be explained by an overflow of transmitter from neighbouring release sites to receptor sites which were not previously activated (Telgkamp et al., 2004).

3.3.4. Effects of pentobarbital on STD

3.3.4.1. EPSC behaviour in trains

Pentobarbital enhanced STD in a dose-dependent manner in the 2.5-20 Hz stimulation range (Figure 3.25). The maximal enhancement of STD was at a concentration of 200 μM which lowered the plateau producing a greater rundown of EPSCs (Plateau/S\textsubscript{1}; Table 3.2.4A). At this concentration, pentobarbital increased the ratio of EPSC amplitudes around the intra-train gap (S\textsubscript{12}/S\textsubscript{10}; Table 3.2.4A). A parallel effect on the quantal content ratio around the intra-train gap (Table 3.2.4B), suggested an increase in P\textsubscript{0}. Pentobarbital also produced an apparent reduction of the product QN (Table 3.2.4A). A correction of
Figure 3.25. Dose-dependence of pentobarbital enhancement of STD. 

A) Pentobarbital, applied at 50 and 200 μM, enhanced STD producing a greater and faster rundown of EPSCs with increasing stimulation frequencies. Pentobarbital did not abolish the post-gap increase in the 12th EPSC amplitude. 

B) A decrease in the amplitude of the first EPSC amplitude was only observed after 4 min of pentobarbital application. The data were obtained from samples within less than 4 min of drug application. 

C) Dose dependence of STD at 2.5, 5, 10, and 20 Hz. Pentobarbital, at 100-200 μM, enhanced STD at 10 - 20 Hz more than at lower stimulation frequencies ($P < 0.05$, ANOVA). Data represent averages obtained from 6 neurons.
Table 3.2.4A: Summary of pentobarbital effects on parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1) (nA)</td>
<td>1.21 ± 0.35</td>
<td>1.15 ± 0.29</td>
<td>1.11 ± 0.36</td>
</tr>
<tr>
<td>S(_2) (nA)</td>
<td>0.74 ± 0.19</td>
<td>0.66 ± 0.11</td>
<td>0.52 ± 0.13</td>
</tr>
<tr>
<td>S(<em>{12}/S</em>{10})</td>
<td>1.20 ± 0.16</td>
<td>1.21 ± 0.21</td>
<td>1.67 ± 0.19*</td>
</tr>
<tr>
<td>S(_2/S_1)</td>
<td>0.63 ± 0.07</td>
<td>0.58 ± 0.08</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Plateau (S(<em>{15}-S</em>{20}) (nA)</td>
<td>0.51 ± 0.10</td>
<td>0.34 ± 0.09</td>
<td>0.23 ± 0.07*</td>
</tr>
<tr>
<td>Plateau/S(_1)</td>
<td>0.42 ± 0.12</td>
<td>0.30 ± 0.08</td>
<td>0.21 ± 0.07*</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>4.31 ± 0.13</td>
<td>3.52 ± 0.21*</td>
<td>2.70 ± 0.32*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6; * P < 0.05, ANOVA. Data are from 10 Hz trains.
Figure 3.26. Quantal alterations mediated pentobarbital effects on STD. A) Left: apparent quantal sizes during corticothalamic STD in response to 10 Hz stimulation. During application of 200 μM pentobarbital, quantal sizes became significantly smaller starting at the 3rd response until the end of the train (P < 0.01, ANOVA test). Right: Effects of pentobarbital on quantal contents during corticothalamic STD. A low dose of pentobarbital (50 μM) decreased whereas a high dose increased the quantal content throughout the EPSC train. Note the significant increase in quantal content after the omitted 11th stimulus. During application of 50 or 200 μM pentobarbital, quantal content were significantly different than control starting at the 2nd response until the end of the train (P < 0.01, ANOVA test). Neurons held at -80 mV. Data obtained from 6 neurons. Error bars in 1st response show between neuron variations. Jump after gap of quantal content implied high P0. B) Pentobarbital reduced the amplitude of evoked minEPSCs without affecting pre-stimulation minEPSC amplitude. (a) Histograms of spontaneous minEPSCs obtained 5 s prior to the onset of stimulation (6 repeats, 30 s in total). (b) Histograms of minEPSCs obtained 1 s after the end of the stimulus train (6 repeats, 6 s in total). Evoked minEPSC sizes were obtained after subtraction of pre- from poststimulation evoked minEPSCs (b – a). Total minEPSC counts ('minis') are indicated above histograms. Values next to black arrows are mean size ± SEM. Data are from 1 neuron.
Table 3.2.4B: Effect of pentobarbital on derived parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S\textsubscript{1},S\textsubscript{2})</td>
<td>- 0.022 ± 0.011</td>
<td>- 0.025 ± 0.008</td>
<td>- 0.031 ± 0.013</td>
</tr>
<tr>
<td>Q'(S\textsubscript{1}) (pA)</td>
<td>35.2 ± 2.5</td>
<td>36.3 ± 3.0</td>
<td>29.6 ± 5.0</td>
</tr>
<tr>
<td>Q'(S\textsubscript{2}) (pA)</td>
<td>29.4 ± 6.4</td>
<td>31.3 ± 8.6</td>
<td>21.6 ± 7.4</td>
</tr>
<tr>
<td>Q'(S\textsubscript{15}-S\textsubscript{20}) (pA)</td>
<td>25.1 ± 5.7</td>
<td>19.8 ± 4.2</td>
<td>9.7 ± 5.1*</td>
</tr>
<tr>
<td>Q'(S\textsubscript{15}-S\textsubscript{20})/Q'(S\textsubscript{1})</td>
<td>0.71 ± 0.13</td>
<td>0.55 ± 0.16</td>
<td>0.37 ± 0.11*</td>
</tr>
<tr>
<td>Var/mean (S\textsubscript{15}-S\textsubscript{20}) (pA)</td>
<td>27.2 ± 4.9</td>
<td>22.3 ± 4.2</td>
<td>13.5 ± 5.2*</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>32.0 ± 5.3</td>
<td>20.4 ± 8.1</td>
<td>11.3 ± 7.1*</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.1 ± 3.4</td>
<td>12.1 ± 3.1</td>
<td>10.8 ± 2.9</td>
</tr>
<tr>
<td>m\textsubscript{l}</td>
<td>34 ± 8</td>
<td>31 ± 5</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>m\textsubscript{15-20}</td>
<td>20 ± 7</td>
<td>17 ± 6</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>m\textsubscript{12}/m\textsubscript{10}</td>
<td>1.18 ± 0.24</td>
<td>1.21 ± 0.19</td>
<td>1.85 ± 0.17*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6; data from neurons of Table 3.2.4A; * P < 0.05, ANOVA.
QN for $\alpha ((QN/1+\alpha))$ showed a similar effect of pentobarbital ($\alpha$ values were in control $0.17 \pm 0.03$ and in $200 \mu M$ pentobarbital $0.09 \pm 0.02$, $P < 0.05$, $t$-test; the corrected QN values were $3.68 \pm 0.17 \, nA$ in control and $2.45 \pm 0.26 \, nA$ in $200 \mu M$ pentobarbital, $P < 0.05$, $t$-test). These effects were use-dependent and, hence, did not affect the 1st EPSC in the train during application periods of $\leq 4$ minutes (Figure 3.25 B).

With pentobarbital, a major component of STD appeared to be a use-dependent reduction in quantal size (Figure 3.26; Table 3.2.4B). The ratio of the plateau to the 1st apparent quantal size ($Q'(S_{15-20})/Q'(S_1)$) decreased from 71 % in the control to 37% during pentobarbital application. The same effect was seen in reduction in the size of evoked minEPSC without changes in the spontaneous pre-stimulation minEPSCs (Figure 3.26; Table 3.2.4B). The modulation of quantal parameters contingent on stimulation, and lack of effects on pre-stimulation minEPSCs, suggested that pentobarbital produced smaller size quanta either by a presynaptic action of selecting sites with small quanta, or a postsynaptic action confined to activated synaptic sites.

3.3.4.2. STD in raised $Ca^{2+}$ concentration

The next set of experiments examined if pentobarbital effects on STD could be modulated during conditions of high release probability. As previously, raising $[Ca^{2+}]_e$ from 2 to 8 mM produced a greater rundown of EPSCs. Under these conditions, pentobarbital (200 $\mu M$) produced an even greater rundown, reducing the plateau to the 1st EPSC ratio (plateau/$S_1$) to $0.19 \pm 0.08$ ($P < 0.05$, ANOVA; Table 3.2.5A). At this concentration of pentobarbital, the amplitude ratio of the EPSCs around the intra-train
Table 3.2.5A: Pentobarbital effects on parameters of STD in raised [Ca\(^{2+}\)]_e

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2 mM Ca(^{2+}))</th>
<th>High Ca(^{2+}) (8 mM)</th>
<th>Pentobarbital 50 μM (8 mM Ca(^{2+}))</th>
<th>Pentobarbital 200 μM (8 mM Ca(^{2+}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1) (nA)</td>
<td>1.11 ± 0.42</td>
<td>1.66 ± 0.35</td>
<td>1.59 ± 0.39</td>
<td>1.54 ± 0.41</td>
</tr>
<tr>
<td>S(_2) (nA)</td>
<td>0.69 ± 0.19</td>
<td>0.94 ± 0.23</td>
<td>0.71 ± 0.15</td>
<td>0.55 ± 0.21</td>
</tr>
<tr>
<td>S(_2)/S(_1)</td>
<td>0.63 ± 0.11</td>
<td>0.57 ± 0.14</td>
<td>0.45 ± 0.10</td>
<td>0.36 ± 0.16</td>
</tr>
<tr>
<td>S(<em>{12}/S</em>{10})</td>
<td>1.20 ± 0.10</td>
<td>1.35 ± 0.08</td>
<td>1.38 ± 0.17</td>
<td>1.91 ± 0.21***</td>
</tr>
<tr>
<td>Plateau ((S_{15-20}))</td>
<td>0.49 ± 0.10</td>
<td>0.58 ± 0.13</td>
<td>0.45 ± 0.15</td>
<td>0.27 ± 0.09**</td>
</tr>
<tr>
<td>Plateau/ S(_1)</td>
<td>0.44 ± 0.10</td>
<td>0.36 ± 0.11</td>
<td>0.28 ± 0.09</td>
<td>0.19 ± 0.08*</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>3.90 ± 0.13</td>
<td>5.35 ± 0.19*</td>
<td>3.96 ± 0.21**</td>
<td>3.20 ± 0.17***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6; * (relative to control) ** (relative to high Ca\(^{2+}\)) *** (relative to control and Ca\(^{2+}\)) P < 0.05, ANOVA. Data are from 10 Hz trains.
Table 3.2.5B: Pentobarbital effects on derived values of STD in raised [Ca\(^{2+}\)]_e

<table>
<thead>
<tr>
<th></th>
<th>Control (2 mM Ca(^{2+}))</th>
<th>High Ca(^{2+}) (8 mM)</th>
<th>Pentobarbital 50 μM (8 mM Ca(^{2+}))</th>
<th>Pentobarbital 200 μM (8 mM Ca(^{2+}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S(_1),S(_2))</td>
<td>-0.017 ± 0.012</td>
<td>-0.023 ± 0.009</td>
<td>-0.026 ± 0.013</td>
<td>-0.031 ± 0.017</td>
</tr>
<tr>
<td>Q'(S(_1)) (pA)</td>
<td>34.9 ± 8.1</td>
<td>35.4 ± 9.7</td>
<td>32.7 ± 8.1</td>
<td>30.5 ± 7.7</td>
</tr>
<tr>
<td>Q'(S(_2)) (pA)</td>
<td>33.8 ± 9.2</td>
<td>32.6 ± 8.6</td>
<td>29.2 ± 7.3</td>
<td>24.7 ± 9.1</td>
</tr>
<tr>
<td>Q'(S(<em>{15})-S(</em>{20})) (pA)</td>
<td>29.4 ± 7.6</td>
<td>28.9 ± 6.9</td>
<td>25.6 ± 8.2</td>
<td>18.5 ± 10.3</td>
</tr>
<tr>
<td>Q'(S(<em>{15})-S(</em>{20})) / Q'(S(_1))</td>
<td>0.84 ± 0.14</td>
<td>0.81 ± 0.10</td>
<td>0.78 ± 0.12</td>
<td>0.60 ± 0.08*</td>
</tr>
<tr>
<td>Var/mean (S(<em>{15})-S(</em>{20})) (pA)</td>
<td>30.8 ± 6.7</td>
<td>29.7 ± 8.5</td>
<td>27.6 ± 9.1</td>
<td>22.1 ± 7.9</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>33.1 ± 5.3</td>
<td>31.8 ± 9.4</td>
<td>28.3 ± 6.5</td>
<td>25.2 ± 8.4</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>12.3 ± 2.9</td>
<td>11.5 ± 4.3</td>
<td>10.6 ± 3.1</td>
<td>9.6 ± 3.7</td>
</tr>
<tr>
<td>(m_1)</td>
<td>31 ± 9</td>
<td>46 ± 11</td>
<td>48 ± 13</td>
<td>50 ± 15</td>
</tr>
<tr>
<td>(m_{15-20})</td>
<td>16 ± 5</td>
<td>21 ± 8</td>
<td>17 ± 9</td>
<td>14 ± 13</td>
</tr>
<tr>
<td>(m_{12}/m_{10})</td>
<td>1.17 ± 0.13</td>
<td>1.36 ± 0.17</td>
<td>1.40 ± 0.12</td>
<td>1.95 ± 0.16*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5; data from neurons of Table 3.2.5A; * P < 0.05, ANOVA test.
### Table 3.2.6A: Pentobarbital effects on parameters of STD in low $[Ca^{2+}]_e$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2 mM $Ca^{2+}$)</th>
<th>Low $Ca^{2+}$ (0.1 mM)</th>
<th>Pentobarbital 50 µM (0.1 mM $Ca^{2+}$)</th>
<th>Pentobarbital 200 µM (0.1 mM $Ca^{2+}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$ (nA)</td>
<td>1.15 ± 0.42</td>
<td>0.41 ± 0.37</td>
<td>0.39 ± 0.22*</td>
<td>0.35 ± 0.27*</td>
</tr>
<tr>
<td>$S_2$ (nA)</td>
<td>0.72 ± 0.31</td>
<td>0.29 ± 0.20</td>
<td>0.24 ± 0.19</td>
<td>0.19 ± 0.15*</td>
</tr>
<tr>
<td>$S_2/S_1$</td>
<td>0.63 ± 0.14</td>
<td>0.73 ± 0.11</td>
<td>0.64 ± 0.12</td>
<td>0.50 ± 0.15</td>
</tr>
<tr>
<td>$S_{12}/S_{10}$</td>
<td>1.20 ± 0.11</td>
<td>1.01 ± 0.05</td>
<td>1.21 ± 0.08</td>
<td>1.67 ± 0.09*</td>
</tr>
<tr>
<td>Plateau ($S_{15-20}$) (nA)</td>
<td>0.50 ± 0.15</td>
<td>0.23 ± 0.11*</td>
<td>0.19 ± 0.09*</td>
<td>0.12 ± 0.10*</td>
</tr>
<tr>
<td>Plateau/$S_1$</td>
<td>0.43 ± 0.09</td>
<td>0.56 ± 0.06</td>
<td>0.48 ± 0.10</td>
<td>0.34 ± 0.08**</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>4.10 ± 0.15</td>
<td>1.75 ± 0.24*</td>
<td>1.43 ± 0.19*</td>
<td>1.17 ± 0.15**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; $n = 5$, * (relative to control) ** (relative to low $Ca^{2+}$) $P < 0.05$, ANOVA. Data are from 10 Hz trains.
### Table 3.2.6B: Pentobarbital effects on derived parameters of STD in low [Ca\textsuperscript{2+}]\textsubscript{e}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2 mM Ca\textsuperscript{2+})</th>
<th>Low Ca\textsuperscript{2+} (0.1 mM)</th>
<th>Pentobarbital 50 μM (0.1 mM Ca\textsuperscript{2+})</th>
<th>Pentobarbital 200 μM (0.1 mM Ca\textsuperscript{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S\textsubscript{1},S\textsubscript{2})</td>
<td>-0.023 ± 0.011</td>
<td>-0.014 ± 0.008</td>
<td>-0.017 ± 0.009</td>
<td>-0.021 ± 0.013</td>
</tr>
<tr>
<td>Q(S\textsubscript{1}) (pA)</td>
<td>32.3 ± 9.1</td>
<td>14.2 ± 6.8*</td>
<td>13.8 ± 8.8*</td>
<td>12.7 ± 7.9*</td>
</tr>
<tr>
<td>Q(S\textsubscript{2}) (pA)</td>
<td>30.3 ± 8.1</td>
<td>13.7 ± 9.2*</td>
<td>12.9 ± 7.5*</td>
<td>12.1 ± 6.9*</td>
</tr>
<tr>
<td>Q'(S\textsubscript{15-S\textsubscript{20}}) (pA)</td>
<td>28.1 ± 7.7</td>
<td>14.4 ± 6.9</td>
<td>11.8 ± 8.3*</td>
<td>9.4 ± 7.0*</td>
</tr>
<tr>
<td>Q'(S\textsubscript{15-S\textsubscript{20}})/Q(S\textsubscript{1})</td>
<td>0.87 ± 0.17</td>
<td>1.01 ± 0.13</td>
<td>0.85 ± 0.14</td>
<td>0.74 ± 0.11**</td>
</tr>
<tr>
<td>Var/mean (S\textsubscript{15-S\textsubscript{20}}) (pA)</td>
<td>29.3 ± 9.0</td>
<td>14.5 ± 7.4</td>
<td>13.7 ± 8.2</td>
<td>11.5 ± 7.3*</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>35.2 ± 6.1</td>
<td>15.1 ± 8.8</td>
<td>13.4 ± 5.1</td>
<td>10.8 ± 4.7*</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.7 ± 4.6</td>
<td>12.4 ± 5.8</td>
<td>11.4 ± 3.7</td>
<td>11.6 ± 4.1</td>
</tr>
<tr>
<td>m\textsubscript{1}</td>
<td>35 ± 10</td>
<td>28 ± 12</td>
<td>29 ± 9</td>
<td>27 ± 13</td>
</tr>
<tr>
<td>m\textsubscript{15-20}</td>
<td>17 ± 8</td>
<td>16 ± 9</td>
<td>16 ± 7</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>m\textsubscript{12}/m\textsubscript{10}</td>
<td>1.19 ± 0.09</td>
<td>0.99 ± 0.12</td>
<td>1.21 ± 0.14</td>
<td>1.60 ± 0.11**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5; data from neurons of table 3.2.6A; * (relative to control) ** (relative to low Ca\textsuperscript{2+}) P < 0.05, ANOVA.
gap ($S_{12}/S_{10}$) nearly doubled. The reduction in apparent QN was similar to that observed in normal $Ca^{2+}$ (cf. Table 3.2.4A). Hence, pentobarbital effects on STD were additive to the increased EPSC rundown caused by raised $Ca^{2+}$.

Raising $[Ca^{2+}]_e$ did not greatly alter pentobarbital effects on quantal size and content (Table 3.2.5B). In raised $[Ca^{2+}]_e$, pentobarbital decreased the ratio of the plateau to the 1st apparent quantal size and increased the ratio of the quantal contents around the intra-train gap, similar to the effects observed in 2 mM $[Ca^{2+}]_e$ (Table 3.2.5B, cf. Table 3.2.4B). However, reduction in $Q'$ was much less than in 2 mM $[Ca^{2+}]_e$.

3.3.4.3. STD in reduced $Ca^{2+}$ concentration

Reducing $[Ca^{2+}]_e$ from 2 to 0.1 mM did not affect the pentobarbital enhancement of STD (Table 3.2.6A, B). In low $[Ca^{2+}]_e$, pentobarbital still produced a greater rundown then it did in normal $[Ca^{2+}]_e$ media. The effects of low $[Ca^{2+}]_e$ to reduce the apparent quantal size, variance-mean ratio, and evoked minEPSC size were further modified by pentobarbital, which caused further rundown in $Q'$, and an increased ratio of the quantal contents around the intra-train gap. This unexpected latter effect implicates a presynaptic action on the high release pool of quantal packets which mediate STD in low $[Ca^{2+}]_e$ media. In summary, pentobarbital enhancement of STD was resistant to reductions in $[Ca^{2+}]_e$. 
3.3.4.4. Combined cyclothiazide and kynurenate blockade

In order to unmask the presynaptic actions of pentobarbital, it was necessary to reduce the postsynaptic contributions to STD. In other neurons, pentobarbital has been reported to have postsynaptic actions of promoting AMPA receptor desensitization (Jackson et al., 2003), which would contribute to STD. Receptor saturation is also a possible contributor to STD (Chen et al., 2002). For these reasons, pentobarbital actions on STD were re-examined during pharmacological blockade of receptor desensitization and saturation.

Pentobarbital enhancement of STD was unaffected by a combined blockade of receptor desensitization and saturation. During co-application of CTZ (50 µM) with KYN (50 µM), pentobarbital (200 µM) still increased the rundown of EPSCs in a use-dependent manner (Figure 3.27; Table 3.2.7A). The apparent quantal size plateau \( (Q'(S_{15-20})) \), ratio of \( Q(S_{15-S20})/Q'S_1 \), variance-mean ratio, and evoked minEPSC size all decreased in response to pentobarbital (Table 3.2.7B). In addition, the quantal content plateau \( (m_{15-20}) \) also increased significantly relative to control or co-applied CTZ + KYN (Table 3.2.7B). The above data implicated an enhancement of STD by a presynaptic action that reduced the quantal size.

3.3.5. Effects of altered extracellular K\(^+\) concentration ([K\(^+\)]\(_e\)) on STD

Transmitter release is sensitive to [K\(^+\)]\(_e\) alterations, which modify the membrane potential in the nerve terminal (Hatt and Smith 1976; Saint et al. 1987). This provided a rationale to examine the effects of altering [K\(^+\)]\(_e\) on STD and their modulation by pentobarbital.
Figure 3.27. Pentobarbital enhancement of STD during combined blockade of receptor desensitization and saturation. Co-application of CTZ (50 μM) with KYN (50 μM) reduced the amplitude of EPSCs (left) and abolished the decrease in quantal size throughout the train (middle). The quantal content (right) had a time course similar to that of EPSC amplitude (left) in the presence of co-applied CTZ and KYN. Under these conditions, pentobarbital application enhanced EPSC depression (left) and reduced the quantal size (middle) throughout the duration of EPSC train. In parallel, pentobarbital increased the quantal content (right) during the train at a rate similar, and possibly due to the decrease in quantal size. Data are from 5 neurons. Holding potential was -80 mV. Error bars of 1st response show between neuron variations.
Table 3.2.7A: Pentobarbital effects on parameters of STD during co-applied CTZ and KYN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CTZ + KYN (50 µM)</th>
<th>Pentobarbital + CTZ + KYN (200 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(50 µM)</td>
<td>(50 µM)</td>
<td>(50 µM)</td>
</tr>
<tr>
<td>S₁ (nA)</td>
<td>1.29 ± 0.17</td>
<td>0.89 ± 0.13*</td>
<td>0.85 ± 0.14*</td>
</tr>
<tr>
<td>S₂ (nA)</td>
<td>1.04 ± 0.13</td>
<td>0.62 ± 0.14*</td>
<td>0.60 ± 0.18*</td>
</tr>
<tr>
<td>S₂/S₁</td>
<td>0.85 ± 0.10</td>
<td>0.88 ± 0.17</td>
<td>0.70 ± 0.11</td>
</tr>
<tr>
<td>S₁₂/S₁₀</td>
<td>1.28 ± 0.19</td>
<td>1.21 ± 0.10</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>Plateau (S₁₅₋₂₀)</td>
<td>0.61 ± 0.16</td>
<td>0.49 ± 0.14</td>
<td>0.34 ± 0.11</td>
</tr>
<tr>
<td>Plateau/S₁</td>
<td>0.47 ± 0.08</td>
<td>0.55 ± 0.05</td>
<td>0.40 ± 0.06**</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>3.99 ± 0.18</td>
<td>2.63 ± 0.41</td>
<td>3.42 ± 0.27</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6; * (relative to control)
** (relative to CTZ + KYN) P < 0.05 ANOVA test. Data are from 10 Hz trains.
Table 3.2.7B: Pentobarbital effects on derived parameters of STD during co-applied CTZ and KYN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CTZ + KYN (50 µM)</th>
<th>Pentobarbital + CTZ + KYN (50 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S₁,S₂)</td>
<td>-0.024 ± 0.013</td>
<td>-0.011 ± 0.008</td>
<td>-0.019 ± 0.011</td>
</tr>
<tr>
<td>Q'(S₁) (pA)</td>
<td>37.5 ± 5.7</td>
<td>25.3 ± 4.5</td>
<td>24.5 ± 5.3</td>
</tr>
<tr>
<td>Q'(S₂) (pA)</td>
<td>32.6 ± 3.9</td>
<td>26.1 ± 2.1*</td>
<td>17.6 ± 1.9*</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀) (pA)</td>
<td>27.3 ± 2.8</td>
<td>24.8 ± 3.1</td>
<td>4.1 ± 3.8*</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀) / Q'(S₁)</td>
<td>0.71 ± 0.13</td>
<td>0.98 ± 0.10</td>
<td>0.17 ± 0.11*</td>
</tr>
<tr>
<td>Var/mean (S₁₅-S₂₀) (pA)</td>
<td>29.4 ± 3.7</td>
<td>25.2 ± 5.1</td>
<td>6.9 ± 4.4*</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>31.0 ± 2.6</td>
<td>27.6 ± 4.6</td>
<td>10.3 ± 3.8*</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>12.3 ± 1.9</td>
<td>8.9 ± 2.4</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td>m₁</td>
<td>34 ± 14</td>
<td>37 ± 16</td>
<td>36 ± 10</td>
</tr>
<tr>
<td>m₁₅₂₀</td>
<td>24 ± 4</td>
<td>15 ± 5</td>
<td>78 ± 9*</td>
</tr>
<tr>
<td>m₁₁₂/m₁₁₀</td>
<td>1.21 ± 0.18</td>
<td>1.28 ± 0.21</td>
<td>1.09 ± 0.17</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 6; data from same neurons as Table 3.2.7A; * (relative to control) ** (relative to CTZ + KYN) P < 0.05, ANOVA.
3.3.5.1. High $[K^+]_e$ perfusion

Raising $[K^+]_e$ from 2.5 to 10 mM caused significant changes in the shape of STD (Figure 3.28A; Table 3.2.8A). The amplitude of the 2nd EPSC facilitated relative to the 1st EPSC (Figure 3.28A). The EPSC plateau increased whereas the rundown (Plateau/S1 ratio) was raised compared to control $[K^+]_e$ conditions (Table 3.2.8A). The apparent QN nearly doubled in response to high $[K^+]_e$ application (Table 3.2.8A), indicating an enhancement of refill or recruitment of sites previously with low fractional release, now to be included in the releasable pool (Quastel, 1997).

Unexpectedly, high $[K^+]_e$ application abolished the covariance between the 1st and 2nd EPSC, yielding a small positive value (Table 3.2.8B). This might or might not be a statistical aberration. The apparent quantal size, variance-mean ratio and evoked minEPSC size were unaffected by high $[K^+]_e$ (Table 3.2.8B). These data implicated a presynaptic action that increased the fractional release and pulse to pulse facilitation.

In 10 mM $[K^+]_e$, pentobarbital had a marked effect on the shape of EPSC train. Pentobarbital effects included: 1) transforming the facilitation between the 1st and 2nd EPSC into depression; 2) decreasing the plateau; and, 3) increasing the rundown of EPSCs, (Figure 3.28 A; Table 3.2.8A). The apparent QN also decreased, similar to the effects in normal $[K^+]_e$ media (cf. Table 3.2.4A). A similar effect of pentobarbital was observed, after correcting QN for $\alpha$ values ($\text{QN}/(1+\alpha)$; $\alpha$ values were 0.15 ± 0.04 in control, 0.16 ± 0.02 in high $[K^+]$, and 0.12 ± 0.03 in pentobarbital/high $[K^+]$ ($P > 0.05$, t-test). The corrected QN values were (in nA) 5.11 ± 0.22 in control, 9.38 ± 0.41 in high
[K⁺]e, and 5.04 ± 0.37 in pentobarbital and high [K⁺]e (\(P < 0.05, t\)-test). Pentobarbital also restored the negative covariance at the beginning of the train. The use-dependent effects of pentobarbital on the decline of the apparent quantal size early in the train (\(Q(S_{15}-S_{20})/Q(S_1)\) ratio) were similar to the effects in normal [K⁺]e (Table 3.2.8B). The total pentobarbital suppression of the increase in \(S_2/S_1\) produced by 10 mM [K⁺]e indicated a presynaptic effect of pentobarbital distinct from what was seen in other experiments.

The apparent increase in QN by 10 mM K⁺ reflects the raised \(S_2, S_3, S_4\) – presumably reflecting a combination of high \(\alpha\) and rising \(P_0\). The unchanged \(S_1\) in high [K⁺]e indicated that QN (~6 nA) did not change at the beginning of the train. The data indicate fractional release of about 0.3 for \(S_1\) and \(S_2\) under control conditions, with 10 mM [K⁺]e raising fractional release to about 0.9 or more at \(S_2\). Hence, hypothetically, fractional release could have increased greatly if \(\alpha\) after the first pulse remained low, or could have remained nearly unchanged, if \(\alpha\) were to become near 1, as suggested by the absence of negative covariance, or anywhere in between, depending upon how \(\alpha\) is postulated to have changed.

3.3.5.2. Low [K⁺]e perfusion

Reducing [K⁺]e concentration did not affect STD (Table 3.2.9 A, B). However, the mean ratio of EPSC amplitudes and quantal contents around the intra-train gap decreased compared to normal [K⁺]e (Table 3.2.9A, B). This observation suggested that the post-gap increases in EPSC size and quantal content were attenuated as a result of the low [K⁺]e.
Figure 3.28. Effects of altered $[K^+]_e$ on pentobarbital enhancement of STD.  

A) Effects of raised $[K^+]_e$.  
Top: EPSC traces during application of 2.5 mM $[K^+]_e$ (left), 10 mM $[K^+]_e$ (middle), and 200 µM pentobarbital in 10 mM $[K^+]_e$ (right).  
Bottom: Mean EPSC amplitudes (left), quantal size estimates (middle), and quantal contents (right) from control, raised $[K^+]_e$, and pentobarbital in raised $[K^+]_e$.  
Data are from 5 neurons.  

B) Effects of reduced $[K^+]_e$.  
Top: EPSC traces during application of 2.5 mM $[K^+]_e$ (left), 0.1 mM $[K^+]_e$ (middle), and 200 µM pentobarbital in 0.1 mM $[K^+]_e$ (right).  
Bottom: Mean EPSC amplitudes (left), quantal size estimates (middle), and quantal contents (right) from control, reduced $[K^+]_e$, and pentobarbital in reduced $[K^+]_e$.  
Traces in A and B are responses to single trains from one neuron each.  
Other data are averages from 5 neurons.  
Error bars in 1st response show between-neuron variations.
Table 3.2.8A: Summary of effects of high [$K^+$_c], pentobarbital on parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2.5 mM $K^+$)</th>
<th>High $K^+$ (10 mM)</th>
<th>Pentobarbital 200 µM (in high $K^+$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$ (nA)</td>
<td>1.78 ± 0.25</td>
<td>1.75 ± 0.12</td>
<td>1.73 ± 0.11</td>
</tr>
<tr>
<td>$S_2$ (nA)</td>
<td>1.08 ± 0.15</td>
<td>1.92 ± 0.19*</td>
<td>1.24 ± 0.14**</td>
</tr>
<tr>
<td>$S_2/S_1$</td>
<td>0.60 ± 0.11</td>
<td>1.08 ± 0.17*</td>
<td>0.71 ± 0.15**</td>
</tr>
<tr>
<td>$S_{12}/S_{10}$</td>
<td>1.31 ± 0.10</td>
<td>1.05 ± 0.14*</td>
<td>1.45 ± 0.17**</td>
</tr>
<tr>
<td>Plateau (nA)</td>
<td>0.67 ± 0.15</td>
<td>0.93 ± 0.19*</td>
<td>0.52 ± 0.16**</td>
</tr>
<tr>
<td>Plateau/S_1</td>
<td>0.38 ± 0.10</td>
<td>0.53 ± 0.19*</td>
<td>0.31 ± 0.21**</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>5.91 ± 0.25</td>
<td>10.83 ± 0.33*</td>
<td>5.65 ± 0.47**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5; * (relative to control) ** (relative to high $K^+$) $P < 0.05$ ANOVA test. Data are from 10 Hz trains.
Table 3.2.8B: Derived parameters of STD in high [K$^+$]$_e$, pentobarbital

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2.5 mM K$^+$)</th>
<th>High K$^+$ (10 mM)</th>
<th>Pentobarbital 200 μM (in 10 mM K$^+$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{cov}(S_1, S_2)$</td>
<td>-0.017 ± 0.007</td>
<td>0.003 ± 0.005*</td>
<td>-0.011 ± 0.009</td>
</tr>
<tr>
<td>$Q'(S_1)$ (pA)</td>
<td>34.1 ± 7.4</td>
<td>33.8 ± 3.0</td>
<td>33.6 ± 2.5</td>
</tr>
<tr>
<td>$Q'(S_2)$ (pA)</td>
<td>29.0 ± 6.9</td>
<td>28.7 ± 2.9</td>
<td>28.3 ± 2.6</td>
</tr>
<tr>
<td>$Q'(S_{15}-S_{20})$ (pA)</td>
<td>28.3 ± 6.1</td>
<td>29.9 ± 10.8</td>
<td>18.0 ± 7.1</td>
</tr>
<tr>
<td>$Q'(S_{15}-S_{20}) / Q'(S_1)$</td>
<td>0.83 ± 0.10</td>
<td>0.88 ± 0.17</td>
<td>0.53 ± 0.09*</td>
</tr>
<tr>
<td>$\text{Var/ Mean}$ (S$<em>{15}$-S$</em>{20}$) (pA)</td>
<td>30.1 ± 4.4</td>
<td>32.3 ± 5.1</td>
<td>20.3 ± 6.2</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>32.0 ± 5.3</td>
<td>31.8 ± 6.6</td>
<td>24.3 ± 4.2</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.1 ± 3.4</td>
<td>12.3 ± 4.1</td>
<td>10.9 ± 3.7</td>
</tr>
<tr>
<td>$m_1$</td>
<td>54 ± 8</td>
<td>52 ± 6</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>$m_{15-20}$</td>
<td>23 ± 3</td>
<td>32 ± 4</td>
<td>44 ± 6</td>
</tr>
<tr>
<td>$m_{12}/m_{10}$</td>
<td>1.32 ± 0.07</td>
<td>1.09 ± 0.09</td>
<td>1.15 ± 0.11</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5; data from same neurons as table 3.2.8A; * (relative to control) ** (relative to high K$^+$) $P < 0.05$ ANOVA test.
Table 3.2.9A: Summary of effects of low [K\textsuperscript{+}], pentobarbital on parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2.5 mM K\textsuperscript{+})</th>
<th>Low K\textsuperscript{+} (0.1 mM)</th>
<th>Pentobarbital 200 µM (in 0.1 mM K\textsuperscript{+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S\textsubscript{1} (nA)</td>
<td>1.65 ± 0.20</td>
<td>1.60 ± 0.17</td>
<td>1.55 ± 0.25</td>
</tr>
<tr>
<td>S\textsubscript{2} (nA)</td>
<td>1.10 ± 0.17</td>
<td>0.99 ± 0.10</td>
<td>1.42 ± 0.15</td>
</tr>
<tr>
<td>S\textsubscript{2}/S\textsubscript{1}</td>
<td>0.66 ± 0.13</td>
<td>0.40 ± 0.18</td>
<td>0.91 ± 0.25</td>
</tr>
<tr>
<td>S\textsubscript{12}/S\textsubscript{10}</td>
<td>1.21 ± 0.07</td>
<td>1.01 ± 0.09</td>
<td>1.40 ± 0.11*</td>
</tr>
<tr>
<td>Plateau (S\textsubscript{15-20}) (nA)</td>
<td>0.67 ± 0.18</td>
<td>0.60 ± 0.11</td>
<td>0.39 ± 0.17</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>5.90 ± 0.30</td>
<td>5.25 ± 0.23</td>
<td>6.25 ± 0.43*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5; * (relative to low K\textsuperscript{+})
P < 0.05, ANOVA test. Data are from 10 Hz trains.
Table 3.2.9B: Derived parameters of STD in low $[K^+]_o$, pentobarbital

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (2.5 mM K$^+$)</th>
<th>Low K$^+$ (0.1 mM)</th>
<th>Pentobarbital 200 µM (in 0.1 mM K$^+$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{cov}(S_1, S_2)$</td>
<td>-0.019 ± 0.009</td>
<td>-0.015 ± 0.007</td>
<td>-0.009 ± 0.006</td>
</tr>
<tr>
<td>$Q'(S_1)$ (pA)</td>
<td>33.4 ± 2.5</td>
<td>33.0 ± 11.3</td>
<td>32.3 ± 8.9</td>
</tr>
<tr>
<td>$Q'(S_2)$ (pA)</td>
<td>29.5 ± 2.0</td>
<td>28.7 ± 9.5</td>
<td>27.8 ± 12.1</td>
</tr>
<tr>
<td>$Q'(S_{15-20})$ (pA)</td>
<td>28.5 ± 5.7</td>
<td>28.0 ± 8.1</td>
<td>21.8 ± 6.9</td>
</tr>
<tr>
<td>$\frac{Q'(S_{15-20})}{Q'(S_1)}$</td>
<td>0.85 ± 0.12</td>
<td>0.84 ± 0.19</td>
<td>0.67 ± 0.11</td>
</tr>
<tr>
<td>$\text{Var}/\text{Mean}$ $(S_{15-20})$ (pA)</td>
<td>31.4 ± 6.1</td>
<td>30.5 ± 7.0</td>
<td>23.4 ± 8.2</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>30.1 ± 4.1</td>
<td>29.7 ± 8.0</td>
<td>26.1 ± 5.1</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>9.7 ± 2.4</td>
<td>10.1 ± 3.2</td>
<td>9.9 ± 3.9</td>
</tr>
<tr>
<td>$m_1$</td>
<td>54 ± 9</td>
<td>50 ± 5</td>
<td>51 ± 8</td>
</tr>
<tr>
<td>$m_{15-20}$</td>
<td>23 ± 9</td>
<td>21 ± 6</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>$m_{12}/m_{10}$</td>
<td>1.33 ± 0.23</td>
<td>1.04 ± 0.16</td>
<td>1.43 ± 0.14$^*$</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; data from same neurons as table 3.2.9A; $n = 5$, * (relative to low K$^+$) $P < 0.05$ ANOVA test.
contrary to effect of nerve terminal hyperpolarization at the neuromuscular junction which would theoretically increase release (Hubbard et al., 1967).

In low $[K^+]_o$, pentobarbital had few effects on STD (Table 3.2.9A, B). The sole significant effect was to increase the post-gap jump in EPSC amplitude (Table 3.2.9A). This effect is compatible with a raised fractional release ($P_o$), raised $P_o(1-\alpha)$, and/or lowered $\alpha$, and occurred in conjunction with increases in the quantal content (Figure 28B, Table 3.2.9B). Hence, reducing $[K^+]_o$ revealed release-promoting actions of pentobarbital, distinct from those observed in normal or raised $[K^+]_o$.

3.3.6. Effects of tetrodotoxin (TTX)

The following investigations examined the effects of partial blockade of voltage-gated Na$^+$ channels on STD. Application of 8 to 64 nM TTX caused significant changes in the configuration of STD (Figure 3.29). At 32 nM, TTX reduced the 1$^{st}$ and 2$^{nd}$ EPSC amplitudes and increased the rundown of the EPSC train (Figure 3.29; Table 3.2.10A). At this concentration, TTX also significantly reduced the apparent QN. Unexpectedly, the ratio of EPSCs around the intra-train gap increased to a value $> 2$. This observation was in contrast to the prediction of the binomial model that the amplitude of the EPSC following the intra-train gap could increase only to twice as much as the previous EPSC amplitude. This result suggested that some blockade of presynaptic action potentials reversed the increased gap between stimuli. At 64 nM, TTX irreversibly abolished EPSCs (Figure 3.29), as expected if presynaptic action potentials were completely blocked.
TTX effects on STD also involved a use-independent reduction in quantal size in the entire EPSC train (Figure 3.29; Table 3.2.1B). The decrease in the apparent quantal size was significant already at the 1<sup>st</sup> and 2<sup>nd</sup> responses (Q'(S<sub>1</sub>), Q'(S<sub>2</sub>)). The plateau of the apparent quantal size (Q'(S<sub>15-20</sub>)) and its ratio to the 1<sup>st</sup> quantal size (Q'(S<sub>15-20</sub>)/ Q'(S<sub>1</sub>)) also decreased significantly during TTX (Table 3.2.1B). These effects of TTX coincided with reductions in the variance-mean ratio and the evoked minEPSC size (Figure 3.30; Table 3.2.1B). The amplitude of pre-stimulation minEPSCs was unaffected by TTX application, arguing against a postsynaptic action. Hence, TTX effects on STD likely involved a presynaptic use-dependent action that produced smaller quanta, plus an effect to reduce quantal size that did not reverse in the 20 s between-train period.

In order to unmask the net presynaptic actions of TTX, it was necessary to re-examine its effects in conditions that reduce postsynaptic contributions to STD. For this reason, the experiments were repeated with co-applied CTZ and KYN. As previously observed, co-application of CTZ (50 µM) with KYN (50 µM) decreased the 2<sup>nd</sup> and plateau EPSC amplitudes as well as the apparent QN (cf. Table 3.2.3A). A subsequent application of TTX (32 nM) produced a lower EPSC plateau and apparent QN values and a faster EPSC rundown, similar to the effects without co-applied CTZ + KYN (cf. Table 3.2.10A).

In these experiments, TTX affected quantal parameters nearly the same way as without co-applied CTZ and KYN (cf. Table 3.2.10B). The apparent quantal size decreased throughout the train (Table 3.2.11B). The plateau of apparent quantal size, variance-mean
Figure 3.29. Tetrodotoxin enhanced STD by reducing quantal size. A) Traces of EPSC trains during application of TTX at 8, 32, and 64 nM. At 8 - 32 nM TTX application enhanced STD producing a greater and faster rundown of EPSCs. At 32 nM, TTX decreased the amplitude of the EPSCs early in the train. Application of TTX at 64 nM resulted in irreversible loss of EPSCs. B) Left: Mean EPSC amplitude from 6 neurons before and during application of 8 and 32 nM TTX. Middle: Apparent quantal size estimates during STD. At 32 nM, TTX reduced the quantal size significantly (P < 0.01, t-test). The quantal size decreased and reached a plateau at response 4. Right: Quantal content during TTX enhancement of STD. Note the increase in quantal content following the intra-train gap. Data are from 5 neurons. Holding potential was - 80 mV. Error bars of first response show between-neuron variations.
Figure 3.30. TTX decreased the size of evoked miniature EPSCs without affecting spontaneous miniature EPSC size. A) Amplitude histograms of spontaneous minEPSCs 5 s before the stimulus train (6 repeats, 30 s in total). B) Amplitude histograms of evoked minEPSCs counted 1 s after the stimulus train (6 repeats, 6 s total). Total mini count is indicated above histograms. Evoked minEPSC sizes were obtained after subtraction of spontaneous from evoked minEPSC histogram (B – A). Values next to black arrows pointing at peaks are mean size ± SEM. Data are from 1 neuron.
Table 3.2.10A: Summary of TTX effects on parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>8</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (nA)</td>
<td>1.19 ± 0.33</td>
<td>0.95 ± 0.36</td>
<td>0.49 ± 0.28*</td>
</tr>
<tr>
<td>S2 (nA)</td>
<td>0.87 ± 0.14</td>
<td>0.80 ± 0.34</td>
<td>0.40 ± 0.16*</td>
</tr>
<tr>
<td>S2/S1</td>
<td>0.73 ± 0.07</td>
<td>0.84 ± 0.09</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>S12/S10</td>
<td>1.51 ± 0.11</td>
<td>1.40 ± 0.19</td>
<td>2.52 ± 0.41*</td>
</tr>
<tr>
<td>Plateau</td>
<td>0.39 ± 0.08</td>
<td>0.20 ± 0.12</td>
<td>0.12 ± 0.09*</td>
</tr>
<tr>
<td>(S15-20) (nA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plateau/ S1</td>
<td>0.32 ± 0.11</td>
<td>0.21 ± 0.07</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>4.40 ± 0.17</td>
<td>4.59 ± 0.31</td>
<td>1.82 ± 0.35*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5, *P < 0.05, ANOVA.
Data are from 10 Hz trains.
### Table 3.2.10B: TTX effects on derived parameters of STD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>8</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S₁, S₂)</td>
<td>- 0.026 ± 0.012</td>
<td>- 0.019 ± 0.013</td>
<td>- 0.014 ± 0.009</td>
</tr>
<tr>
<td>Q'(S₁) (pA)</td>
<td>33.5 ± 2.5</td>
<td>34.6 ± 6.3</td>
<td>17.8 ± 5.1*</td>
</tr>
<tr>
<td>Q'(S₂) (pA)</td>
<td>27.8 ± 2.0</td>
<td>30.0 ± 5.3</td>
<td>14.2 ± 3.6*</td>
</tr>
<tr>
<td>Q'(S₁₋₀₋₂₀) (pA)</td>
<td>24.5 ± 3.6</td>
<td>19.8 ± 4.3</td>
<td>7.9 ± 3.8*</td>
</tr>
<tr>
<td>Q'(S₁₋₀₋₂₀) Q'(S₁)</td>
<td>0.73 ± 0.11</td>
<td>0.57 ± 0.09</td>
<td>0.44 ± 0.07*</td>
</tr>
<tr>
<td>Var/Mean (S₁₋₀₋₂₀) (pA)</td>
<td>27.8 ± 4.7</td>
<td>22.9 ± 5.1</td>
<td>9.6 ± 2.8*</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>32.1 ± 4.1</td>
<td>22.3 ± 3.0</td>
<td>12.9 ± 3.3*</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>10.1 ± 2.0</td>
<td>10.7 ± 1.6</td>
<td>9.8 ± 1.9</td>
</tr>
<tr>
<td>m₁</td>
<td>35 ± 6</td>
<td>27 ± 10</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>m₁₋₀₋₂₀</td>
<td>18 ± 3</td>
<td>10 ± 7</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>m₁₂/m₁₀</td>
<td>1.52 ± 0.25</td>
<td>1.42 ± 0.31</td>
<td>2.52 ± 0.27*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; data from same neurons as Table 3.2.10A; n = 5, * P < 0.05, ANOVA.
Figure 3.31. TTX effects on STD during blockade of receptor desensitization and saturation. Co-application of CTZ (50 mM) with KYN reduced the amplitude of EPSCs without affecting the rundown (left). Under these conditions, the quantal size (middle) did not change throughout the train whereas the quantal content (right) followed the time course of the EPSC train. A subsequent application of TTX (32 nM) enhanced STD reducing the plateau and increasing the rundown. TTX decreased the quantal size, already at the 2\textsuperscript{nd} response and increased the quantal content, from the 8\textsuperscript{th} response until the end of the train (right). Data are from 5 neurons. Holding potential was -80 mV. Error bars of 1\textsuperscript{st} response show between-neuron variations.
Table 3.2.11A: TTX effects on parameters of STD during co-applied CTZ + KYN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CTZ + KYN (50 μM)</th>
<th>TTX + CTZ + KYN (32 nM) (50 μM) (50 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁ (nA)</td>
<td>1.29 ± 0.24</td>
<td>0.85 ± 0.18</td>
<td>0.83 ± 0.16*</td>
</tr>
<tr>
<td>S₂ (nA)</td>
<td>1.04 ± 0.13</td>
<td>0.62 ± 0.10*</td>
<td>0.51 ± 0.11*</td>
</tr>
<tr>
<td>S₂/S₁</td>
<td>0.80 ± 0.10</td>
<td>0.73 ± 0.07</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td>S₁₂/S₁₀</td>
<td>1.21 ± 0.13</td>
<td>1.15 ± 0.09</td>
<td>1.22 ± 0.08</td>
</tr>
<tr>
<td>Plateau (S₁₅-₂₀)</td>
<td>0.62 ± 0.10</td>
<td>0.38 ± 0.11*</td>
<td>0.22 ± 0.12*</td>
</tr>
<tr>
<td>Plateau/S₁</td>
<td>0.48 ± 0.09</td>
<td>0.44 ± 0.06</td>
<td>0.26 ± 0.05**</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td>5.04 ± 0.35</td>
<td>3.04 ± 0.25*</td>
<td>2.5 ± 0.31**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5, * (relative to control) ** (relative to control and CTZ + KYN) P < 0.05, ANOVA.
Data are from 10 Hz trains.
Table 3.2.11B: TTX effects on derived parameters of STD during co-applied CTZ and KYN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CTZ + KYN (50 μM)</th>
<th>TTX + CTZ + KYN (32 nM) (50 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S₁, S₂)</td>
<td>-0.026 ± 0.010</td>
<td>-0.013 ± 0.007</td>
<td>-0.018 ± 0.013</td>
</tr>
<tr>
<td>Q'(S₁) (pA)</td>
<td>37.0 ± 6.1</td>
<td>24.7 ± 5.1*</td>
<td>25.6 ± 4.3*</td>
</tr>
<tr>
<td>Q'(S₂) (pA)</td>
<td>32.6 ± 5.0</td>
<td>25.6 ± 3.7</td>
<td>19.1 ± 4.3*</td>
</tr>
<tr>
<td>Q'(S₁₅-S₂₀) (pA)</td>
<td>27.1 ± 3.7</td>
<td>24.8 ± 5.1</td>
<td>5.1 ± 2.9**</td>
</tr>
<tr>
<td>(\frac{Q'(S₁₅-S₂₀)}{Q'(S₁)})</td>
<td>0.73 ± 0.19</td>
<td>1.03 ± 0.11*</td>
<td>0.20 ± 0.13**</td>
</tr>
<tr>
<td>Var/mean (S₁₅-S₂₀) (pA)</td>
<td>29.4 ± 3.3</td>
<td>26.1 ± 2.8</td>
<td>7.2 ± 2.5**</td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>32.0 ± 5.3</td>
<td>27.6 ± 4.2</td>
<td>9.4 ± 3.7**</td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.1 ± 3.4</td>
<td>10.9 ± 2.4</td>
<td>10.4 ± 3.1</td>
</tr>
<tr>
<td>(m₁)</td>
<td>34 ± 11</td>
<td>33 ± 14</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>(m₁₅-2₀)</td>
<td>23 ± 9</td>
<td>15 ± 8</td>
<td>50 ± 11**</td>
</tr>
<tr>
<td>(\frac{m₁₂}{m₁₀})</td>
<td>1.23 ± 0.12</td>
<td>1.27 ± 0.14</td>
<td>1.43 ± 0.18</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; data from same neurons as table 3.2.11A; n = 5, * (relative to control) ** (relative to control and CTZ + KYN) \(P < 0.05\), ANOVA test.
ratio, and evoked minEPSC size decreased as without CTZ and KYN. Also, TTX co-applied with CTZ and KYN did not affect the amplitude of pre-stimulation minEPSCs. Thus, receptor desensitization or saturation did not mediate TTX effects on quantal size.

3.3.7. Effects of glucose deprivation on STD

Because of the extensive literature relating barbiturate actions to effects on metabolism (see Introduction), the next experiments explored how STD might be altered during conditions of limited energy supply, for which brief glucose deprivation was used.

Overall, graded reductions in glucose concentrations caused a gradual nullification of STD. Stepping the glucose concentration to values lower than 25 mM caused a decrease in the amplitude of EPSCs both at the beginning of trains and subsequently (Figure 3.32; Table 3.2.12A). This was accompanied by an increase of the Plateau/S₁ ratio and a reduction in the apparent QN. The post-gap jump was also reduced with decreasing glucose concentrations (Figure 3.32; Table 3.2.12A). Hence, low glucose produced an apparent reduction in fractional release.

The gradual nullification of STD due to glucose deprivations was accompanied by a loss of the negative covariance terms early in the train (Figure 3.32 E). Figure 3.32E shows that for control conditions in 25 mM glucose, the covariance term, equal to - 1/N, decreased from cov(S₁,S₂) to cov(S₁,S₃); The data suggest that either between-train nonstationarity becomes relatively high, and/or fractional release becomes so small that covariances become nearly undetectable. In principle, the variance is related to p by
Var(S) = <S>·Q·(1 – p), which is scarcely affected by p when p is very small, with p = m/N. The covariance, -<S₁><S₂>/N = p·Q·<S₂> is correspondingly small and therefore readily obscured by between-train non-stationarity, e.g., random failure of some nerve terminals to participate in the response.

As glucose concentrations were stepped down to below 10 mM, the apparent quantal sizes, the variance-mean ratios as well as the size of evoked minEPSCs decreased significantly to values in the 9-12 pA range (Figure 3.32, 3.33; Table 3.2.12B). The ratio of quantal contents around the intra-train gap also decreased, with lowering glucose concentrations. Glucose deprivation did not alter the size of pre-stimulation minEPSCs (Figure 3.33; Table 3.2.12B). The effects on apparent quantal size and evoked minEPSC size, and lack of effects on pre-simulation minEPSC size, implicate a presynaptic site (or sites), sensitive to glucose deprivation, particularly during periods of intense synaptic activity, with little or no recovery in the 20 s between train period.

The effects of nominally zero glucose conditions were examined on evoked and spontaneous EPSCs. Glucose omission from the perfusion media after 1 min resulted in no change in the holding current but an irreversible loss of both spontaneous and evoked EPSCs. In 3 neurons, large, irreversible increases in holding current and input conductance that occurred at 10 min signified a loss of cell viability. Despite 15-40 min periods of observation in these neurons, re-establishing control perfusion after the 3 min omission did not result in any recovery of the EPSCs.
Figure 3.32. Effects of glucose deprivation on STD. A) Current traces of corticothalamic EPSCs during STD evoked during brief glucose deprivations. *Top:* Lowering glucose concentration from 25 to 10 mM (middle trace) decreased the amplitude of the entire EPSC train without changing the rundown. *Middle:* Lowering glucose concentration from 25 to 5 mM further decreased the amplitude of the entire EPSC train and also increased the rundown. *Bottom:* Reducing glucose from 25 to 2.5 mM abolished STD (middle trace). B) Average EPSCs amplitudes (n = 6). C) Apparent quantal size estimates during glucose deprivation. D) Quantal content estimates from the neurons in B and C. E) Covariance estimates obtained by pairing the 1st EPSC with the 2nd – 5th EPSC at different glucose concentrations. The linear regression line is proportional to the rate of vesicular replenishment.
Table 3.2.12A: Summary of parameters of STD at different glucose concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[glucose] (mM)</th>
<th>25</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.20 ± 0.16</td>
<td>0.93 ± 0.13</td>
<td>0.63 ± 0.19*</td>
<td>0.20 ± 0.08*</td>
</tr>
<tr>
<td>S1 (nA)</td>
<td></td>
<td>0.73 ± 0.07</td>
<td>0.42 ± 0.08</td>
<td>0.26 ± 0.06*</td>
<td>0.16 ± 0.08*</td>
</tr>
<tr>
<td>S2/S1</td>
<td></td>
<td>0.61 ± 0.11</td>
<td>0.45 ± 0.08</td>
<td>0.42 ± 0.06</td>
<td>0.79 ± 0.09</td>
</tr>
<tr>
<td>S12/S10</td>
<td></td>
<td>1.27 ± 0.08</td>
<td>1.29 ± 0.13</td>
<td>0.95 ± 0.09</td>
<td>1.04 ± 0.07*</td>
</tr>
<tr>
<td>Plateau (S15-20) (nA)</td>
<td></td>
<td>0.54 ± 0.16</td>
<td>0.40 ± 0.19</td>
<td>0.30 ± 0.11</td>
<td>0.21 ± 0.13*</td>
</tr>
<tr>
<td>Plateau/S1</td>
<td></td>
<td>0.45 ± 0.10</td>
<td>0.43 ± 0.09</td>
<td>0.47 ± 0.09</td>
<td>1.05 ± 0.13**</td>
</tr>
<tr>
<td>Apparent QN (nA)</td>
<td></td>
<td>4.31 ± 0.33</td>
<td>2.69 ± 0.31*</td>
<td>1.76 ± 0.29*</td>
<td>1.09 ± 0.41*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; n = 5, * (relative to glucose 25 mM) ** (relative to all other groups) P < 0.05, ANOVA test. Data are from 10 Hz trains.
Figure 3.33. Evoked and spontaneous miniature EPSCs during glucose deprivation. A) Amplitude histograms of spontaneous minEPSCs counted 5 s before the stimulus train (6 repeats, 30 s total) during glucose deprivation produced by stepping glucose concentration from 25 to 10, 5, and to 2.5 mM. B) Amplitude histograms of evoked minEPSCs counted 1 s after the stimulus train (6 repeats, 6 s total) during the same conditions as in A. Total minEPSC count ('minis') is indicated above histograms. Evoked minEPSC sizes were obtained after subtraction of spontaneous from evoked minEPSC histogram (B - A). Values next to black arrows are mean size ± SEM. Data are from 1 neuron.
Table 3.2.12B: Derived parameters of STD at different glucose concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[glucose] (mM)</th>
<th>25</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>cov(S₁,S₂) (nA²)</td>
<td>-0.022 ± 0.013</td>
<td>-0.009 ± 0.006</td>
<td>0.002 ± 0.003</td>
<td>4x10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>Q'(S₁) (pA)</td>
<td>35.9 ± 8.1</td>
<td>30.1 ± 9.1</td>
<td>18.2 ± 10*</td>
<td>10.7 ± 4.8*</td>
<td></td>
</tr>
<tr>
<td>Q'(S₂) (pA)</td>
<td>31.6 ± 7.9</td>
<td>29 ± 5.1</td>
<td>16.3 ± 3.8</td>
<td>9.2 ± 6.2*</td>
<td></td>
</tr>
<tr>
<td>Q'(S₁5-S₂0) (pA)</td>
<td>29.2 ± 3.8</td>
<td>25.6 ± 4.3</td>
<td>12.9 ± 2.1*</td>
<td>8.9 ± 3.3*</td>
<td></td>
</tr>
<tr>
<td>Q'(S₁5-S₂0) / Q'(S₁)</td>
<td>0.81 ± 0.12</td>
<td>0.85 ± 0.15</td>
<td>0.70 ± 0.11</td>
<td>0.83 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>Var/Mean (S₁5-S₂0) (pA)</td>
<td>28.3 ± 3.2</td>
<td>26.7 ± 2.5</td>
<td>14.6 ± 4.1*</td>
<td>11.4 ± 2.1*</td>
<td></td>
</tr>
<tr>
<td>Evoked minEPSC size (pA)</td>
<td>30.5 ± 3.3</td>
<td>28.1 ± 3.4</td>
<td>18.6 ± 2.1*</td>
<td>10.2 ± 2.8**</td>
<td></td>
</tr>
<tr>
<td>Pre-stimulation minEPSC size (pA)</td>
<td>11.8 ± 4.1</td>
<td>11.4 ± 2.8</td>
<td>10.8 ± 3.6</td>
<td>10.2 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>m₁</td>
<td>33 ± 4</td>
<td>26 ± 3</td>
<td>34 ± 7</td>
<td>19 ± 8</td>
<td></td>
</tr>
<tr>
<td>m₁5-20</td>
<td>19 ± 2</td>
<td>14 ± 3</td>
<td>23 ± 8</td>
<td>23 ± 7</td>
<td></td>
</tr>
<tr>
<td>m₁2/m₁₀</td>
<td>1.23 ± 0.07</td>
<td>1.26 ± 0.08</td>
<td>1.00 ± 0.05*</td>
<td>0.98 ± 0.07*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM between neurons; data from same neurons as table 3.2.12A; n = 5, * (relative to glucose 25 and 10 mM) ** (relative to all other groups) P < 0.05, ANOVA test.
3.8. Discussion

The present studies have demonstrated that pentobarbital produced changes in quantal parameters during short-term depression of corticothalamic EPSCs. In control conditions, the quantal aspects of synaptic transmission was found to behave similarly to that at neuromuscular and calyx of Held synapses (Scheuss and Neher, 2001; Scheuss et al., 2002). In particular, there were significant negative covariances between responses to the first and second stimuli, as predicted by the binomial/depletion model. In addition, STD, at different stimulation frequencies, was modulated in much the same way as at the neuromuscular junction (Elmqvist and Quastel, 1965a). The plateau phase of EPSCs subsequent to their rundown can be explained by refill eventually matching release characteristics, rather than a different population of release sites becoming involved. This proposal applies to the stimulation frequencies studied here but may not apply for higher frequencies where refill rates remain unaltered (Wesseling and Lo, 2002).

This agreement with the binomial/depletion model provided a validation for using variance/mean ratios, corrected using covariances, to obtain estimates of apparent quantal size $Q'$ (theoretically $Q(1 + CV_Q^2)$), at each stimulus. The results then showed that a substantial part of STD arises from decline in $Q'$, as at calyx of Held (Scheuss et al., 2002), another glutamatergic (AMPA) synapse, and unlike the neuromuscular junction (Elmqvist and Quastel, 1965a).

The validity of the statistical estimates of $Q'$ was also established by their agreement (late in trains) with the size of minEPSCs evoked by the stimulation (cf. Otsu et al., 2004).
When combined with a pharmacological approach, the statistical method provided estimates for changes in release parameters during corticothalamic STD and their modulation by pentobarbital and other drugs or changes in the bathing medium. A combination of postsynaptic receptor desensitization and saturation contributed to STD by reducing the apparent quantal size. Presynaptically, depletion of readily-releasable quanta mediated the STD that remained in the absence of receptor desensitization and saturation. These findings validate, for the first time, a depletion-based model at a relatively small central synapse and suggest a link between pentobarbital depressant effects and the metabolic requirement during intense processing tasks in the thalamus.

3.3.8.1. Behaviour of EPSCs in trains

Repetitive stimulation produced a frequency-dependent rundown of EPSCs in thalamocortical neurons, illustrating a short-term form of depression, similar to other observations at corticothalamic synapses (Li et. al. 2003; Reichova and Sherman 2004). The detection of negative covariances between successive pairs of EPSCs at the onset of repetitive stimulation validated the application of the depletion model, similar to neuromuscular and calyx of Held synapses (cf. Elmqvist and Quastel, 1965a; Scheuss and Neher, 2001), to obtain estimates of apparent quantal size at each stage in the train. The negative covariances were necessary for corrections of the variance to mean ratios from the initial responses in a train of EPSCs, which provided an estimate of the apparent quantal size. This method also corrected for deviations in the variance to mean ratio attributable to failure of axonal conduction and/or action potential invasion into nerve terminals that might occur during prolonged train stimulation.
However, the corrected variance-mean model estimated the apparent quantal size and not the "true" quantal size. In other words, this approximation, which is based on assumption of binomial characteristics, relates to the true quantal size by additional contributions of within- and between-site variabilities \((1 + CV_B^2)\) and \((1 + CV_W^2)\), respectively; reviewed by Auger and Marty 2000). In other neurons, such variabilities have been estimated by recording at single synapses (Forti et al. 1997) or by comparing distributions of minEPSCs originating at distant terminal branches (Bekkers and Clements 1999). If incorporated into the corrected variance-mean ratio, a future assessment of within- and between site variability at corticothalamic synapses would approximate the true quantal size alterations during STD.

Corticothalamic STD occurred at a frequency range that allows thalamic relay neurons to filter high frequency cortical inputs (cf. Reinker et. al., 2004). At other CNS synapses that receive high frequency inputs, STD occurs preferentially at frequencies > 100 Hz, whereas, low frequency inputs induce primarily facilitation (von Gersdorff and Borst 2002). Thalamic relay neurons receive a variety of cortical inputs in the 2.5-20 Hz range that drive or modulate their firing and firing patterns (Reichova and Sherman 2004). Corticothalamic STD may function as a low-pass filter (Fortune and Rose 2000) and contribute to thalamic processing of somatosensory inputs particularly during conditions of intense stimulation and various waking and sleep states.
3.3.8.2. Effects of $[Ca^{2+}]_e$ on STD

Corticothalamic STD was influenced by but did not critically depend on extracellular $[Ca^{2+}]$. In high $[Ca^{2+}]_e$-containing media, STD behaved as expected, producing a faster rundown of EPSCs and higher plateau (cf. Elmqvist and Quastel, 1965a; Scheuss et al., 2002). The heightened plateau of EPSCs was attributable to an increased quantal content throughout the EPSC train, indicating a presynaptic origin with high $[Ca^{2+}]_e$ increasing $P_0$'s. The greater EPSC size subsequent to an intra-train gap also increased in agreement with raised $P_0$. These observations demonstrate a modulatory presynaptic effect of raised $[Ca^{2+}]_e$ on STD, closely resembling that seen at neuromuscular junction (Elmqvist and Quastel, 1965a).

Surprisingly, reducing $[Ca^{2+}]_e$ did not abolish STD, disproving the assumption of a strict $Ca^{2+}$ dependence. This persistent STD was not characterized by changes in quantal content but rather was attributable to a decrease in quantal size. This apparent dependence of quantal sizes on $[Ca^{2+}]_e$ might implicate the existence of a heterogeneous population of release sites with varying transmitter contents. The concomitant persistence of STD and reductions in quantal size suggest that reducing $[Ca^{2+}]_e$ caused a shift between populations of release sites. Alternatively, the smaller quanta could result from a partial release of transmitter content due to a reduced spread of the local $Ca^{2+}$ signals. Such effects could provide means to regulate corticothalamic plasticity.
3.3.8.3. Receptor desensitization and saturation during STD

Receptor desensitization and saturation contributed to but did not exclusively mediate corticothalamic STD, consistent with observations in calyx of Held neurons (cf. Scheuss et al. 2002). Pharmacological blockade of desensitization and saturation slightly reduced but did not abolish the rundown of corticothalamic EPSCs during train stimulation. The effects demonstrated that STD is attributable to depletion of readily-releasable quanta, which is likely a major component.

However, these results imply a paradox. The binomial/depletion model (and negative covariances) indicate that the signals early in the train after the first response are from sites where there was no previous release of a quantum of transmitter, especially at frequencies $\geq 10\text{Hz}$, where refill is relatively small. But those responses show lowered quantal size (Table 3.2.1B; Figure 3.20). A possible explanation is that desensitization and receptor saturation occur because of overflow of neurotransmitter from sites where quanta were released to neighbouring sites where quanta are subsequently released (Rossi and Hamann, 1998; Isaacson, 1999; DiGregorio et al., 2002; Telgkamp et al., 2004). This is in keeping with the observed morphology of corticothalamic synapses in the cat (Jones and Powell, 1969).

3.3.8.4. Pentobarbital effects on STD

Pentobarbital enhanced STD by a mechanism that involved a use-dependent decrease in quantal size, that was not sensitive to blockade of AMPA receptor desensitization and saturation. The reduction in the apparent quantal size, and evoked minEPSCs occurred
without effects on ongoing minEPSC size observed before stimulation. There are several possibilities which can explain this result. First, the reduction in quantal size is presynaptic, there being a lowered amount of transmitter (glutamate) in each quantum. Second, it could be that with pentobarbital some sites no longer become stimulated presynaptically (e.g. because of action potential failure) and those that continue to be stimulated are associated with smaller quanta, because of filtering at distally located dendrites. Thirdly, it is possible that with stimulation there develops a preferential release of pre-existing quanta that have less than normal amounts of neurotransmitter. Lastly, pentobarbital might enhance or produce a kind of desensitization not seen normally and not blocked by a combination of cyclothiazide and kynurenate.

Other studies have demonstrated a preferred action of pentobarbital to promote the desensitization of the GluR2 subtype of AMPA receptors (Taverna et al. 1994). However, the actions of pentobarbital at this receptor subtype expressed in thalamic neurons (Spreafico et al. 1994), are sensitive to blockade by cyclothiazide (Jackson et al. 2003).

3.3.8.5. Effects of extracellular $K^+$ alterations on STD

High $[K^+]_e$ increased whereas low $[K^+]_e$ reduced the number of released quanta without producing significant changes in quantal size, which indicated a predominantly presynaptic effect. Unexpectedly, raising $[K^+]_e$ to 10 mM, while having no effect on the first EPSC's in trains, greatly increased the second and subsequent EPSCs (Figure 3.28; Table 3.2.8A, B). In other words, facilitation from pulse 1 to pulse 2 was greatly increased. There was also a loss of the negative covariance between $S_1$ and $S_2$, suggesting
a large increase in the rate of replenishment (refill, \( \alpha \)) of the readily releasable store (Kuromi and Kidokoro, 2004). Increasing \([K^+]_e\) may have promoted the back propagation of an action potential which is normally limited by activation of Ca\(^{2+}\)-gated K\(^+\) channels subsequent to a local rise in presynaptic \([Ca^{2+}]\). These results imply that local increases in \([K^+]_e\) subsequent to activity at nerve terminals could modify transmitter release, transforming a depressed into a facilitated synaptic response (cf. Poolos et al., 1987; Nishimura et al., 1993; Kamiya and Zucker, 1994).

In 10 mM \([K^+]_e\), pentobarbital at 200 \(\mu M\) completely blocked the pulse-pulse facilitation produced by raised \([K^+]_e\). This result suggests that whatever the mechanism by which \([K^+]_e\) has this effect (note it is opposite to presynaptic depolarization – see Hubbard et al., 1967) it should be sensitive to lower concentrations of pentobarbital which reduced fractional release without affecting quantal size (cf. Table 3.2.4B). A possible explanation for this is that pentobarbital activated a Ca\(^{2+}\)-gated K\(^+\) channel (Sailer et al., 2004) which blocked the propagation of an antidromic action potential in raised \([K^+]_e\). This possibility could be the focus of further studies.

In low \([K^+]_e\), although STD was apparently unaffected, pentobarbital increased the amplitude of the EPSC after the intra-train gap, indicating an increase in \(P_o\), or \((1 - \alpha)\), which possibly reflected a selection of high probability release sites. In summary, altering \([K^+]_e\) unmasked actions of pentobarbital on parameters of quantal release as well as apparent quantal size.
3.3.8.6. Effects of tetrodotoxin

Because pentobarbital might at least partially act by modifying the presynaptic action potential (Blaustein, 1968), it was worthwhile to study the effect(s) of tetrodotoxin (TTX), an agent considered to act only by blockade of voltage-gated Na$^+$ channels. At a concentration (32 nM) half that producing block of EPSCs, TTX equally reduced the apparent quantal size and evoked minEPSC size, without affecting pre-stimulation minEPSC size (Table 3.2.10B; Figure 3.30). TTX effects presumably resulted from a reduced Na$^+$ entry into the nerve terminal rather than a decrease in depolarization per action potential, which would reduce effective m's, numbers of quanta released per stimulus.

3.3.8.7 Effects of lowered glucose

In low ambient glucose (5 or 2.5 compared to 25 mM; Table 3.2.12A, B), there were two major effects, lowered quantal size and lowered rundown of quantal numbers (m's) in trains (abolition of STD in 2.5 mM). Reductions in the jump after the omitted stimulus also were consistent with reduced P$_0$, compared to controls. This reduction in P$_0$ could be related to the observation that interference with ATP hydrolysis impairs the transition of packets from the resting/non-releasable to the release-competent pool (Heidelberger et al. 2002). However, this would amount to a reduction in $\alpha$ (refill of releasable quanta) rather than reduction in P$_0$. It is therefore likely that energy supply may be critical for both release, per se, and for maintenance of the readily releasable pool of packets during periods of intense stimulation.
As with TTX, quantal sizes did not recover much between trains. Since a specific kind of desensitization produced by low glucose is hardly likely, there are only two remaining possibilities for the lowered Q' (and evoked minEPSC size), namely, reduced amount of transmitter per quantum and/or selection of synapses with small quanta (pre- or postsynaptically). Of these possibilities, the first, lowered neurotransmitter per quantum (or packet) seems by far the more likely, since the uptake of glutamate into synaptic packets critically depends on glucose (Ikemoto et. al., 2002). The present data (Table 3.2.12, A, B) suggest that glucose deprivation impairs the maintenance of transmitter content in the transmitter packets, which constitute the pool or store of quanta from which outputs are evoked.
4. General Discussion

This thesis provides for the first time, evidence for dual pre- and postsynaptic actions of the barbiturate, pentobarbital. The most notable actions were to promote oscillogenesis in a thalamic network and to cause short-term plastic modifications of cortical inputs to thalamic neurons. The details of these actions have received discussion at the end of each Results section. Therefore, I will succinctly summarize the most pertinent findings and then, discuss their relevance to a context of anesthetic mechanisms.

Summary of results

Pentobarbital oscillogenesis. A subanesthetic concentration of pentobarbital induced thalamic oscillations in in vitro preparations. Sustained oscillations at 1-15 Hz required electrical stimulation of the internal capsule, but not elevated temperature or low extracellular [Mg$^{2+}$]. Receptors for glutamate and glycine mediated oscillations in ventrobasal nuclei, disconnected from nRT. Receptors for glutamate and GABA mediated oscillations in nRT, disconnected from ventrobasal nuclei. Pentobarbital oscillogenesis occurred in isolated networks of the ventrobasal and reticular nuclei, mediated by glutamate receptors with frequency modulation by GABA$_{A^-}$, GABA$_{B^-}$, and glycine-receptors.

Spermine modulation. Extracellular spermine acted on the polyamine site of NMDA receptors, to increase membrane rectification on depolarization, to reduce firing threshold, and to slow the decay of corticothalamic EPSPs. The heightened excitability of thalamocortical neurons increased tonic firing evoked by depolarizing current pulses.
and EPSP bursts of action potentials. Spermine increased the rates of rise and amplitudes of low threshold Ca\^{2+} spikes by an unknown mechanism, not mediated by NMDA receptors. By increasing the efficacy of corticothalamic excitation, spermine actions take on importance in the transformation of somatosensory signals to tonic and burst discharge patterns during the juvenile stage of rat brain development.

**Pentobarbital-spermine interactions.** Pentobarbital at an anesthetic concentration reversed spermine actions that prolonged corticothalamic EPSPs in thalamocortical neurons. This effect involved postsynaptic interactions at the polyamine site on NMDA receptors. Pentobarbital shortened the EPSP duration and reversed the prolongation caused by spermine. The opposing effects of pentobarbital and spermine on corticothalamic transmission provide a model for anesthetic modulation of glutamate receptors in thalamic hyperexcitability.

**Pentobarbital effects on short-term depression (STD).** Pentobarbital enhanced STD of corticothalamic EPSCs by decreasing quantal size in a use-dependent manner. These actions were presumably presynaptic because they were insensitive to pharmacological blockade of desensitization and saturation of AMPA receptors. Pentobarbital affected the statistically estimated quantal size (apparent quantal size) and the amplitude of evoked minEPSCs. Prior to electrical stimulation, pentobarbital did not affect the amplitude of ongoing minEPSCs, which reaffirmed a lack of postsynaptic action on spontaneous minEPSCs. The effects of pentobarbital to promote STD, may have resulted from
preferred release of quanta with a small transmitter content, activation of a K$^+$ conductance-mediated shunt of the presynaptic action potential, or impairment of evoked release due to blockade of Ca$^{2+}$ channels (see below).

Alterations in extracellular [K$^+$] on short-term depression. High [K$^+$]$_e$ increased, whereas low [K$^+$]$_e$ reduced the number of released quanta. Significant changes in quantal size did not accompany these effects, indicating that pentobarbital acted predominantly at presynaptic sites. Pentobarbital completely blocked the high [K$^+$]-induced facilitation early in a stimulus train. Low [K$^+$]$_e$ by itself did not affect STD. In combination with low [K$^+$]$_e$, pentobarbital increased the amplitude of the EPSC after the intra-train gap, implicating an increased fractional release due to preferred release from high probability sites.

Effects of tetrodotoxin (TTX) on short-term depression. Application of TTX at a concentration that was half that producing blockade of evoked action potentials, reduced the apparent quantal size and evoked minEPSC size to the same extent. TTX did not affect pre-stimulation minEPSC amplitude, suggestive of a minimal postsynaptic action. The effects of TTX presumably resulted from a reduced Na$^+$ entry into the nerve terminal and not from a decreased depolarization by each action potential. This interpretation was in agreement with a lack of effect on numbers of quanta released per stimulus (effective $m$'s).
**Glucose deprivation and short-term depression.** Glucose deprivation had two major effects (Table 3.2.12A, B) – a reduction in quantal size and lesser rundown of quantal contents (m's). A ten-fold reduction in the extracellular glucose concentration abolished STD and the post-gap jump in EPSC amplitude. These combined effects were consistent with reduced output probability of release, P₀. The combined effects coupled with a reduced quantal size imply that glucose deprivation impaired the maintenance of transmitter content in the packets. It is plausible that the induced energy shortage modifies the pool of quanta from which outputs are normally evoked because glucose deprivation did not affect spontaneous minEPSCs.

**Initiation of pentobarbital oscillations**

Induction of oscillations by pentobarbital seems paradoxical for a drug usually viewed as a CNS depressant. Pentobarbital hyperpolarizes thalamocortical neurons, acting by multiple mechanisms over a subanesthetic concentration range (Wan and Puil, 2002; Wan et al., 2003). Hyperpolarizing activation and deinactivation of intrinsic currents would promote oscillogenesis in a thalamic slice network (cf. McCormick and Pape, 1990), modulated by receptor-gated currents (cf. Steriade et al., 1997).

In the present study, GABAₐ, GABAₜ, and glycine receptors modulated, but none was essential for pentobarbital-induced oscillations (cf. Table 4.1). Glycine receptors likely were critical for oscillations in a dorsal thalamic network, divested of GABAergic inhibition. Glycine is not a recognized neurotransmitter in the thalamus, where GABA
Table 4.1: Receptor involvement in pentobarbital-induced oscillations

<table>
<thead>
<tr>
<th>Recording Site</th>
<th>Glutamate</th>
<th>GABA</th>
<th>Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventrobasal nuclei</td>
<td>Essential</td>
<td>Modulate frequency</td>
<td>Modulate frequency</td>
</tr>
<tr>
<td>Ventrobasal nuclei, without nRT</td>
<td>Essential</td>
<td>No apparent role</td>
<td>Essential</td>
</tr>
<tr>
<td>nRT</td>
<td>-</td>
<td>Essential</td>
<td>No apparent role</td>
</tr>
<tr>
<td>nRT, without ventrobasal nuclei</td>
<td>-</td>
<td>Essential</td>
<td>No apparent role</td>
</tr>
</tbody>
</table>

- not tested
mediates transmission by reticulothalamic and some interneuronal pathways (Steriade et al., 1997). The thalamus exhibits strychnine-binding sites, mRNA for glycine receptor subunits and expresses $\alpha$ subunit protein (Zarbin et al., 1981; Malosio et al., 1991; Naas et al., 1991). Conceivably, activation of glycine receptors may mediate synaptic inhibition in ventrobasal nuclei of rat (cf. Tebecis, 1974; Ghavanini et al., 2005).

A subanesthetic concentration of pentobarbital induced oscillations in a corticothalamic network. These oscillations required glutamatergic excitation, but not GABAergic inhibition from nRT. Glycine receptors were essential for the oscillations in ventrobasal nuclei, isolated from nRT. GABA receptors were essential for the oscillations in nRT, isolated from ventrobasal nuclei. Thus, pentobarbital can induce oscillogenesis in either ventrobasal nuclei or nRT, independent of their reciprocal synaptic connectivity.

The pentobarbital-induced oscillations in slices may have some relevance to the genesis of spindling in the EEG during barbiturate administration (see Introduction). The stimulation may have mimicked the slow rhythm that neocortex imposes through glutamatergic pathways (Steriade et al., 1991, 1997; von Krosigk et al., 1993). The insensitivity to temperature was possibly due to compromised network behavior in the slice (cf. Andersen and Andersen, 1968). The sustained thalamocortical oscillations observed in the slice network may reflect pentobarbital actions that produce sedative-hypnotic effects in vivo (Nelson et al., 2002). Substances that mimic this oscillogenesis may have potential as sedative-hypnotic drugs. The stationary oscillations may represent a model of sedation-hypnosis, amenable to pharmacological analysis.
Pentobarbital likely acted on Mg\(^{2+}\) independent sites. In either normal or low external [Mg\(^{2+}\)], an anesthetic concentration (200 µM; cf. Sato et al., 1995) first increased and then abolished oscillations. Jacobsen et al., (2001) observed that at lower concentrations (100 µM) pentobarbital eliminated ventrobasal oscillations in low [Mg\(^{2+}\)]. Disappearance of oscillations may have resulted from a GABA\(_A\) receptor shunt of glutamate excitation (Wan et al., 2003 and Sawada and Yamamoto, 1985). The new and previous results are consistent with the marked CNS depression that occurs during deep anesthesia in vivo (Sato et al., 1995).

**Spermine modulation of thalamic excitability**

The endogenous polyamine spermine applied in the 1-200 µM range enhanced the excitability of thalamocortical neurons in specific ways that were consistent with a neuromodulator role at P12–P15 stage of development. Spermine actions on NMDA receptors produced a heightened state of excitability which were viewed as prolonged EPSPs, and increased bursting and tonic firing of action potentials. To a large extent, these effects resulted from increased membrane rectification on depolarization and a reduced threshold for action potential genesis. Spermine also modulated the burst firing mode by increasing the rate of rise and amplitude of low threshold Ca\(^{2+}\) spikes (LTSs). This unusual effect did not involve interaction with glutamate receptors. The modulation of corticothalamic excitation and LTSs of MGB neurons may be critical in the transformation of auditory signals in gerbil thalamus at the P14 stage of development.
Spermine is widely distributed in rodent and human brain (Harman and Shaw, 1981; Morrison et al., 1995). A membrane transporter appears to maintain low extracellular concentrations of <1 μM (Dot et al., 2000). These concentrations may increase on NMDA stimulation to > 50 μM in striatal neurons of adult rat brain (Fage et al., 1992). The effects of spermine on NMDA receptors and low threshold Ca\(^{2+}\) spikes in juvenile MGB neurons (ED\(_{50}\) = ~100 μM) are consistent with neuromodulatory actions at high micromolar concentrations (Williams, 1997). Given the role of NMDA receptors during development, such modulation by spermine is likely important for learning processes (Chida et al., 1992).

The present results are relevant to the normal function of the central auditory system. The NMDA receptor-mediated effects of spermine would enhance the ability of thalamic neurons to detect simultaneous inputs, as in coincidence detection. For example, an overexpression of the spermine-sensitive NR2B subunit (Williams et al., 1994) prolongs EPSPs and shortens the time window between two coincident signals in hippocampal neurons (Tang et al., 1999). In thalamic neurons, the generation of synchronous activity may involve coincidence detection (Roy and Alloway, 2001) as well as amplitude selectivity in the MGB neurons (Kuwabara and Suga, 1993).

The effects of spermine on the LTSs of MGB neurons may have relevance for conscious or sleep states and disorders of consciousness. The LTS is essential in the generation of
bursting and oscillatory activity in the auditory nuclei (Hu, 1995; Tennigkeit et al., 1996). By increasing the rate of rise and amplitude of the LTS and slowing its decay, spermine modulation may increase an MGB neuron’s responsiveness of neurons at hyperpolarized potentials (Hu et al., 1994) to inputs during these states (He and Hu, 2002). Modulation by spermine may have importance for bursting behavior during sleep states whereas excessive modulation may occur in absence epilepsy as in audiogenic seizures (Porta et al., 1981), sensitive to blockade by polyamine antagonists (Kotlinska and Liljequist, 1996).

**Pentobarbital reversal of spermine modulation**

Pentobarbital, at an anesthetic concentration, modulated corticothalamic transmission by postsynaptic actions at the polyamine site on NMDA receptors. Pentobarbital effects included shortening of the duration of EPSPs and reversal of their prolongation by extracellular spermine. Consistent with pentobarbital shortening of burst durations and mean open times of NMDA-mediated single channel currents (Charlesworth et al., 1995), these actions on NMDA-mediated transmission may contribute to its depressant effects on oscillogenesis.

Like other polyamines, spermine increases the potency of barbiturates to induce general anesthesia (Daniell, 1992). The basis for this enhancement is presently unclear because of the observed opposing actions of pentobarbital and spermine on the prolongation of NMDA mediated EPSPs. Indeed, pentobarbital depression of transmission prevented the
prolongation of NMDA responses caused by spermine. The effects and interactions of pentobarbital might have relevance for various forms of epilepsy. For example, NMDA receptors are activated in thalamocortical neurons during the development of spike-and-wave discharges in a strain of genetic absence epilepsy rats (Koerner et al., 1996). Similarly, an injection of NMDA into subthalamic nuclei of rats generates audiogenic seizure behaviour (Faingold el al. 1989) and increases neuronal firing in MGB neurons (N’Gouemo and Faingold, 1997). Hence, pentobarbital actions that shorten corticothalamic EPSPs might alleviate NMDA mediated epileptic seizures.

**Presumed presynaptic actions of pentobarbital**

An anesthetic concentration of pentobarbital enhanced STD at corticothalamic synapses. The increased STD can be attributed to a presynaptic use-dependent action that produced small size quanta. The effects persisted during blockade of AMPA receptor desensitization and saturation and were not accompanied by alterations in spontaneous minEPSC size. The rapid fall in quantal size implies a rather small population of preformed quanta. This was evident in the fast reduction in quantal size after the 3rd stimulus in the train. The reduction can also be explained by use-dependent inactivation of Ca\(^{2+}\) channels which reduces Ca\(^{2+}\) entry into terminals early in the train and impairs the formation of releasable quanta. Pentobarbital, at a similar concentration, reduces the amplitude of miniature EPSPs by impairing Ca\(^{2+}\) entry into nerve terminals (Baudoux et. al., 2003). By reducing Ca\(^{2+}\) entry into corticothalamic terminals, pentobarbital could have interfered with glutamate release by selectively affecting a population of small size and/or partially refilled quanta.
Similar effects of tetrodotoxin and pentobarbital

Tetrodotoxin mimicked the pentobarbital enhancement of short-term depression. The effects of TTX to reduce apparent quantal size (Q') in a use-contingent manner differed from that of pentobarbital in that recovery from TTX between iterated trains was incomplete (Table 3.2.10B). TTX had a progressive effect between trains, reducing the number of participating release sites. The same interpretations of a lowered Q' (see Section 3.3.8.4., page 159), apply for TTX as for pentobarbital, except that a specific kind of TTX-induced desensitization seems implausible. The effects of TTX on Q' occurred at half the concentration which blocked action potentials, and likely resulted from reduced Na$^+$ entry into the nerve terminal, rather than from blockade of depolarization. The similar amplitude of pre-stimulation and evoked minEPSCs observed here during TTX application suggests a close resemblance to minEPSCs, or true minEPSCs, observed in other studies during complete blockade of action potentials at TTX concentrations $\geq 1 \mu$M (cf. Edwards et al., 1990).

Given the high selectivity of TTX, these results may imply that these drugs modulate Na$^+$-dependent processes that pump glutamate into releasable packets at corticothalamic terminals. There was a large difference in the concentrations of TTX and pentobarbital that affected transmitter release. Unlike pentobarbital, TTX would likely block axonal invasion of the presynaptic action potential. In axons, such blockade by pentobarbital is evident only at millimolar concentrations (Blaustein, 1968). Pentobarbital depression of thalamic firing occurs at much lower, subanesthetic doses and is attributable to a K$^+$ conductance shunt (Wan et al., 2003). Hence, pentobarbital in the present experiments
could have shunted the presynaptic action potential by activating K\(^+\) conductances in the nerve terminal.

*Effects of glucose deprivation on short-term depression*

The similar effects of glucose deprivation imply that enhancement of short-term depression by pentobarbital was, in part, attributable to its depressant actions on metabolism (see Introduction). The reduction in fractional release during glucose deprivation could be explained by a drop in the ATP content, which impairs the maintenance of a release-competent pool of transmitter vesicles (Heidelberger et al. 2002). By reducing the energy supply, pentobarbital may impair both release, *per se*, and the maintenance of the readily releasable pool of transmitter packets, particularly during periods of increased activity-dependent release of transmitter.

*Apparent redundancy in pentobarbital effects on transmitter release*

The mimicry of action potential blockade and energy deprivation as well as multiple actions on quantal release (cf. Wan et al., 2003), suggests that pentobarbital may have reduced the size of the store and transmitter content of quantal packets. Pentobarbital inhibits glycolysis which provides most of the ATP at synaptic terminals (Crane et al., 1978). A decrease in the ATP content at nerve terminals impairs processes that maintain the content (Ikemoto et al., 2003) and availability (Heidelberger et al. 2002) of readily-releasable quanta. It is also possible that conditions of glucose deprivation or blockade of Na\(^+\) entry by TTX affected separate processes, each impaired by high micromolar concentrations of pentobarbital.
The effects of pentobarbital, TTX, and energy deprivation on transmitter release have an alternative explanation. These effects may involve impairment of action potentials at specific sites, sparing distal sites of transmitter release. Selective blockade would result in a preferred release of the remaining, highly releasable small quanta, and reduced quantal size as observed postsynaptically. This process is compatible with the observations that pentobarbital depletes docked packets at synaptic active zones (Jones and Devon, 1978; Hajos et al. 1978).

Other studies have suggested that heterogeneity in contents of transmitter packets contributes to variation in quantal amplitude (Edwards, 1990). This proposal is tantamount to the proposition that release of partially filled quanta occurs normally. The lowered quantal size due to incomplete filling in the low glucose condition would then be seen as an exaggeration of something that also occurs normally. However, it is also possible that small quanta are preferentially released when energy supply is low and the same could be true in the presence of TTX and pentobarbital.

**Mechanisms of pentobarbital enhancement of short-term depression**

There are at least four distinct mechanisms that could explain the ability of pentobarbital to reduce quantal size: 1) a selective process whereby pentobarbital depleted large size quanta, sparing the smaller size quanta. For example, pentobarbital can interfere with Ca\(^{2+}\) entry (Charlesworth et al., 1995) into active zones critical for transmitter release at nerve terminals. The selective process is consistent with known actions of pentobarbital that deplete a subset of docked packets in active zones (Jones and Devon, 1978; Hajos et
al. 1978); 2) a pentobarbital induced suppression of glycolysis and associated ATP- and 
Na\(^+\) -dependent glutamate uptake (see Introduction) which may reduce transmitter 
content per packet (cf. Gueldry et. al. 1987; Ikemoto et al. 2002); 3) a pentobarbital 
interference with Ca\(^{2+}\) entry (Charlesworth et al., 1995) that alters the kiss-and-run 
(Pocock and Richards, 1987; An and Zenisek, 2004) or porocytotic (Kriebel et al., 2001) 
type openings of fusion pores. This blockade would limit the amount of released 
transmitter; and, 4) a pentobarbital-evoked desensitization of postsynaptic cyclothiazide-
insensitive receptors. This desensitization would reduce the postsynaptically observed 
quantal size, in response to a given amount of transmitter (see Introduction). At present, 
there is no evidence for such receptors on thalamic neurons (cf. Chen et. al. 2002).

In principle, there are many known pre- and postsynaptic sites or targets for barbiturate 
action that could be involved in these experiments on corticothalamic STD (Figure 4.1).
For example, one might expect pentobarbital to activate a K\(^+\) conductance-mediated 
shunt of presynaptic action potentials (Wan et al., 2003), limiting the activation of 
voltage-gated Ca\(^{2+}\) channels and therefore release probability (P\(_0\)). Also \textit{a priori}, one 
might expect that, by interfering with metabolism, pentobarbital might inhibit the transfer 
of resting packets to the releasable store (Heidelberger et al. 2002).

The present results, however, contradict these expectations, particularly those from 
experiments with raised [K\(^+\)]\(_e\). In raised [K\(^+\)]\(_e\), pentobarbital completely inhibited a novel 
action of [K\(^+\)]\(_e\) to increase quantal release only after the first stimulus in the train. This 
finding suggests that a major effect of pentobarbital at lower concentrations might be to
suppress normal modulation of transmitter release by changes in local \([K^+]_e\) at synapses, produced by activity at nearby synapses. An elevated \([K^+]_e\) would arise from release sites/boutons that have not released quanta after the first stimulus. Then, it follows from the depletion model that the transmitter released from nearby synapses would have contributed to the observed greater responses to the second stimuli in a raised \(K^+\) condition (Figure 3.28 A; Table 3.2.8A). The inferred 'cross-talk' between neighbouring synapses, and its modification by drugs, represent an interesting area for further study.

**Limitations**

The methods and experimental approaches in this thesis possess a number of limitations. The \textit{in vitro} oscillations induced by pentobarbital did not include concomitant measurement of the intracellular changes in electrical membrane properties that participate in thalamocortical oscillogenesis (von Krosigk et al., 1993). Simultaneous intra- and extracellular recording during pentobarbital application and oscillogenesis would enable identification of ionic conductances that are essential for producing synchronous rhythmic activity.

The study of extracellular application of spermine on excitability did not exclude possible intracellular interactions. Spermine may have gained access to the cytoplasm through membrane transporters (Fage et al., 1992; Dot et al., 2000). For example, an intracellular action of spermine occurs at \(Ca^{2+}\)-permeable AMPA receptors and inward rectifier \(K^+\) channels (Williams, 1997), which boosts thalamic excitability. Unlike inward rectifier
K⁺ channels, however, Ca²⁺-permeable AMPA receptors have not been identified in the thalamus.

The present study involved stimulation of multiple converging corticothalamic fibres which could result in conduction failure at axonal branches, especially late in the stimulus train. Future studies using focal stimulation (Dunant and Muller, 1986; Quastel and Saint, 1988) may attempt to minimize conduction failure which also may be assessed by simultaneous or independent extracellular recording of fibre volleys (Poolos et al., 1987).

The binomial model is limited by an assumption of independence between release sites (cf. Vere-Jones, 1966). Not surprisingly, many studies have not reported the existence of negative covariances. The lack of negative covariances in these studies may be explained by a positive correlation between active release sites which nullifies the negative covariance that would occur early in the train. Hence, a high level of branch conduction failure may explain the lack of negative covariances in other studies. The negative covariances, here, implicates a minimal contribution of conduction failure to the data.

In the present thesis, the variance and covariance were obtained from sequential repeats of responses. This procedure minimized slow trends that developed between trains throughout the experiment. On the other hand, this approach prevented assessment of slow changes due to drug effects. The procedure may have resulted in an increased sampling error or caused an underestimation of quantal parameters.
The identification of minEPSC size was difficult to estimate within a train and hence, restricted to pre- and post-train periods. The difficulty is attributable to the low signal-to-noise ratio of intra-train minEPSCs. A method that would correct for the time course of the decay of EPSC may enable accurate detection of intra-train minEPSCs. The bin size in the present study resulted in a signal resolution with a minimum value of 5 pA. Studies that have an improved signal-to-noise ratio may provide information on smaller changes in quantal size. This information then could be combined with rise times for estimating the distance between release sites and the synapse.

Unequivocal demonstration of a drug effect requires observations of full reversibility. In most cases, the drug effects were reversible. However, TTX application at concentrations $\geq 64$ nM or the omission of glucose from the ACSF, produced effects that were irreversible during the period of observation, followed by loss of neuronal viability. An inability to observe excitation did not allow a more exact determination of pentobarbital’s effects on spontaneous minEPSCs, normally studied in the presence of $\geq 1 \mu M$ [TTX]. Future studies that incorporate techniques for differentiating evoked and spontaneous transmitter release (cf. Xu and Sastry, 2003), may address this issue. Extensive studies are required to examine reversible effects of glucose deprivations over a much wider concentration range than used here. The new studies may facilitate a distinction between pentobarbital effects on spontaneous and evoked release, for an assessment of its long-term effects on transmission.
Conclusion

The present studies have provided several new contributions to understanding pentobarbital actions on thalamic neurons. A subanesthetic concentration of pentobarbital induced network oscillations in vitro, which would modulate thalamic inhibition and facilitate corticothalamic excitation in vivo. Another original contribution is the validation of the binomial depletion model of transmitter release at a conventional CNS synapse. The analysis has led to novel interpretations about pre- and postsynaptic anesthetic actions of pentobarbital on short-term plasticity during repetitive corticothalamic excitation. These studies also showed interesting similarities in its actions that reduced quantal size, to imposed conditions of impaired Na⁺ entry into nerve terminals and energy shortage due to glucose deprivation. It seems significant that pentobarbital reversed the facilitating effect of elevated \([K^+]_o\) which typically promotes a plastic change in transmission. This action represents a new type of synaptic modulation by barbiturate, complementing known anesthetic actions on thalamic neurons. Many of the actions of pentobarbital, including actions on quantal parameters, are summarized in the schematic diagram of Figure 4.1. The method and analysis technique described for pentobarbital actions in this thesis provide a model for examining the pre- and postsynaptic effects of drugs on transmission in the CNS.
Figure 4.1. Possible synaptic targets for pentobarbital actions during corticothalamic STD. Pentobarbital enhancement of STD might involve ion channel modulation, inhibition of Na\(^+\)-dependent glutamate uptake into readily releasable packets, suppression of glycolytic ATP, and impairment of glutamate exocytosis. Actions on ion channels include: induction of K\(^+\) channel-mediated shunt, blockade of voltage-gated Ca\(^{2+}\) channels, blockade of Na\(^+\) channel-mediated action potentials. Pentobarbital might also inhibit carrier-mediated glucose and Na\(^+\) transport into the terminal cytoplasm. Pentobarbital suppression of glycolysis could reduce ATP-dependent uptake of glutamate into packets. In the case of a heterogeneous population of packets, pentobarbital's inhibition of voltage gated Ca\(^{2+}\) channels might select for small size quanta. Pentobarbital may also shorten the formation of fusion pores thereby reducing exocytotic release of glutamate. Postsynaptically, pentobarbital could promote desensitization of AMPA or NMDA receptors.
Bibliography


Elmqvist D, Quastel DM. Presynaptic action of hemicholinium at the neuromuscular junction. *J Physiol* 1965b, 177: 463 - 482.


Madl JE, Royer SM. Glutamate in synaptic terminals is reduced by lack of glucose but not hypoxia in rat hippocampal slices. *Neuroscience* 1999, 94: 417 - 430.


Bibliography


Roberts EB, Ramoa AS. Enhanced NR2A subunit expression and decreased NMDA receptor decay time at the onset of ocular dominance plasticity in the ferret. *J Neurophysiol* 1999, 81: 2587 - 2591.


Steriade M. The corticothalamic system in sleep. *Front Biosci* 2003, 8: 878 - 899.


von Gersdorff H, Borst JG. Short-term plasticity at the calyx of held. *Nat Rev Neurosci* 2002, 3: 53 - 64.


